INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

UNIVERSITY OF ALBERTA

TOXICOKINETICS OF NON-STEROIDAL ANTIINFLAMMATORY DRUG-INDUCED GASTROINTESTINAL DAMAGE

By

NEAL MAYNARD DAVIES

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

in

Pharmaceutical Sciences (Pharmacokinetics)

Faculty of Pharmacy and Pharmaceutical Sciences

EDMONTON, ALBERTA

Spring, 1996

UNIVERSITY OF ALBERTA

Library Release Form

NAME OF AUTHOR: **NEAL MAYNARD DAVIES**

TITLE OF THESIS: **TOXICOKINETICS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUG-INDUCED GASTROINTESTINAL DAMAGE**

DEGREE: **DOCTOR OF PHILOSOPHY**

YEAR THIS DEGREE GRANTED: **1996**

(SIGNED) Neal M. Davies

PERMANENT ADDRESS: **2912 University Place N.W.**
Calgary, Alberta
Canada, T2N - 4H5

Date: March 20, 1996

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled **"TOXICOKINETICS OF NON-STEROIDAL ANTIINFLAMMATORY DRUG-INDUCED GASTROINTESTINAL DAMAGE"** submitted by **NEAL MAYNARD DAVIES** in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY in PHARMACEUTICAL SCIENCES (PHARMACOKINETICS)**

.....

Dr. E. Jamali, Professor (Supervisor)

.....

Dr. F.M. Pasutto, Professor (Chair)

.....

Dr. Y.K. Tam, Professor

.....

Dr. A.S. Russell, Professor

.....

Dr. J.A. Rogers, Professor

.....

Dr. J.B. Meddings, Associate Professor

External Examiner

DATED: March 19, 96

"Information is not as important as imagination."

"Think the thoughts that have not been thought."

-Albert Einstein

Dedication

This thesis is dedicated to my mother Beth Davies for her unconditional love and support throughout my studies, and to my father Dr. Ronald W. Davies for science is not only his life, it is a way into life.

ABSTRACT

Whereas, NSAIDs demonstrate equivalent antiinflammatory efficacy they may differ in their toxicological profiles. Assessment of gastrointestinal (GI) toxicity has been limited by the diagnostic techniques available. The objectives of this work were to (1) examine the suitability of sucrose and ^{51}Cr-EDTA as non-invasive probes of GI permeability induced by NSAIDs in an animal model; (2) examine the influence of protective agents in the distal intestine; (3) determine the possibility of concentration-effect relationships and NSAID-induced GI damage; (4) evaluate GI permeability of modified release formulations of NSAIDs and, (5) determine the influence of enantiomers on intestinal permeability.

Sprague-Dawley rats were established as a suitable animal model capable of quantifying NSAID-induced permeability throughout the GI tract. Rats were dosed with NSAIDs followed by ^{51}Cr-EDTA and sucrose and urinary excretion of the markers measured. Single therapeutically equivalent doses of NSAIDs increased GI permeability dose-dependently by different extents with indomethacin eliciting the maximum effect. E_{max} type dose-effect and concentration-effect relationships were elucidated. Administration of misoprostol, sulfasalazine, metronidazole, and a free radical scavenger (tempo) significantly reduced NSAID-induced intestinal permeability. A formulation of indomethacin in a vehicle of D-glucose and citrate (G/C) reduced the indomethacin-induced intestinal permeability, however, this was not evident when administered subcutaneously or for other NSAIDs. Pharmacokinetic studies indicated that a significant reduction in bioavailability of the formulation may have accounted for the reduced intestinal permeability.

The R enantiomers of several chiral NSAIDs increased intestinal permeability which was expected for R-ketoprofen and R-ibuprofen due to substantial chiral R to S inversion. Increased intestinal permeability induced by R-etodolac and R-flurbiprofen

was unexpected because these NSAIDs undergo little or no inversion. S-etodolac and S-flurbiprofen at one half the dose of the racemate had greater than or equal potency to the racemate. These findings suggest that prostaglandin-independent mechanisms may be involved in GI permeability, and that certain stereochemically pure "active" enantiomers are not less potent than the racemate.

Gastroduodenal and intestinal permeability were absorption rate dependent with regular release > sustained release (RR>SR and SR>RR), respectively. A linear relationship and anti-clockwise hysteresis were found for the RR and SR formulations from a plot of S-flurbiprofen concentration versus intestinal permeability suggesting that the longer intestinal residence time after SR is responsible for its increased intestinal permeability as compared to the RR formulation.

Acknowledgments

I would like to sincerely thank my supervisor Dr. F. Jamali for his guidance, and interest in these studies. I would also like to thank you for your encouragement and understanding during the initial difficulties in conducting this work. Finally, I am indebted for the plethora of examples of constructive criticism, pertinacious attention to detail, and a deeper understanding of the pharmaceutical sciences that you have set for me.

Thanks to my committee members, Dr. Franco Pasutto, Dr. Yun Tam, Dr. Anthony Russell and Dr. James Rogers for their interest and guidance over the period of these studies. I would also like to express my thanks to my external examiner Dr. Jon Meddings who as a pioneer in the field of gastroduodenal permeabilty inspired several of the studies contained in this thesis.

I also express my sincere appreciation to my colleagues in the laboratory Dr. Brian Corrigan, Dr. Jaber Emamibafrani, Dr. Majid Vakilynejad, Dr. Kenneth Skeith, Dr. Ray Lovlin, and Patrick Mayo for their friendships and many constructive scientific exchanges and suggestions throughout my studies. I also express my gratitude to Dr. Rezaul Mannan, Keith Anderson, Dr. Len Wiebe and Dr. Bruce Stevenson who I have collaborated with on separate projects that have been derived from the work presented in this thesis. I acknowledge the friendship and technical assistance of Dr. Derrick Freitag for the media preparation and microbial work contained in this thesis. I would also like to acknowledge Dr. Glen Kwon for his friendship, and for stimulating and broadening the scope of my scientific interests. Lastly, I would like to express my sincere gratitude to Dr. Matthew Wright, for his many suggestions, discussions, patience, encouragement, and friendship during the initial course of my work.

This work was made possible by grants received from the Arthritis Society of Canada. I also gratefully acknowledge the receipt of my graduate teaching studentship from the University of Alberta.

Table of Contents

List of Tables

List of Figures

List of Symbols and Abbreviations

AA	Arachidonic Acid
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AUC	Area under the plasma concentration-time curve
α	Level of significance
2-APA	2-arylpropionic acid
β	Terminal elimination rate constant
b.i.d.	Twice daily administration
$^{\circ}$ C	Degrees celsius
cAMP	Cyclic adenosine monophosphate
Cl_{TB}	Apparent total body clearance
cm	Centimeters
CNS	Central nervous system
Conc.	Concentration
C_{max}	Maximum plasma concentration after oral doses
COX	Cyclooxygenase
C.V.	Coefficient of variation
^{51}Cr-EDTA	^{51}Chromium ethylenediaminetetraacetate
EC_{50}	Plasma concentration required to elicit a 50% of maximal response

EC	Enteric coated
ED_{50}	Dose required to elicit a 50% of maximal response
E_{max}	Maximal pharmacological effect
ET	Etodolac
F	Bioavailability
Fig.	Figure
FL	Flurbiprofen
g	Centrifugal force
g	Gram
GD	Gastroduodenal
GI	Gastrointestinal
h	Hours
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
H_2SO_4	Sulfuric acid
IB	Ibuprofen
ID	Internal diameter
In	Indium
I.P.	Intraperitoneal
I.S.	Internal standard
I.V.	Intravenous
Kg	Kilograms
KT	Ketoprofen

L	Litre
M	Molar
mg	Milligrams
min.	Minutes
mL	Milliliters
mmol	Millimoles
mM	Millimolar
n	Number of samples
N	Normal
N.D.	Not detectable
ng	Nanogram
NaOH	Sodium hydroxide
nm	Nanometer
NSAID	Non-steroidal antiinflammatory drug
pH	Negative logarithm (base 10) of the hydrogen ion concentration
PEG	Polyethylene glycol
PG	Prostaglandin
qd	Once daily administration
r^2	(Correlation coefficient)^2
R	latin: Rectus
RA	Rheumatoid arthritis
RLQ	Right lower quadrant

r.p.m.	Revolutions per minute
RR	Regular release
RUQ	Right upper quadrant
s	Seconds
S	latin: Sinister
s.d.	Standard deviation
S-D	Sprague-Dawley
sec	Second
s.e.m.	Standard error of the mean
SR	Sustained release
σ^2	Variance
$t_{1/2}$	Half-life
TEA	Triethylamine
t.i.d.	Three times a day
t_{max}	Time to maximal plasma concentration
µg	Micrograms
µl	Microliter
USA	United States of America
USP	United States Pharmacopoeia
UV	Ultraviolet
Vd	Volume of distribution
ZO	Zona occludens
≈	Approximately

1. Introduction

1.1 Non-Steroidal Anti-inflammatory Drugs (NSAIDs)

Salix derivatives were used from Pliny and Hippocrates through the ages until the active ingredient salicylic acid was discovered by Gerhardt at the end of the last century. Phenylbutazone was subsequently introduced in 1952, followed by indomethacin in 1963. During the last fifty years a plethora of NSAIDs have been introduced on the market indicative of the commercial potential for such compounds and their efficacy as antiinflammatory / analgesic agents. There are currently more than 35 NSAIDs available for clinical use worldwide (Blower, 1993). Previous estimates of their use include >100 million prescriptions written throughout the world for these drugs in 1985 at a cost well over $1 billion (Baum *et al.*, 1985).

The anti-inflammatory and analgesic properties of NSAIDs make this therapeutic class of drug particularly useful in the management of rheumatic diseases and musculoskeletal disorders. NSAIDs are also widely used as analgesics in the treatment of pain of varying origin such as dental extraction, trauma, surgery, dysmenorrhea and postpartum / episiotomy. NSAIDs encompass a wide variety of chemical structural classes (Table 1). The vast majority of NSAIDs are weak acids with pka's ranging from 3 to 6.

All NSAIDs when given in equipotent doses have comparable efficacy (*Epstein et al.*, 1984; Wilkens, 1992). Given the apparent equivalent efficacy, the safety or tolerability profile of individual NSAIDs is becoming a principal criterion for therapeutic selection. NSAID prescriptions is currently commonplace and with the

increased introduction of over-the-counter NSAIDs there is a potential for NSAID-associated side-effects to increase.

Table 1. Chemical Classification of NSAIDs

Acetic Acids	Carboxylic Acids	Enolic Acids	Fenamic Acids	Non-Acidic	Oxicams	Propionic Acids	Pyrano carboxylic Acids
Diclofenac	Aspirin	Oxyphenbutazone	Flufenamic	Nabumetone	Tenoxicam	Ibuprofen	Etodolac
Indomethacin	Diflunisal	Phenylbutazone	acid		Piroxicam	Naproxen	Ketorolac
Tolmetin		Trimethazone	Mefenamic		Meloxicam	Fenoprofen	
Sulindac			acid		Isoxicam	Ketoprofen	
			Meclofenamic			Flurbiprofen	
			acid			Tiaprofenic	
						Acid	

1.1.1 Mechanisms of Action of NSAIDs

1.1.1.1 Inhibition of Endogenous Prostaglandin Synthesis

Traditionally, the therapeutic effects and major toxic side-effects of NSAIDs have been attributed to the ability of these drugs to inhibit the synthesis of stable prostaglandins (PGs), through the direct inhibition of prostaglandin H synthetase which serves both as a cyclooxygenase and as a peroxidase (Vane, 1971). Inhibition of the 'cytoprotective' PGs has become regarded as a major factor accounting for the development of GI ulceration and hemorrhage (Whittle and Vane, 1984). In addition, it has recently been determined that cyclooxygenase exists in various isoenzyme forms (Vane *et al.*, 1994). GI toxicity and the highly individualistic response often seen in NSAID therapy may be due in part to the nature of the isoenzymes involved in a specific patient's inflammatory condition and the differential efficacy of various NSAIDs to inhibit that isoenzyme form.

The predominant presentation, particularly in the biomedical literature, has been that prostaglandin H synthetase inhibition is the main mechanism of therapeutic relevance attributable to NSAIDs. Cyclooxygenase inhibition has been widely discussed as being responsible for GI damage attributed to these compounds. However, the lack of a specific link between inhibition of prostaglandin synthesis and development of GI damage is exemplified by the observation that administration of various NSAIDs in rats can reduce mucosal concentrations of PGE_2 and/or PGI_2 *in vivo* without causing appearance of gastric lesions (Whittle and Vane, 1984; Ligumsky *et al*, 1987; Redfern *et al*, 1987).

The mechanisms of induction of GI toxicity by NSAIDs may involve a variety of pathways including inhibition of PG synthesis, alteration of neutrophil function and reduced mucosal blood flow (Levy and Shaw-Smith, 1994). It is generally believed, however, that the GI abnormalities are mainly due to NSAID-induced inhibition of PG synthesis. The inhibitory effect not only causes loss of cell integrity at the GI tract level, but it is assumed to be responsible for the beneficial effects of NSAIDs. Very recently, however, it has been discovered (Mitchell *et al.*, 1994) that the enzymatic system involved in these processes is more complicated than previously thought (Vane, 1971): Cyclooxygenase (COX) metabolizes arachidonic acid (AA) to PGH_2 which in turn is metabolized to various other PGs, prostacyclin and thromboxanes. NSAIDs inhibit synthesis of PGs via inhibition of COX resulting in anti-inflammatory and cytotoxic effects. Interestingly, however, different NSAIDs have different *in vitro* therapeutic / toxicity ratios, which if extrapolated to the *in vivo* situation may lead to much higher cytotoxicity at the GI tract level but almost equal anti-inflammatory activities. A very plausible explanation recently provided (Mitchell *et al.*, 1994) is that COX consists of at least two very different isozymes, a house keeping isozyme called COX_1 and an inducible isozyme called COX_2. It is postulated that inhibition of COX_1 reduces synthesis of cytoprotective compounds such as prostacyclin while inhibition of COX_2 reduces inflammation. Hence, NSAIDs with high EC_{50} COX_2/COX_1 ratios (Table 2) are expected to cause more GI cytotoxicity as the concentration required to elicit beneficial effects exceeds that which yields toxicity.

Table 2. Plasma concentration (mg/L) of NSAIDs required to achieve 50% *in vitro* inhibition (EC_{50}) of COX activity.

NSAID	COX_1	COX_2	COX_2/COX1
Ticlopidine[a]	0.15	inactive	
Piroxicam[a]	0.0005	0.3	600
Tolmetin[a]	0.04	7.0	175
Aspirin[a,b]	0.3	50	166
Sulindac Metabolite[a]	0.4	40	100
Indomethacin[a,b]	0.01	0.6	60
Ibuprofen[b]	1	15	15
Na Salicylate[b]	35	100	2.8
Carprofen[a]	3	3	1
Diclofenac[a,b]	0.5	0.35	0.7
Naproxen[a,b]	2.2	1.3	0.6
BF389[a,b]	0.15	0.03	0.2
6-MNA[*,c]	240	35	0.14
NS398[d]	inactive	potent	

*6-methoxy-2-naphthyl acetic acid, the active metabolite of nambumetone.
Data from a) Akarasereenont et al (1994), b) Mitchell et al (1994), c) Meade et al (1991) and d) Masferrer et al (1994).

As expected, the very important discovery of the COX isozymes has prompted many investigators to search for molecules effective in inhibiting COX_2 with little or no effect on COX_1. Ranking of NSAIDs based on their *in vitro* COX_2/ COX_1 may be more complicated than initially thought as this ranking of NSAIDs does not take into account the possibility of site-specific damage of NSAIDs within the GI tract or the impact of modified release formulations on GI toxicity.

COX_2/ COX_1 *in vitro* inhibition studies may be both tissue and species specific which may lead to discrepancies in many of the reported literature EC_{50} values and ratios. Therefore, in the interpretation of relative inhibition of COX_2/ COX_1 the type

of tissue preparation, species, and the laboratory from which the results are derived must be considered.

Aspirin has been shown to induce gastroduodenal permeability changes (Meddings *et al.*, 1993; Rabassa *et al.*, 1994; Irvine *et al.*, 1995) and is well-known to induce gastroduodenal damage. However, *in vivo* observations of more distal intestinal damage induced by aspirin as measured by increased intestinal permeability, have shown damage to be minimal (Bjarnason *et al.*, 1986a), and little or no intestinal inflammation was evident (Bjarnason and MacPherson, 1989), compared to any other NSAID despite its high COX_2/COX_1 ratio.

1.1.1.2 Prostaglandin Independent Processes

As recently suggested by Malmberg and Yaksh (1992), the observation of the powerful antiinflammatory effects of NSAIDs has likely diverted attention away from many other properties of therapeutic relevance. NSAIDs can activate physicochemical reactions other than the arachidonic acid pathway, such as metabolic functions in the cell membranes (Abramson and Weissmann, 1989). For example, indomethacin is twice as potent in the inhibition of cyclic AMP dependent protein kinase than in the inhibition of prostaglandin H synthetase (Kantor and Hampton, 1978). Subsequently, many other non-prostaglandin dependent mechanisms, including uncoupling of oxidative phosphorylation (Miyahara and Karler, 1985), inhibition of renal anion transport (Snow and Maass, 1979), decreased rheumatoid factor production (Ceuppens *et al.*, 1982), impaired the ability of chondrocytes to synthesize proteoglycans (Ghosh, 1988), changes in neutrophil and leukocyte function (Abramson

and Weissmann, 1989), and interruption of signal transduction through G proteins (Abramson *et al.*, 1991) have been identified as relevant to the therapeutic actions of these compounds (Abramson, 1991). In addition, other therapeutic effects of NSAIDs are independent of arachidonic acid metabolism; for example, inhibition of superoxide generation in neutrophils, inhibition of phospholipase C in macrophages, inhibition of neutrophil aggregation and disruption of protein-protein interactions in the neutrophil plasmalemma (Vane and Botting, 1990; Abramson and Weismann, 1989). More recently, the antiinflammatory action of NSAIDs has also been suggested to be attributable to inhibition of inducible nitric oxide (NO) synthase gene expression in addition to inhibition of prostaglandin synthesis (Aeberhard *et al.*, 1995). The ratio of the inducible NO isozyme to the NO constitutive isozyme may also prove to be a surrogate indicator of therapeutic efficacy or toxicity of NSAIDs.

It is, therefore, plausible that inhibition of COX_1 may not be the only mechanism by which NSAIDs induce GI damage. NSAID-induced side-effects to the GI mucosa have previously been suggested to result from competitive inhibition of specific enzymatic steps in the anaerobic glycolytic pathway and the tricarboxylic acid cycle thereby reducing adenosine triphosphate (ATP) production leading to cell death (Rainsford and Whitehouse, 1980; Somasundaram *et al.*, 1992). NSAIDs have also been suggested to uncouple oxidative phosphorylation, resulting in depletion of cellular adenosine triphosphate (ATP) (Brody, 1956; Glaborg-Jorgensen *et al.*, 1976). The effect of indomethacin on the GI tract in both human (Bjarnason *et al.*, 1992a) and the rat (Rainsford and Whitehouse, 1980; Rainsford and Whitehouse, 1987) can be

reversed by co-administration of a glucose/citrate mixture. This may indicate a mechanism completely independent of COX_1 inhibition.

Furthermore, the R enantiomer of flurbiprofen has been shown to produce antinociceptive activity and, hence, it has been proposed that R enantiomers of NSAIDs be used clinically as analgesics (Brune *et al.*, 1991). As the R enantiomers do not have significant cyclooxygenase activity the effect of these stereochemically pure NSAIDs cannot be completely explained by peripheral prostaglandin synthesis inhibition in inflammed tissue (Geisslinger *et al.*, 1994).

Inhibition of the cyclooxygenase enzyme may also cause diversion of the arachidonic acid metabolism into the lipoxygenase pathway, producing vasoconstrictor peptido-leukotrienes C4 and D4 and potentially cytotoxic peroxy-derived oxyradicals (Rainsford, 1987; Peskar, 1986). Preliminary studies have indicated that 5-lipoxygenase inhibitors and leukotriene antagonists may possess protective properties in gastric mucosa (Rainsford, 1987; Rogers *et al.*, 1986). Whether this cytoprotective effect also applies to the lower GI tract has not been addressed.

More recently, Lagenbach (1995) developed a mouse strain that is unable to synthesize COX_1, however, suprisingly this strain had no gastric pathology, and appears to be less sensitive to NSAID-induced gastric ulceration. These observations also suggest that NSAID-induced GI side-effects may be due to mechanisms other than (or in addition to) COX_1 inhibition.

1.1.2 NSAID-Induced Upper Gastrointestinal Side-Effects

The clinical use of NSAIDs has been associated with numerous side-effects, the most important in frequency and clinical impact being GI disturbances (Rainsford, 1989). The GI side-effects of NSAIDs were recognized more than a century ago (Myers, 1876). NSAID GI pathology accounts for more than 70,000 hospitalizations and 7,000 deaths annually in the United States (Fries, 1991). Adverse effects in the GI tract have contributed to the termination of clinical studies and the withdrawal from the market of at least seventeen individual NSAIDs (Rainsford, 1987). Epidemiological studies indicate significant differences in the incidence of various NSAID-induced adverse effects including those occurring in the upper GI tract (Wilkens, 1992; Fries *et al.*, 1991; Giercksky *et al.*, 1989; Zeidler, 1991; Committee on Safety of Medicines, 1986). The most apparent side-effect of NSAID use is irritation of the upper GI mucosa, which may manifest itself as gastric pain, heartburn, nausea, vomiting, bleeding, dyspepsia, ulceration, perforation, and in severe cases, hemorrhage and death (Rampton, 1987). The cause for these symptoms is often unknown although in a proportion of patients these are attributed to gastritis or to aggravation of pre-existing peptic ulcer disease. A number of studies have identified a dose-dependency for NSAID-induced upper GI tract toxicity (Griffen *et al.*, 1991; Carson *et al.*, 1987). Although gastroduodenal toxicity is believed to be the principal adverse reaction of NSAID use, there is still insufficient data to accurately rank the safety profile of individual NSAID therapy.

1.1.3 NSAID-Induced Intestinal Damage

Numerous articles examining the gastric and duodenal damage caused by NSAIDs have been published (Beard *et al.*, 1987; Haslock, 1990). However, there is often a lack of correlation between gastric symptoms and gastroscopic evidence of damage with as many as one third of patients being completely asymptomatic (Silvoso *et al.*, 1979). Moreover, amongst patients with gastroduodenal lesions and blood loss, healing of the lesions is not always accompanied by an improvement of anemia (Upadhyay *et al.*, 1990). This may suggest the occurrence of GI afflictions in more distal sites of the GI tract.

Evidence that NSAIDs induce ileal manifestations comes from several case reports (Shack, 1966; Day, 1983; Langman *et al.*, 1985; Madhok *et al.*, 1986; Saw *et al.*, 1990) (Table 3). Recently, the more distal intestinal disturbances caused by NSAIDs have received closer attention (Aabakken, 1992; Bjarnason et al, 1993a). It has been suggested that the prevalence of distal intestinal side-effects may exceed that detected in the gastroduodenum and may be of major toxicological significance (Bjarnason et al, 1986a). In an epidemiological study the expected incidence of lower bowel perforations and bleedings was determined to be 10 and 7 per 100, 000, respectively (Langman *et al.*, 1985). Considering the magnitude of worldwide NSAID use these clinical manifestations undoubtedly contribute to significant morbidity in many patients.

There is a growing body of evidence that distal intestinal damage may be more widespread and of greater serious consequence than previously thought. Allison *et al.*

(1992) have recently reported the increased incidence of small intestinal ulceration in patients prescribed NSAIDs and, although these ulcers are less common than those in the stomach or duodenum, these may be life-threatening. Morris *et al.* (1992) have also reported that 41% of RA patients taking NSAIDs with iron deficiency anemia and undiagnosed GI blood loss had evidence of small intestinal lesions, erosions and ulcers iatrogenically attributed to NSAIDs upon small bowel enteroscopy. Furthermore, it has also been demonstrated that preterm infants may develop an increased risk of necrotizing enterocolitis and intestinal perforation when receiving indomethacin for closure of a patent ductus arteriosus (Nagaraj et al, 1981; Alpan et al, 1985; Marshall, 1985).

Table 3 Case Reports of NSAID-Induced Small Intestinal Toxicity

Authors	NSAID(s)	#	Age	Gender	Duration of Use	Toxicological Effect
Lennert and Kootz (1967)	phenylbutazone	1	54	M	NR	luminal stenoses, ulceration, peritonitis
Davies and Brightmore (1970)	phenylbutazone	1	46	M	NR	obstruction of small intestine annular stricture
	phenylbutazone and aspirin+	1	36	F	NR	obstruction of mid-jejunum. perforation, abscess in left iliac fossa
	indomethacin	1	57	F	NR	perforation of ileocecal valve
	phenylbutazone+	1	76	F	NR	perforation of antimesenteric border
Sturges and Krone (1973)	indomethacin	1	45	M	5 years	jejunal strictures, nausea vomiting, weight loss, jejunal ulceration, subnucosal fibrosis
Chadwick et al. (1976)	mefenamic acid*	1 1	44 69	F F	NR	steatorrhea, abdominal distention, weight loss
Nagaraj et al (1981)	indomethacin+	21	PI	NR	1-14 days	necrotozing enterocolitis, focal perforation of the GI tract
Marlow and Chiswick (1982)	indomethacin+	1	3 months	F	12 weeks	bloody stools, peritonitis, cecal perforations, necrotising enterocolitis
Neoptolemos et al. (1983)	phenylbutazone	1	73	F	2-5 years	abdominal colic, distension, diarrhea, anemia, multiple strictures, ulcers
Hall et al. (1983)	mefenamic acid*	1	43	M	3 years	diarrhea, abdominal colic, weight loss, jejunal dilation, inflammatory infiltrate
	mefenamic acid	1	69	M	NR	diarrhea, weight loss

Alpan et al (1985)	indomethacin+	1	2 days	M	1 days	abdominal distension bloody stools, jejunal perforation
	indomethacin+	1	4 days	M	3 day	abdominal distension, perforation of teminal ileum
	indomethacin	1	1 day	F	3 days	abdominal distension, jejunal-ileal junction perforation
	indomethacin	1	2 days	F	3 days	abdominal distension, perforated terminal ileum
Kühl et al (1985)	indomethacin	1	30weeks	M	7 days	abdominal distension, vomiting, enterocolitis, pneumo-peritoneum, peritonitis, perforations
	indomethacin	1	28	F	11 days	intestinal perforation, peritonitis
	indomethacin+	1	26	M	8 days	intestinal perforation, septic shock, peritonitis
Stewart et al (1985)	indomethacin	1	38	M	4 days	abdominal pain, diarrhea, peritonitis, perforated sigmoid colon and multiple sigmoid diverticulae and small bowel perforations
Hervás et al (1986)	indomethacin	1	2 days	F	6 days	vomiting, abdominal distension,perforation of terminal ileum
Madhok et al. (1986)	phenylbutazone naproxen	1	51	F	2 weeks	bleeding, peri-umbilical pain, melena, malaise, ileal bleeding,
	indomethacin	1	62	M	10 years	diarrhea, ulceration, anemia
	indomethacin	1	70	M	14 months	iron deficiency anemia, ileal stricture
Saverymuttu et al. (1986)	indomethacin	1	53	F	5 years	anemia, stricture, intestinal obstruction, ulceration
Freeman (1986)	sulindac*	1	64	F	3 days	diarrhea, small bowel lesion, abdominal cramping, iron deficiency anemia

Bjarnason et al (1987)	various NSAIDs	39	NR	NR	various	small intestinal inflammation, ulceration strictures, bile acid malabsorption
Johnston (1987)	azapropazone	1	62	M	5 years	small bowel dilation, diaphragm strictures
Isaacs et al. (1987)	mefenamic acid	1	66	F	14 years	diarrhea, weight loss,villous atrophy steatorrhoea, anemia, abnormal vitamin B12 absorption
	mefenamic acid	1	73	F	1.25 years	steatorrhoea, weight loss, abnormal vitamin B12
Sukumar (1987)	piroxicam	1	75	F	3 years	weight loss, abdominal pain, vomiting, anemia, strictures, ulceration
Bem et al (1988)	Various NSAIDs	42	NR	NR	NR	intestinal perforation
Bjarnason et al (1988)	aspirin and azapropazone	1	40	F	5 years	weight loss, fever, anemia, diarrhea, hypoalbuminemia, right iliac fossa inflammation, jejunal and ileal strictures, and ileal ulcerations
	piroxicam	1	70	F	1.5 years	anemia, fatigue, weight loss, colicky abdominal pain, diarrhea, fever, hypoalbuminemia, small intestinal obstruction and diaphragms
	piroxicam	1	64	F	3 years	small intestinal obstruction ileal strictures
	naproxen	1	65	F	4 months	hypoalbuminemia, weight loss, inflammation of right iliac fossa, ileitis, small intestinal strictures

Lang et al. (1988)	phenylbutazone	1	NR	NR	NR	ileal stricture, submucosal fibrosis
	piroxicam	1	70	NR	18 months	anemia, subacute obstruction submucosal fibrosis
	azapropazone	1	40	NR	3 years	anemia, subacute obstruction submucosal fibrosis
	piroxicam ketoprofen brufen	1	75	NR	NR	subacute obstruction, mid-ileum submucosal fibrosis
	piroxicam	1	64	NR	3 years	subacute obstruction submucosal fibrosis
	piroxicam	1	72	NR	5 years	submucosal fibrosis
	various	1	78	NR	NR	submucosal fibrosis
Levi et al. (1990)	phenylbutazone	1	40	M	2 weeks	diarrhea, vomiting stricture, intestinal obstruction, and ulcers inflammation, weight loss, anemia, bloating
Saw et al. (1990)	piroxicam	1	67	F	3 weeks	intestinal obstruction, bleeding, haemorrhage, perforation, multiple mucosal ulcer throughout ileum, cecum and ascending colon
Zanardo et al (1990)	indomethacin	1	31w	F	NR	intestinal perforation, pneumo-peritoneum
Morris et al (1991)	various NSAIDs	10	NR	NR	NR	anemia, jejunal ileal ulceration, red spot
Morris et al (1992)	various NSAIDs	19	NR	NR	NR	anemia, 7 red spot lesions, 6 erosions, 13 ulcers
McCune et al (1992)	azapropazone and piroxicam	1	62	F	5 years	obstruction, diaphragms, small bowel herniation, sub mucosal fibrosis, abdominal pain

Hershfield (1992)	various NSAIDs	1	64	F	1.5-15 years	abdominal pain, distention, vomiting, anemia, hypoalbuminemia, strictures,
Matsuhashi et al. (1992)	diclofenac piroxicam	1	60	F	18 years	ulcers, anemia, multiple ileal ulcers and strictures, melena, abdominal pain
Keating and Rees (1992)	piroxicam	1	47	M	1 year	weight loss, anemia, diaphragm strictures, abdominal pain
Fellows et al. (1992)	flurbiprofen	1	69	F	7 years	anorexia, weight loss, nausea, vomiting, abdominal bloating, anemia, strictures
Going et al. (1993)	indomethacin	1	60	F	15 years	circumferential linear ulcers, peritonitis, death
Morris et al (1994)	various NSAIDs	26	NR	NR	various	red sopt lesions, erosions, ulcers, anemia
Kwo and Tremaine (1995)	ibuprofen	1	37	F	2 years	diarrhea, ileocecal ulcer, anemia, multiple diaphragm-like strictures

NR= not reported, * rechallenge, + death

The most apparent side-effects of NSAID use in the lower intestine which is manifest as increased intestinal permeability, bleeding, diaphragm like-strictures, ulceration, perforation, and in severe cases hemorrhage and death (Bjarnason *et al.*, 1993a). Blood loss from the lower GI tract may result in significant morbidity contributing to the anemia of patients with RA taking NSAIDs. Indeed it has been postulated that bleeding from lower intestinal ulcers and not from the stomach or duodenum may be the major source of blood loss from the GI tract (Bjarnason and MacPherson, 1989). Additionally vitamin B_{12} and bile acid absorption may also be impaired contributing to anemia and increased morbidity (Morris *et al.*, 1992; *Davies et al.*, 1996). Studies have shown that up to 70% of patients taking NSAIDs chronically develop intestinal inflammation associated with blood and protein loss, and on discontinuation of NSAIDs this intestinal inflammation may persist for up to 16 months (Bjarnason et al, 1987a,b,c). Further studies have shown that long-term NSAID treatment leads to enhanced migration of ^{111}Indium-labelled leucocytes to the ileum indicating small bowel inflammation. This, together with evidence of increased fecal ^{111}Indium excretion provides further evidence that NSAIDs cause intestinal inflammation in a substantial number of patients. (Bjarnason *et al.*, 1984a; Seagal *et al.*, 1986; Rooney *et al.*, 1986). The possibility of a heterogenous nature in the ability of NSAIDs to induce intestinal inflammation has been suggested as there was a trend for aspirin to induce little or no intestinal inflammation and to be relatively less toxic in the intestine than in the stomach, as compared to other NSAIDS. However, a limited

sample size limits a firm conclusion from these observations (Brodie *et al*, 1970; Shriver *et al*, 1975; Bjarnason and MacPherson, 1989).

'Diaphragmatic' like strictures induced by NSAIDs after long term administration to patients have been reported by several investigators (Sturges and Krone, 1973; Bjarnason *et al.*, 1988a; Matsuhashi *et al.*, 1992; Saverymuttu *et al.*, 1986). NSAID also induce narrow-based ileal stenoses (Deakin, 1988; Lang *et al.*, 1988). The pathology may be characteristic, if not pathognomic, for NSAIDs and has been termed 'diaphragm disease' (Lang *et al.*, 1988). These are multiple (3-70), thin (2 to 4 mm), concentric, septate-like mucosal projections that narrow the lumen to 0.1 to 2 cm. These are mainly located in the midsmall intestine and are histologically characterized by prominent submucosal fibrosis with no evidence of vascular involvement (Levi *et al.*, 1990).

While the clinical presentation of NSAID-induced gastropathy - bleeding - may be more dramatic than enteropathy, short or long-term pathogenic importance of this adverse effect of NSAIDs on the intestine has not been fully characterized. Consequently, the more distal intestinal disturbances induced by NSAIDs are receiving closer attention (Madok, 1986, Aabakken, 1992).

1.4 **Techniques for Assessing NSAID-GI Damage**

Gastroduodenal (GD) endoscopy has been the gold standard method for assessing the gross toxicological manifestations of NSAIDs because it has been generally believed that side-effects of NSAIDs are usually confined to the upper GI tract. Expectedly, therefore, a great deal of concern has been directed towards gastric

and duodenal damage by NSAIDs and many modified release formulations (i.e. enteric coated and sustained release formulations) have been prepared to prevent or minimize direct contact of NSAIDs with the upper GD tract.

Endoscopy for diagnosis of upper GD damage induced by NSAIDs is unsuitable as a screening test as it is time-consuming and expensive. Detection of NSAID-induced upper GD abnormalities is also associated with difficulties such as the determination of the site of affliction, distinctions between degrees of damage, and interference by other pathophysiological complications. Upper GD lesions associated with NSAIDs may be described as edema, erythema, mucosal hemorrhage, erosions, or ulcers. Investigators often give numerical values to each type of lesion and total them up to get a score for each patient. However, these scoring systems are subjective and therefore susceptible to error (McCarthy, 1989). Furthermore, detection by endoscopy is usually only obtained after one of the complications (obstruction, perforation, or hemorrhage) becomes clinically apparent (Davies and Brightmore, 1970).

Since the early 1980s, however, substantial efforts have been made to develop non-invasive methods of detecting GI tract abnormalities. Intercellular junctions of GI epithelial cells appear to be particularly susceptible to a variety of noxious agents. Consequently, they may be the first organelle to suffer when the energy production of the enterocyte is compromised (Bjarnason and Peters, 1989). This results in disruption of intercellular integrity and allows permeation of macromolecules into the GI tract mucosa. The degree of GI tract penetration by passively absorbed, water soluble, molecules is referred to as called permeability. Tests of GI tract permeability are

designed to assess the functional integrity of the intestine. This is done non-invasively by measurement of urinary recovery of orally-administered probes. Methods based upon measurement of GI tract permeability have been found to be extremely useful in measuring gastroduodenal (GD) and intestinal damage induced by NSAIDs in human studies (Meddings *et al*, 1993; Bjarnason *et al*, 1984a).

1.4.1 Methods of Assessing Gastrointestinal Permeability

1.4.1.1 Intestine

In vivo intestinal permeability has been assessed by a number of analytical techniques. The three most commonly employed markers are the urinary excretion following oral ingestion of carbohydrates (i.e. lactulose, cellobiose, and mannitol), ethylene glycol polymers [i.e. polyethylene glycol (PEG)], and non-degradable radionuclide probes such as ^{51}Cr-EDTA (Tagesson *et al.*, 1984; Juby *et al.*, 1986; Aabakken, 1989).

The movement of molecules across biological membranes may occur via three series of processes, 1) simple diffusion 2) perorption and 3) specific transport mechanisms. The specific transport mechanisms include those that are carrier-mediated such as, 1) facilitated diffusion, 2) exchange diffusion (counter-transport), 3) active transport 4) pinocytosis (Csáky, 1981).

Permeation of the intestine is a complex function reflecting at least 3 distinct unmediated permeation pathways of transmucosal diffusion including, 1) the intercellular junction between adjacent enterocytes (tight junctions), 2) aqueous transmembrane pores in the enterocyte brush border membrane, 3) the lipid rich

hydrophobic pathway in the brush border. *In vivo* permeability tests assess the functional integrity of the intestinal barrier.

All of the current intestinal permeability markers have advantages and disadvantages and none possess all the criteria of an ideal marker (Table 4). The requirements of an ideal passive permeability marker include, a) that it should be non-toxic, b) absorbed entirely by passive diffusion, c) not modified by enzymes nor metabolized, d) not found in the diet, e) not produced endogenously, f) cleared rapidly and completely, g) be hydrophilic and limited to the extracellular compartment, h) be non-immunogenic, i) be easily and rapidly measurable in biological fluids with high precision and accuracy (Cooper, 1984; Chadwick *et al.*, 1977).

Other than their non-invasive nature, a distinct advantage of permeability tests is reflecting the functional state of a major area of the intestinal mucosa, whereas morphological analysis may suffer due to sampling error, particularly if abnormalities are randomly distributed.

Table 4. Some Ideal Requirements for an Intestinal Permeability Marker

	^{51}Cr-EDTA	Monosaccharides	Oligosaccharides	PEG
No Exposure to Radiation	a	+	+	+
Non degradable	+	a	a	+
Non toxic	+	+	+	+
Non metabolized	+	+	+	+
No Endogenous Production	+	a	+	+
Complete Renal Excretion	+	a	+	a
Easily and Reliably Assayed	+	a	a	a

Hydrophilic	+	+	+	+
Lipophobic	+	+	+	a
First order Permeation Kinetics	+	+	+	+

(a) See discussion in text

1.4.1.1.1 Polyethyleneglycol (PEG)

Current interest in measurement of intestinal permeability originated with the initial work undertaken using polyethylene glycol (PEG) (Chadwick *et al.*, 1977). Pegs are a viscous mixture of slightly hygroscopic water soluble polymers of a wide range of molecular weights ranging from 200 (average molecular weight) to 600 as liquids and up to 6000 as solids. PEG 400 has been extensively employed as a permeability probe (Smith and Brooks, 1984; Parke *et al.*, 1983).

After an overnight fast, PEG (1-5 grams in 50-100 milliliters of water) is ingested and urine is collected generally from 0-6 hours (Maxton *et al.*, 1986; Fälth-Magnuson *et al.*, 1989). However, it has become evident that PEG is an unsatisfactory probe for measuring intestinal permeability (Peters and Bjarnason, 1988). Urinary recovery of PEG polymers is low and variable after intravenous instillation in man indicating loss to peripheral body compartments (Tagesson *et al.*, 1984).

PEG is relatively lipophilic and this is reflected in permeation extensively through the GI mucosal membranes, and thus it appears to be an insensitive marker to small increases in mucosal permeability (Chadwick *et al.*, 1977; Ukabam and Cooper, 1984). Additionally the use of PEG to assess intestinal permeability in RA has given results that are contradictory and difficult to interpret (Smith and Brooks, 1984; Parke *et al.*, 1983; Skoldstam *et al.*, 1979; Sundquist *et al.*, 1980). Some studies of PEG 400

intestinal permeability studies are conflicting as both increased and decreased permeability have been attained (Hollander *et al.*, 1986; Magnusson *et al.*, 1983). Furthermore, laborious specimen preparation, interbatch variation, an unpleasant taste, and demanding analytical techniques that lack sensitivity limits the use of PEG as a permeability marker (*Jenkins et al.*, 1987; Bjarnason, 1986b).

1.4.1.1.2 Carbohydrates

A number of carbohydrates have been used in studies of permeability including the hexoses (i.e. L-rhamnose), sugar alcohols (i.e. D-mannitol), and the disaccharides (e.g. lactulose, cellobiose). D-xylose has been used in permeability studies but it can be transported into the enterocyte by a carrier-mediated process, therefore it is not an indicator of passive permeability. Xylose absorption also appears to be reduced after indomethacin treatment in non-arthritic subjects and in indomethacin-treated patients with RA (Kendall *et al.*, 1971; Dyer *et al.*, 1971; Struthers *et al.*, 1985).

Most currently utilized carbohydrate permeability studies employ differential sugar absorption tests, in which two sugars (usually a mono-and disaccharide) are given concomitantly and urinary recovery of each is determined. In differential sugar tests after an overnight fast patients swallow a mixture of hypertonic sugars (1330 mmol/kg of water) and urine is collected for 5 hours (Juby et al, 1989; Struthers et al, 1985). The monosaccharide shows the extent of permeation through aqueous pores, and the disaccharide reflects the permeation of the intercellular pathway. The ratio of the two gives an index of relative function of the pathways (Bjarnason *et al.*, 1986b).

There are some inherent problems with sugar permeability markers. It seems that osmotic fillers may affect mucosal permeability since hypertonic lactulose test solutions may decrease permeability (Bjarnason *et al.*, 1984b). Many carbohydrates are metabolized by intestinal flora and the brush border membrane has some cellobiose activity. Rhamnose is incompletely excreted after an oral load which may underestimate its intestinal permeability. Lactulose and mannitol may be present in some foodstuffs and some patients may excrete minute quantities of endogenously produced mannitol which may overestimate intestinal permeability (Hamilton *et al.*, 1982; Maxton *et al.*, 1986). Furthermore, sugar loads can cause abdominal distention, diarrhea, and flatulence in some patients (Hamilton et al, 1982). Additionally, quantitation of carbohydrates generally requires specific chemical analysis or lengthy and time-consuming extraction and chromatographic procedures (Bjarnason *et al.*, 1986b).

Studies using carbohydrate markers have produced apparently contradictory results. For example some investigators have reported that the permeability measurements for the disaccharide lactulose, agree closely ($r^2 = 0.82$-0.98) with the permeability measurements obtained with ^{51}Cr-EDTA (Maxton *et al.*, 1986; Behrens *et al.*, 1987), while others have noted that NSAID-induced permeability of ^{51}Cr-EDTA did not result in corresponding increase in mono or disaccharide permeation (Bjarnason *et al.* 1983a; Jenkins *et al.*, 1991; Struthers *et al.*, 1983). Subsequently, the results of Juby *et al.* (1986) and O'Mahoney and Ferguson (1991) using urinary sugar excretion have also confirmed the results of Bjarnason (1984a, 1986ac, 1990a,

1991a,b, 1992a) and have demonstrated abnormalities of intestinal permeability in RA patients taking NSAIDs. However, the use of an *in vivo* carbohydrate permeability marker appears practically less reliable than that of the ^{51}Cr-EDTA.

In addition to the theoretical requirements of an ideal permeability probe (Table 4) the use of excretory ratios of markers (i.e. lactulose / mannitol; ^{51}Cr-EDTA / mannitol; ^{51}Cr-EDTA / L-rhamnose; cellobiose/ L-rhamnose) to indicate the presence of enteropathy may have advantages over a single marker (Jenkins *et al.*, 1991; Bjarnason *et al.*, 1992a) by minimizing some of the extramural factors which would affect the markers identically so their urinary excretion ratios are unchanged, decreasing the variability and increasing the reliability of the intestinal permeability data. The urine excretion ratio value specifically reflects alterations in intestinal permeability and is largely unaffected by premucosal and postmucosal determinants of the overall permeability of the intestine as each probe acts as an internal standard for the other.

1.4.1.1.3 ^{51}Cr-EDTA

^{51}Cr-EDTA was initially utilized as a marker of glomerular filtration and subsequently as a screening test for coeliac disease (Bjarnason *et al.*, 1983a,b).^{51}Cr EDTA incorporates the analytical advantages of a γ-ray emitting isotope in a water-soluble, highly stable, chelated compound with pharmacodynamic inertness (Lökken and Sögnen, 1967). The compound is stable and has a half-life of one month so that aliquots of the radioactive material can be easily stored.

At 8:00 AM, after an overnight fast, subjects drink a test solution containing 100 μCi of ^{51}Cr -EDTA in 10-100 mL of distilled water, followed by 300 mL of water (Bjarnason *et al.*, 1983b; Davies and Rampton, 1991). Alcohol and spicy foods are prohibited for 3 days before, and throughout, each study. Subjects are required to fast for an additional 2 h, after which, they are allowed food and fluid. Adequate sensitivity of the ^{51}Cr-EDTA absorption test seems to require a 24 h urine collection (Aabakken, 1989). Results are expressed as a percentage of the orally-administered test dose excreted in the urine during the same time interval.

The permeation of ^{51}Cr-EDTA has been shown to be relatively specific to the small intestine and a comparison of peroral and intraduodenal instillation showed no significant differences in the extent of urinary excretion (Simpson, 1985; Aabakken and Osnes, 1990). However, more recent studies have suggested that there may be some degree of colonic permeation (Elia *et al.*, 1987; Jenkins *et al.*, 1991). Nevertheless, Bjarnason *et al.* (1984a) have suggested that this colonic permeation is small as ^{51}Cr-EDTA is incorporated into feces and therefore unavailable for colonic permeation. The ^{51}Cr-EDTA test has been shown to be reproducible and the safety, simplicity, and accuracy of the procedure meet all the requirements for a permeability test (Bjarnason *et al.*, 1983a,b).

The ^{51}Cr-EDTA test has received criticism for high interindividual variability and low sensitivity (Mielants and Vey, 1985; Simpson, 1985; Peled *et al.*, 1985). However, these studies used small sample numbers and test sensitivity is increased with a urinary collection of 24 h (Aabakken, 1990). One may also question the

theoretical possibility that membrane integrity could be affected by EDTA itself, however, experiments on animal tissue suggest concentrations required would need to be 1000 times that employed in permeability tests (Aronson and Rogers, 1972; Tibdall, 1964). Alcohol may profoundly effect the results of this permeability test, however, its effect is reversible upon abstinence (Bjarnason *et al*, 1984a,b).

^{51}Cr-EDTA has also been criticized for being a radioactive pharmaceutical. The estimated radiation dose of a 100μCi (3.7 Mbq) dose is 0.12 milli sieverts (mSv) (Jenkins *et al.*, 1987). In comparison a chest X-ray gives 0.05 mSv, abdominal X-ray 1.4 mSv and the total radiation dose from natural sources is about 2 mSv/year (Bjarnason *et al.*, 1986). Therefore, the effective dose equivalent is comparable to other standards currently routinely employed in nuclear medical sciences.

1.4.1.2 Upper Gastroduodenum

1.4.1.1 Sucrose

More recently, sucrose has been introduced as a marker of NSAID-induced gastroduodenal (GD) damage (Meddings *et al.*, 1993; Sutherland *et al.*, 1994). Sucrose permeability, unlike endoscopy, is simple, cheap and readily accepted by patients (Steinhart *et al*, 1996). Increased urinary excretion of sucrose after an oral dose, indicates abnormalities to the epithelium of the upper GI tract. When entering the lower intestine sucrose is readily cleaved to monosugars due to sucrase activity in the brush border membrane. This makes sucrose unsuitable for assessing intestinal permeability since absorption of intact sucrose is restricted to the upper GI tract. Hence, detection of sucrose in the urine indicates leakage of the GI segments proximal

to enzyme activity (i.e. stomach and duodenum). It has been demonstrated that sucrose permeability correlates with severity of the upper GI damage and increases with repetitive exposure of NSAIDs (Meddings *et al.*, 1993; Rabassa *et al.*, 1994; Sutherland *et al.*, 1994; Irvine *et al.*, 1995; Meddings *et al.*, 1995). Increased sucrose permeability in man may be useful in predicting the presence of clinically significant gastric disease seen upon endoscopy. Furthermore, healing of gastric epithelial damage can also be monitored through the use of sequential measurements of sucrose permeability (Meddings *et al.*, 1995).

1.5 Regulation of Intestinal Permeability

The importance of the GI tract lies in its dual function as an absorptive organ responsible for selectively transporting nutrients, and electrolytes into the systemic circulation while conversely acting as a barrier to bacteria, enzymes, and noxious material between the lumen and the blood stream. In order to perform these divergent functions the GI tract contains numerous specialized cell types with differing organizations and functions. The barrier function is, however, largely a property of the epithelial cells lining the lumen and particularly the tight junctions formed between adjacent enterocytes (Powell, 1981). It is apparent that the permeability of the GI tract varies from region to region suggesting not only a high degree of regulation but also a balance between the absorptive and barrier requirements. Numerous studies, both *in vivo* and *in vitro*, have indicated that the permeability of the tight junctions can be altered, particularly in GI segments, by a wide variety of exogenous compounds and pathological processes (Milks *et al.*, 1986; Jenkins *et al.*, 1988; Citi, 1993; Mielants,

1991a). In addition, Bjarnason and co-workers (1986a and b) and Meddings and co-workers (1993) have demonstrated that NSAIDs cause increased intestinal and gastroduodenal permeability at the level of the mucosal tight junction and that this effect may precede clinical gastroenteropathy. Therefore, increased GI permeability may be used as sensitive indicator of gastroenteropathy induced by exogenous compounds such as NSAIDs.

The GI tract is the initial defense barrier exposed to xenobiotics administered by the oral route. Among the regions of the GI tract, the intestine displays the most marked balance between absorptive and barrier functions. The mucosa of the intestine is composed of three distinct layers, lamina propria, muscularis mucosa, and epithelium. The epithelial layer forms a barrier between the lumen and the rest of the body. Simultaneously, the epithelial cells serve to keep most of the gut contents out of the body while allowing selected nutrients to be transported. The absorption of molecules across the GI epithelium may occur via two routes, either across the epithelial cells themselves (transcellular) and/or across the intercellular spaces (paracellular) (Bjarnason *et al.*, 1986b).

The enterocytes of the intestine barricade the paracellular spaces from the epithelial cell surface by means of intercellular occluding junctions, located at the apical portion of the lateral membrane. The intercellular occluding or tight junctions conjoins intestinal epithelial cells, separates the intestinal lumen from the paracellular space and serves as the major barrier which restricts passive paracellular flow of molecules. The occluding junction consists of a series of fusions which occur between

the lateral membrane of adjacent epithelial cells and are seen as a net-like mesh of strands or grooves in freeze-fracture replicas (Madara and Trier, 1980). In general, the larger the number of junctional strands, the more capable is the epithelial cell to restrict passive transjunctional flow of ions. The tight junctions have a finite degree of ion permeability that varies with the number of strand barricades which they contain (Claude, 1978). Individual elements of the perijunctional cytoskeleton, including actin microfilaments, associate with the tight junction by means of plaque-like densities that intimately associate with the lateral membrane at the site of the tight junction (Powell, 1981). These cytoskeletal-tight-junction associations may represent the anatomical basis for cytoskeletal control of tight junction permeability (Madara *et al.*, 1987). The major pathway for molecules with relatively large molecular masses is passive absorption via the paracellular route while flow of these compounds via the transcellular pathway is negligible unless cell death occurs (i.e., in ulceration) (Bjarnason *et al.*, 1986b). Brush border abnormalities and widening of intercellular junctions have been reported in tissue from untreated patients with coeliac disease who also show appreciable increases in intestinal permeability (Madara and Trier, 1980).

Intestinal permeability of the tight junctions appears to be a multifactorial series of processes finely regulated by a variety of inter- and intracellular mediators (Powell, 1981; Schneeberger and Lynch, 1992). A number of studies have examined the control of the permeability of tight junctions in a variety of cell lines (Duffey *et al.*, 1981; Claude, 1978; Milks *et al.*, 1986; Winter *et al.*, 1991; Kubes, 1992). The mediators that regulate the permeability of the tight junction have not been clearly delineated but

may be influenced by a number of factors including 1) cyclic AMP, 2) arachidonic acid cascade, 3) tricarboxylic acid cycle and anaerobic glycolysis 4) cytoskeleton, 5) neutrophils, 6) calcium, 7) nitric oxide, and 8) cytokines.

A number of studies have examined the control of the permeability of tight junctions and it has been demonstrated that this is largely regulated by cAMP-dependent mechanisms (Duffy *et al.*, 1981). The concentration of cAMP in the epithelial cell, and in turn the permeability of tight junctions, may be influenced by levels of endogenous prostaglandins (Stevenson *et al.*, 1988; Powell, 1984). Thus it is reasonable to assume that changes in endogenous prostaglandin levels by NSAIDs may result in changes in the function of tight junctions. In addition, there are a number of other mechanisms by which cAMP can be altered as this secondary messenger is linked to protein kinase function which is also modulated by prostaglandins (Powell, 1984)

NSAID-induced side-effects to the GI mucosa have been suggested to result from competitive inhibition of specific enzymatic steps in the anaerobic glycolitic pathway and the tricarboxylic acid cycle thereby reducing adenosine triphosphate (ATP) production leading to enterocyte death (Rainsford and Whitehouse, 1980; Rainsford, 1991).

Multiple observations have suggested important links between the tight junction and cytoskeletal structures. Contraction of the perijunctional actin-myosin ring may be elicited in intestinal epithelia (Madara *et al.*, 1987). Regulation of the cytoskeleton may be assessed through the use of microfilament inhibitors, kinase activators, and protein inhibitors. Activation of a kinase enzyme which controls

epithelial permeability has been previously suggested (Winter *et al.*, 1991). Identification of the tight junction proteins ZO-1, ZO-2, and cingulin have also been characterized within the tight junctions. Direct manipulation of proteins has also been demonstrated to effect tight junction regulation (Citi, 1993).

The migration of neutrophils has also been shown to increase permeability of epithelial cells (Milks *et al.*, 1986). However, no studies to date have examined the effect of neutrophil migration on epithelial permeability, and the influence of NSAID exposure.

Ca^{2+} plays an important role in almost any type of cell-cell interaction, it is not surprising that changes in its regulation produces drastic changes in the structure and function of tight junctions. The integrity of epithelial cell tight junctions has been shown to depend on extracellular Ca^{2+} (Gumbinger, 1987). The effect of NSAIDs on calcium regulation within epithelial cells has yet to be evaluated.

Nitric oxide may be an important endogenous modulator of the sequelae associated with inflammation of the GI tract. Nitric oxide is inactivated by superoxide anions which are induced by NSAIDs (Rainsford, 1987). Endogenous production of nitric oxide is believed to play an important role in modulation of permeability across the epithelial barrier (Kubes, 1992).

As neutrophils and macrophages migrate through the paracellular pathway, they simultaneously release a plethora of inflammatory mediators. This has been shown to increase endothelial cell permeability in a time- and dose-dependent fashion. Although inflammatory endpoints have been shown to be expressed and released

during inflammation few studies have examined the role of inflammatory mediators on the permeability of organ epithelia such as the GI epithelium (Madara and Stafford, 1989). The pathogenesis of NSAID-induced GI inflammation may involve the expression of cytokines from neutrophils which would also contribute to GI permeability changes, and epithelial cytotoxicity.

1.6 Inflammatory Diseases and Permeability

Permeability changes may play an important role in the pathogenesis of inflammatory joint disorders by exposing the mucosa and the circulation to exogenous macromolecules, luminal antigens, toxins, and bacteria (O'Mahoney and Ferguson, 1991; Cooper, 1984). Associations between intestinal disease and various forms of arthropathies have been known for some time (Rooney *et al.*, 1990; Zaphiropoulos, 1986). Therefore, presently it appears that bowel permeability in RA patients may be inherently subnormal as compared with that of controls. There is evidence that other physiological abnormalities in the GI mucosa also occur in RA patients including processing of amino acids and mucosal enzymes such as histidine methyl transferase (Bergstrom and Havermark, 1976; Bett, 1966). Thus in the therapy of chronic arthropathies drug or disease related inflammatory reactions or even a combination of both may be responsible for the apparent intestinal manifestations (Morris *et al.*, 1992).

The clinical significance of increased permeability tests are speculative, however, permeability changes may be important factors in the development of intestinal disease and RA (Rooney *et al.*, 1990). Tests of intestinal permeability have

been employed in a wide array of applications in the investigation of intestinal disease. These tests are safe, well tolerated, reproducible, and easy to perform and because of their non-invasive nature can be easily applied to diagnostic screening and research, and could possibly replace the need for invasive investigations of intestinal disease such as radiology, biopsy and enteroscopy. An implicit advantage of permeability tests is that they reflect the functional integrity over a major area of the intestinal mucosa, whereas biopsy may suffer from sampling error if damage is distributed randomly or to inaccessible areas (Lim *et al.*, 1993).

Intestinal permeability tests are objective guides to response to therapy, and may be useful as adjuncts in the diagnosis and evaluation of several disease states including: Crohn's disease (Teahon *et al.*, 1992), chronic alcoholism (Bjarnason *et al,*, 1983b), coeliac disease (Turck *et al*, 1987), atopic eczema (Bjarnason *et al.*, 1985a, RA (Bjarnason *et al.*, 1984a), cystic fibrosis (Leclerq-Foucart *et al.*, 1986) carcinoma of the GI tract, giardiasis, systemic sclerosis, graft versus host disease, acquired immune deficiency syndrome, (Lim *et al.*, 1993) diabetic diarrhea, tropical enteropathy, and drug-induced side-effects to the small intestine (Table 5) including NSAID-induced side-effects (Bjarnason *et al.*, 1986a; Pearson *et al.*, 1986; Selby *et al.*, 1984).

Table 5 Drugs With Reported Association With Lower Gastrointestinal Tract Toxicity

Antibiotics	Cytotoxic Agents	Gold	Penicillamine	Vasopressin
Cetrimide	Ergotamine	Methotrexate	Potassium	
Cimitedine	Estrogen	NSAIDs	Progesterone	
Corticosteroids	Ethanol	Methyldopa	Salazopyrin	

1.7 Studies of NSAID-Induced Intestinal Permeability: Human

NSAID-induced increases in intestinal permeability have been extensively studied in humans (Bjarnason *et al.*, 1984a; Bjarnason *et al.*, 1986a; Aabakken and Osnes, 1990; Jenkins *et al.*, 1987; Davies *et al.*, 1993). These permeability changes have been detected by the oral administration of probes such as ^{51}Cr-EDTA, lactulose, cellobiose and polyethylene glycol (PEG) (Smith and Brooks, 1984; Parke *et al.*, 1983; Sundquist *et al.*, 1980; Juby *et al.* 1986; O'Mahoney and Ferguson, 1991). ^{51}Cr-EDTA has been the most frequently employed probe in NSAID-induced permeability studies and is absorbed to a limited extent, exclusively through the intercellular junctions (Bjarnason *et al.*, 1986b).

Table 6 summarizes all of the available NSAID-induced permeability data in humans. Uniformly, the human permeability studies have assumed a homogenous nature for NSAID-induced permeability changes. The majority of these studies use indomethacin as the prototypical NSAID. In general the studies show an increase from baseline of ^{51}Cr-EDTA excretion in urine which is dose-dependent and appears to be antagonized by concomitant misoprostol, glucose / citrate, metronidazole, and sulfasalazine but not by H_2-antagonists in healthy volunteers and in arthritic patients (Banerjee *et al.*, 1986; Bjarnason *et al.*, 1986a; Bjarnason *et al.*, 1992a; Davies *et al.*, 1993). Additionally, the permeability effects appear to be independent of age and gender (Bjarnason *et al.*, 1991a).

Intestinal permeability was initially estimated in healthy subjects after ingestion of two doses of aspirin (1.2 and 1.2 g), ibuprofen (400 and 400 mg) and indomethacin (75 and 50 mg) at midnight and the other one hour before a ^{51}Cr-EDTA permeability test the next day (Bjarnason *et al.*, 1986b). Intestinal permeability increased significantly from control levels following each drug and, in this limited example, the effect was correlated to NSAID potency to inhibit cyclooxygenase. Intestinal permeability also increased to a similar extent after oral and rectal administration of indomethacin, suggesting that this effect of NSAIDs may be systemically mediated (Bjarnason *et al.*, 1984a).

It is postulated that NSAIDs inhibit cyclooxygenase and therefore there is inadequate prostaglandin generation from fatty acids by the damaged cell membranes. (Bjarnason *et al.*, 1993a). Bjarnason and co-workers first examined the influence of concomitant prostaglandin administration on indomethacin induced permeability changes first with prostin E_2, a naturally-occurring prostaglandin, which did not seem to reverse permeability changes perhaps due to the instability of the preparation. However, prostaglandin E_2 itself significantly decreased baseline permeation of ^{51}Cr-EDTA (Bjarnason *et al.*, 1986a). Misoprostol alone had no effect on permeation of ^{51}Cr-EDTA (Bjarnason *et al.*, 1989a; Davies *et al.*, 1993), however, co-administered with indomethacin, misoprostol in high doses for short periods protected the small bowel mucosa from the effects of indomethacin by decreasing the indomethacin-induced increased intestinal permeation of ^{51}Cr-EDTA. Rioprostol, a prostaglandin analogue given in small doses at the same time as indomethacin had a maximally

protective action (Bjarnason *et al.*, 1986b), however, the excretion values were still significantly higher than the baseline values (Bjarnason *et al.*, 1989b). The permeation of other markers (i.e. 3-O-methyl glucose, D-xylose, and L-rhamnose) was not effected by indomethacin. However, Davies *et al.* (1993) saw a lack of reduction of indomethacin-induced intestinal permeability with misoprostol. In this study only 800 µg misoprostol was administered every 24 hours for 150 mg doses of indomethacin, whereas in the Bjarnason study (1990a) 1200 µg misoprostol was administered over 20 hours with 125 mg indomethacin given during the last 12 hours. These studies also suggest that indomethacin-induced intestinal permeability may be dose-dependent or that intestinal permeability may only be partially mediated by reduced mucosal prostaglandins.

Sulfasalazine and metronidazole are being clinically used to reduce NSAID-induced enteropathy (Bjarnason *et al.*, 1991a,b, Bjarnason *et al.*, 1992b). In addition, sulfasalazine also prevents acute indomethacin-induced increases in intestinal permeability the effect of indomethacin administered as 75 mg orally twice daily for one day was inhibited by sulfasalazine 1 g orally twice daily for three days (Banerjee *et al.*, 1986). The effects of indomethacin treatment (50 mg three times a day) for one week with co-administered metronidazole 400 mg twice a day significantly reduced indomethacin-induced permeability changes (Davies *et al.*, 1993). This may imply a role for intestinal bacteria in the pathogenesis of the initial permeability increase induced by NSAIDs. A two-stage process in NSAID-induced intestinal damage has been suggested (Davies *et al.*, 1993). Initially, there is superficial and reversible

mucosal damage, probably directly related to NSAID effects on local prostaglandin depletion but independent of luminal contents. The second stage is independent of direct drug effects but follows if initial damage to mucosal defenses is not prevented or reversed. Due to the increased epithelial permeability harmful luminal contents, including bacterial flora, have increased access to the GI mucosa and this may pave the way for more severe GI damage (Davies et al, 1993).

The ability of the ^{51}Cr-EDTA test to detect an increase in intestinal permeability which resulted from administration of two different doses of naproxen has been reported (Aabakken and Osnes, 1990). There was a statistically significant difference between the median increase as a percentage of the baseline excretion for 750 mg naproxen (19%), and 1000 mg naproxen (68%). Misoprostol did not appear to protect against naproxen-induced increased intestinal permeability (Jenkins, 1988). However, the time-course of naproxen induced permeability changes was not considered nor was the dose-dependency of misoprostol administration. In addition, the small sample numbers involved in this study (n = 6) may have been inadequate for firm conclusions of these results. Any protective effect of sucralfate is confined to the stomach as sucralfate did not provide protection from naproxen-induced permeability changes in the distal intestine (Aabakken *et al.*, 1989a).

Some NSAIDs are pro-drugs that are inactive as cyclooxygenase inhibitors until after absorption, and therefore it has been suggested that they might cause less intestinal damage than other NSAIDs (Davies and Rampton, 1991). After a one week treatment period of sulindac 200 mg daily there was a lack of an apparent increase in

intestinal permeability above baseline, whereas an indomethacin treatment of 2 mg/kg/day in three divided doses increased ^{51}Cr-EDTA permeation. Similar results have recently been reported with another pro-drug NSAID nabumetone (Bjarnason *et al.*, 1991a). After taking nabumetone, 1 g at midnight for seven days, nabumetone did not appear to induce an increase in ^{51}Cr-EDTA permeability above baseline whereas indomethacin treatment increased ^{51}Cr-EDTA permeation. These results could be interpreted to suggest that the systemically mediated effect of NSAIDs is weak and that the main damage is sustained after drug absorption or excretion in bile. However, a major contribution of local effects of NSAIDs in inducing permeability changes contradicts previous suggestions of a major systemic effect being responsible for NSAID-induced intestinal permeability (Bjarnason *et al.*, 1984a). These results may also be attributed to either intestinal permeability being reflective of mechanisms other than reduction of prostaglandins such as diversion of arachidonic acid metabolism down the lipoxygenase pathway, and oxy-radical production or direct drug cytotoxicity, or the differing pattern of biliary excretion of nabumetone (0%), sulindac (4%), and indomethacin (36%) (Helleberg, 1981; Dujuvone *et al.*, 1983). In addition, the sample numbers in these studies are small and no suggestion of a possible dose-dependency or time course of this effect was mentioned. Moreover, in the Bjarnason study (1991a) the collection period was only 5 hours. Aabakken suggests a 24 h collection period increases the sensitivity of the test (Aabakken, 1990). Furthermore, there was a trend for nabumetone to increase permeability from baseline in several subjects which may reach statistical significance with a larger sample size.

In addition to inhibiting prostaglandin synthesis, NSAIDs may inhibit glycolysis and the tricarboxylic acid cycle resulting in inhibition of oxidative phosphorylation and damage the enterocyte resulting in a depletion of cellular ATP (Somandasarum *et al.*, 1991). The consequence of reduced ATP production is a collapse of the cytoskeleton and therefore intercellular junction regulation is affected. Indomethacin administered as 50 or 75 mg increased intestinal permeability, whereas, a formulation of indomethacin containing 15 mg glucose and 15 mg citrate to each milligram of indomethacin prevented an increase in intestinal permeability above baseline values (Bjarnason *et al.*, 1992a). This protective effect of glucose-citrate was dependent on the ratio of glucose and citrate with an optimal ratio of 1:15:15, when the sugars were given together orally and concomitantly with indomethacin. It has been suggested that the presence of these sugars in the intestinal lumen may modify the reaction to indomethacin or that citrate may be cytoprotective against free radical damage caused by NSAIDs (Bjarnason *et al.*, 1992a).

Although the deleterious effects of NSAIDs in the intestine are coming into focus very few studies have examined the possible protective measures in the distal intestine. Furthermore, differentiation between the gastric and intestinal toxicological manifestations of NSAIDs has been largely ignored. Hence, it is not known if the suggested schemes for upper gastroduodenal protection alleviates NSAID-induced intestinal lesions as well. Aabakken's group have examined the use of the H2-antagonists (cimetidine and famotidine) and sucralfate none of which seem to possess

any apparent protective or antagonizing effect on naproxen-induced intestinal permeability (Aabakken *et al.*, 1989a; 1989b; 1990).

In addition, in a latin-square crossover study, naproxen 500 mg twice daily for 7 days as plain tablets, enteric-coated tablets, or enteric-coated granules in capsules was administered to healthy male volunteers. All drug formulations induced a significant increase in ^{51}Cr-EDTA permeability but no statistical differences between these formulations were detected. A considerable interindividual variation was seen, however it appears that the median urinary excretion values for the enterogranulate capsules and the enterocoated tablets were higher than after plain tablets (Aabakken *et al.*, 1989c). The effect of modified release formulations on NSAID-induced permeability has not been adequately addressed in other clinical studies.

Mielants *et al.* (1991a) report that there was no significant difference in altered intestinal permeability between patients taking NSAID and patients taking corticosteroids. This further suggests that alteration of intestinal permeability may not only be accounted for by an inhibition of mucosal cyclooxygenase activity but that other pathways in the arachidonic acid cascade might be implicated. Corticosteroids are known inhibitors of phospholipase A_2 as is indomethacin which has been used as a positive control in the human intestinal permeability studies (Rainsford, 1988).

It also appears that the effect of NSAIDs on increasing intestinal permeability differs markedly from their potency to cause gastric damage. Thus, aspirin, which is most frequently implicated in causing gastric and duodenal mucosal damage, only increased intestinal permeability slightly above baseline values (Bjarnason, 1986a) and

little or no intestinal inflammation was evident (Bjarnason and MacPherson, 1989). Conversely, it has also been suggested that aspirin did increase intestinal permeability in a limited number of patients (Jenkins *et al.*, 1987). However, the type of formulation and dose administered were not mentioned.

Unfortunately, due to the ethical constraints of repeated administration of radiolabelled compounds in humans and the technical difficulty of access to the human distal intestine these studies in humans have been performed in the absence of any pharmacokinetic considerations and, thus, neither the time-course of these changes nor their relationship to drug concentration in plasma or GI tissues have been established. Furthermore, complete characterization of the dose-effect relationship for these NSAIDs has not been described due to maximum ethical daily dosage regulations. However it appears that the relative effect of indomethacin on intestinal permeability is short lived with restoration of intestinal integrity within a week of the last ingested dose (*Bjarnason et al.*, 1991a). This contrasts with that seen in NSAID enteropathy where inflammation may persist for over 16 months after stopping NSAIDs (Bjarnason *et al.*, 1987a).

The precise mechanisms which cause an increased intestinal permeability to ^{51}Cr-EDTA after NSAID ingestion are uncertain, however, there is evidence that permeability changes are not completely related to the NSAIDs ability to inhibit cyclooxygenase since concomitant prostaglandin administration only partially reverses permeability changes (Bjarnason *et al.*, 1986a; Bjarnason *et al.*, 1989a). It is unknown whether reduced mucosal prostaglandin production, the concomitant metabolic

diversion of arachidonic acid through the lipoxygenase pathway with subsequent formation of leukotrienes and oxy-radicals or even an imbalance between the products of the two pathways is responsible for the observed increased intestinal permeability. The precise mechanisms(s) by which such changes cause damage to the intercellular junction is speculative and could be due to a number of factors such as reduced blood flow, mast cell degranulation, lysosomal release, or other as yet unconsidered possibilities. It has been suggested that NSAIDs disrupt the actions that synergistically regulate the enterocyte in such a way that it fails to maintain the energy dependent intracellular mechanism that regulates and controls the integrity of the intercellular junction (Bjarnason *et al.*, 1991a). The increased intestinal permeability, may then allow dietary macromolecules, luminal toxins, bile acids, pancreatic juices and bacteria access to the mucosa, where, in cooperation with NSAID effects on chemotaxis and neutrophil function, pave the way for bacterial invasion and subsequent inflammation and ulceration (Bjarnason *et al.*, 1993a).

Additionally, NSAID-induced side-effects to the GI mucosa have been suggested to result from competitive inhibition of specific enzymatic steps in the anaerobic glycolytic pathway and the tricarboxylic acid cycle thereby reducing adenosine triphosphate (ATP) production leading to cell death (Rainsford and Whitehouse, 1980; Somasundaram *et al.*, 1992). NSAIDs have been suggested to uncouple oxidative phosphorylation, resulting in depletion of cellular adenosine triphosphate (ATP) (Brody, 1956; Glaborg-Jorgensen, 1976). The reduced ATP production results in a collapse of the cytoskeleton and hence control of the

intercellular junctions (Madara, 1987). Hence, NSAIDs may affect a combination of factors involved in the energy-dependent intracellular mechanism that regulates the integrity of the intercellular junction between adjacent enterocytes (Madara *et al.*, 1987). Additionally, it has been recently reported that glucose/citrate intermediates in the TCA cycle can reduce GI side-effects of indomethacin in rats and intestinal permeability in humans (Rainsford, 1981; Bjarnason, 1992a).

There is also evidence to suggest a role for xanthine oxidase derived reactive oxygen radicals in NSAID-induced GI toxicity (Del Soldato *et al.*, 1985; Rainsford, 1987). Cysteamine (a free radical scavenger) was effective in preventing ulcerative erosions of the intestine induced by indomethacin and aspartic acid seems to provide intestinal protection in animal models (Rainsford and Whitehouse, 1980; Del Soldato *et al.*, 1987). Allopurinol has been shown to reduce the intestinal permeability due to ischemia-repurfusion and when given with aspirin protects the gastric mucosa from injury (Rainsford, 1990; Vaughan *et al.*, 1992). Superoxide anions might be produced by activation of xanthine oxidase by the increased AMP relative to ATP and ADP. Alterations in AMP relative to ATP and ADP could arise from mitochondrial uncoupling effects of NSAIDs on phosporylation (Rainsford, 1988; Glaborg-Jorgensen *et al.*, 1976). The effects of various cytoprotective agents in combination with NSAIDs will be important to elucidate the finer mechanistic detail of the effect of NSAIDs on GI permeability.

Table 6. Human NSAID-Induced ^{51}Cr-EDTA Intestinal Permeability Studies

Reference	No.	Age[a]	Dose (mg)	% ^{51}Cr-EDTA Excretion
Bjarnason et al (1984)	6	NR	A:Controls	1.94±0.51
	6		B:RA Controls	2.07±0.48
	2		C:OA Controls	2.02±0.42
	2		D:Ibuprofen 400mg * 3 Days	4.47±2.27*
	1		E:Naproxen 250 mg * 2 Days	2.83±1.15*
	1		250 mg * 3 Days	5.175±1.01*
	1		500mg nocte	2.02
	1		1000mg	7.21*
	1		750mg	10.92*
	1		E:Indomethacin 25mg * 3 Days	3.57*
	1		50 mg * 2 Days	4.86*
	1		75mg * 2 Days	10.4*
	2		75 nocte	3.59*
	1		75 mg	5.45*
	1		100mg supp	5.4±1.03*
	1		100 mg	10.02*
	1		150 mg	8.02*
	1		175 mg	4.00*
	1		75 mg + salicylate 3g	5.60*
Bjarnason et al (1986)	14	26-39	A:Control	1.9±0.5
			B:Aspirin (1.2g + 1.2 g)	2.3±0.3
			C:Ibuprofen (400mg+400mg)	2.9±1.2*
			D:Indomethacin (75mg + 50 mg)	4.7±1.3*
			E:Indomethacin (100 mg) supp	4.5±0.9*
	7		A:Control	2.2±0.6
			B:Indomethacin (75mg + 50mg)	4.6±1.5*
			C:PGE_2(5mg)	1.5±0.7
			D:B+C	6.6±4.4*
			E:Ranitidine 150mg (-24, -12, -1, +12 h) + D	5.6±3.2*

Banerjee et al (1986)	20	NR	A:Baseline B:Sulphasalazine 1g bid *3 Days C:Indomethacin 75mg bid for 1 day D:B + C	0.75±0.09 0.73±0.08+ 1.96±0.31* 1.21±0.31#^
Jenkins et al (1987)	50(26M:24F) 36(11M:25F)	34 (21-56) 51.9 (17-78)	A:Controls B:RA	1.36±0.54 3.72±2.37*
Jenkins et al (1988)	6 (5M:1F)	37.2 (31-56)	A:Baseline B:Naproxen 375mg tid*7 Days C:Misoprostol 200 µg qid before +during D:B+ Misoprostol 200 µg qid	1.54±0.49 3.25±1.07* 2.06±0.56 3.06±1.39*
Aabakken et al (1989b)	18 (18M)	median 25.5(21-44)	A:Baseline B:Naproxen plain tablets C:Baseline D:Naproxen enteric-coated tablets E:Baseline F:Naproxen coated granules in a capsule	2.63 4.42* 2.86 4.14* 2.06 4.18*
Aabakken et al (1989c)	17M	median 25(19-32)	A:Baseline B:Naproxen 500 mg bid C:Naproxen 500 mg bid + cimetidine 400 mg bid tablets D:Naproxen 500 mg bid + cimetidine 400 mg bid suspension	2.6 3.4* 3.85* 3.9*
Aabakken et al (1989d)	16M	median 21(19-32)	A:Baseline B:Naproxen 500 mg bid C:Naproxen 500 mg bid + sucralfate 2 g bid	2.5 3.7* 4.2*
Bjarnason et al (1990)	20 (20M)	(23-39)	A:Baseline B:Misoprostol 200µg -16,-12, -8.5,-4,-1.5, and 5 h C:Indomethacin 75mg -8h; 50mg at -1h D:Misoprostol and Indomethacin combined	0.76±0.07 0.66±0.08 1.96±0.31* 1.16±0.17#
Aabakken et al. (1990)	18 (18M)	26 (23-32)	A:Naproxen 500mg + placebo *7 Days B:Naproxen 500mg + 20 mg famotidine *7 Days C:Naproxen 500mg + 40 mg famotidine *7 Days	3.1* 3.0* 3.1*

Aabakken and Osnes (1990)	80 (80M) 42 38 27 Within subject data	median 25 (18-44)	A:Baseline B:Naproxen (500 + 250mg)bid*7 Days C:Baseline D:Naproxen (500 +500mg)bid*7 Days A:Baseline B:Naproxen (500 + 250mg)bid*7 Days C:Baseline D:Naproxen (500 +500mg)bid*7 Days	2.44 3.15* 2.26 3.39* 2.67 3.75* 2.64 4.17*
Bjarnason et al (1991b)	16 (10M:6F)	32±10 (20-53)	A:Baseline B:Carbopol (200mg qid*4 Days) C:Indo (75mg + 75 mg) -12, -1 h D:Indomethacin + carbopol	0.51±0.16 0.61±0.20 1.57±0.48* 1.42±0.60*
Bjarnason et al (1991a)	12(6M:6F)	29±2	A:Baseline B:Indomethacin (50mg * 3 Doses) * 7 Days C:Nabumetone 1g * 7 Days	0.63±0.09 1.20±0.14* 0.70±0.10+
Davies and Rampton (1991)	25 16 9	med 23(20-47)	A:Baseline B:Indomethacin 2mg/kg tid*7 Days C:Baseline D:Sulindac 200mg qd*9 Days	1.2±0.4 2.4±0.7# 1.4±0.7 1.8±0.4*+
Jenkins et al (1991)	20 6 9 RA,OA 3 2 2 1 1	(22-28) 28 (33-61)51 (45-72) 48	A:Control B:Ileostomy C:NSAID Diclofenac (50mg*2 Days) Naproxen (500mg*2 Days + 250mg *2 Days) Flurbiprofen (100mg*2 Days+50mg *2 Days) Indomethacin (25mg*3 Days) Piroxicam (20mg *1 Daily)	2.27±0.15 0.45±0.08 4.64±1.20* * * * * *
Bjarnason et al (1992)	12 (6M:6F)	29	A:Control B:Indomethacin 50mg tid*7 Days C:Nambumetone 1 g qd*7 Days	0.63 1.20* 0.70+

Bjarnason et al (1992)	20(12M:8F)	29±3	A:Control 5 hours	0.62±0.3
			B:Indomethacin (50 +75) -12, -1 h	1.54±0.19*
			C:Indomethacin (50 +75) g/c (1:15:15) -12, -1 h	1.15±0.67*+
			D:Indomethacin (50+75) g/c (1:10:10) -12, -1 h	NR
			E:Indomethacin (50+75) g/c (1:5:5) -12, -1 h	NR
			F:Indomethacin (50+75) glucose (45:1) -12, -1 h	NR
			G:Indomethacin (50+75) citrate (45:1) -12, -1 h	NR
	6		D:Control	0.79±0.10+
			E:Glucose/citrate (500 + 750), -12,-1 h	0.75±0.10+
Davies et al (1993)	40 (37M:3F)	(22-57)	Group 1 A: Baseline	1.12±0.29
			B: Indomethacin (50mg tid)+PB*7 Days	2.60±0.78*
			C:Baseline	1.10 ±0.39
			D:Indomethacin (50mg tid) + metronidazole (400mg bid)*7 Days	1.55±0.54*
			Group 2 E:Baseline	1.31±0.51
			F:Indomethacin (50mg tid)+ PB*7 Days	2.21±0.62
			G:Baseline	1.37±0.70
			H:Indomethacin (50 mg tid) + misoprostol (200 µg qid)*7 Days	3.26±1.10*
			Group 3 I:Baseline	1.48±0.54
			J:Metronidazole 400 mg bid*7 Days	1.15±0.70
			K:Baseline	1.30±0.34
			L:Misoprostol 200 µg qid*7 Days	1.08 ±0.28*
Choi et al (1995)	57(28M:29F)	mean 30	A:Baseline	0.42±0.17
	21	(20-51)	B:Diclofenac sodium 50 mg tid * 7 Days	0.51±0.28+
	34		C:Baseline	0.42±0.17
	10		D:Diclofenac sodium SR 50 mg tid * 7 Days	0.66±0.238
	13		E:Baseline	0.39±0.14
			F:Indomethacin 50 mg bid * 7 Days	0.95±0.64*
			G:Baseline	0.39±0.11
			H:Tenoxicam 20 mg qd * 7 Days	0.39±0.10+

a Mean age; range in parentheses, sex (# of M or F), and disease state (OA,RA) when available

Abbreviations: No. = number of subjects or patients in study; tid = 3 times daily; qid = 4 times daily; q6h (q12h) = every 6 (12) hours; qd = daily. ABC:1,2, or 3x50mg D40ml 2.5mg/mL solution a=adult; c=children; m=male; f=female; G/C=glucose/citrate; RA=rheumatoid arthritis; SR=sustained release; OA=osteoarthritis; PB=Placebo SpA=spondylarthropathies; IBD =inflammatory bowel disease; * not significantly different from baseline; + not significantly different from baseline; # significantly different from indomethacin alone

1.8 Corollary Methods of Assessing NSAID-Induced GI Damage

1.8.1 Lower Enteroscopy

Until recently, attempts to examine the small bowel endoscopically have been limited by the use of suboptimal instruments and techniques. Morris *et al.* (1992) have described the use of a balloon driven sonde enteroscope to obtain extended views of the small bowel. Nineteen (41%) of 46 anemic arthritic patients taking NSAIDs and three (27%) of 11 patients with unexplained iron deficiency, were found to have small bowel lesions to account for their anemia. Small bowel lesions were found in 3 of 8 (37%) of patients with acute GI bleeding. The use of small bowel enteroscopy to detect NSAID-induced intestinal strictures has subsequently been reported by several other groups (Hershfield, 1992; Cutler *et al.*, 1993).

1.8.2 ^{111}Indium Leucocytes Scan

The initial and immediate effect of NSAIDs to increase intestinal permeability is a prerequisite for the subsequent development of intestinal inflammation. This intestinal inflammation induced by NSAIDs is associated with blood and protein loss, both of which may contribute to the general ill-health of rheumatic patients (Bjarnason *et al.*, 1987a,b,c). Further, studies have shown that long-term NSAID treatment leads to enhanced migration of ^{111}Indium-labeled leucocytes to the ileum indicating a chronic inflammatory process within the intestine. This is a useful clinical qualitative scintigraphic method for determining the sites of affliction of NSAID damage within the GI tract (Davies *et al.*, 1996).

1.8.3 ^{111}Indium Fecal Counts

The ^{111}Indium labeled leukocytes scan together with a 4 day fecal collection of ^{111}Indium excretion provides a sensitive and quantitative measure of intestinal inflammatory activity (Bjarnason and MacPherson, 1989). Using these techniques it has been shown that NSAIDs cause intestinal inflammation in a substantial number of patients receiving these drugs. Evidence also suggests that small intestinal inflammation occurs predominantly in the terminal jejunum and upper ileum (Bjarnason and MacPherson, 1989). Indeed, it has been observed that up to 70% of patients with rheumatoid and osteoarthritis on long-term treatment with NSAIDs develop intestinal inflammation (Bjarnason, 1987). This, therefore, represents a potentially serious adverse side-effect of NSAIDs. While the clinical presentation of NSAID-gastropathy may be more dramatic than enteropathy, the full impact of the short or long term importance of this adverse effect of NSAIDs on the intestine are relatively unknown.

1.8.4 Intestinal Protein Loss

A small percentage of arthritic patients may have hypoalbuminemia ascribed to protein losing enteropathy. Bjarnason's group have also simultaneously labeled leucocytes with indium-111 and proteins labeled with chromium-51. After a 10 day fecal collection a correlation of chromium-51 activity in feces with serum from the previous day is made. Rheumatoid patients on long-term NSAID therapy with intestinal inflammation have been shown to exhibit a protein losing enteropathy using this technique (Bjarnason *et al.*, 1987c).

1.8.5 Intestinal Bleeding

It has become increasingly evident that there does not appear to be a close

relationship between upper endoscopic findings and evidence of intestinal bleeding in NSAID treated patients (Holt *et al.*, 1993). Several approaches have been developed to evaluate NSAID-induced bleeding in the lower GI as blood loss from this site is an important contributing factor to the anemia of patients with RA taking NSAIDs (Bjarnason *et al.*, 1987a,b,c; Morris *et al.*, 1992). Approximately 47% of patients with occult GI bleeding while on NSAIDs were found to have jejunal or ileal ulceration (Morris, 1991). NSAID-induced enteropathy appears to be associated with low-grade blood loss and protein loss and these toxicological effects may be utilized as an early diagnostic technique for NSAID-induced enteropathy in these patients (Bjarnason *et al.*, 1993a).

1.8.6 ^{99m} Technetium Labeled Erythrocytes

The simultaneous labeling of leucocytes with indium-111 and erythrocytes with technetium-99m revealed identical location of both radioactive blood cells in the right iliac fossa indicative of an inflammatory process iatrogenically attributable to NSAID consumption (Bjarnason *et al.*, 1987a).

1.8.7 ^{51}Cr-EDTA Labeled Erythrocytes

Bjarnason's group have correlated intestinal inflammation with intestinal blood loss. Intestinal inflammation is assessed by indium-111 techniques as described previously (see section 1.8.3). Lower GI blood loss has been assessed by labeling erythrocytes with chromium-51 and correlating the 5 day fecal excretion of the isotope with the radioactivity in the blood from the previous day. The fecal excretion of indium-111 correlates significantly with the mean daily intestinal blood loss ($r = 0.59$,

$p<0.01$) (Bjarnason *et al.*, 1987c). However, these techniques require meticulous methodology to obtain accurate results.

1.9 Prevention and Treatment of NSAID-Induced GI Damage

As NSAIDs are definitely linked to the development of serious GI side-effects, numerous strategies have been employed to reduce this damage before it occurs. There has also been an attempt to develop new NSAIDs in recent years, however, at present although clinically significant differences between the various drugs on the market exist all NSAIDs induce some type of GI manifestation and none has proved convincingly superior.

The most effective means of reducing GI toxicity has been to withdraw the offending NSAID. However, this approach may not be clinically suitable for many RA patients depending on their disease severity. An alternative approach has been the concomitant treatment with protective substances to circumvent these side-effects. Preventative measures evaluated to date have been investigated using a wide variety of agents including antisecretory agents and antacids (H_2-receptor antagonists, omeprazole, and anti-cholinergic agents) and, attempts to increase mucosal defense (sucralfate, misoprostol; Zwitterionic phospholipids) (Rainsford and Whitehouse 1980; Cohen *et al.*, 1985; Lichtenberger *et al*, 1995).

The antisecretory agents are employed as it is believed that acid in the stomach plays a major role in upper GI ulcerogenesis. Each of these approaches has had some measurable success in achieving GI protection from NSAIDs, however, they have not been as effective as to achieve total or ideal GI protection. Attempts to increase

mucosal defense through the use of prostaglandin analogues are effective but there have been considerable problems with side-effects (mainly diarrhea) which limits the clinical utility of the prostaglandin analogues (Boyd and Wormsley, 1987). Sucralfate appears to have physical effects on the protective mucus layer of the upper GI and may also inhibit pepsin activity and stimulate prostaglandin and epidermal growth factor production (Shea-Donohue *et al.*, 1986). The chemical pre-association of NSAIDs with exogenous zwitterionic phospholipids has shown to protect against NSAID-induced GI tract damage in a rat model (Lichtenberger et al, 1995). This intermolecular association has been suggested to be the mechanism by which NSAIDs attenuate the hydrophobic barrier properties of the upper GI tract. However, this concept cannot be fully embraced without further animal testing and corroborative pharmacokinetic data.

A range of other approaches have been employed to treat NSAID-induced GI damage. Protection through selective biochemical mediators has been attempted to establish if selective-5-lipoxygenase inhibitors and antagonists exert inhibitory effects on NSAID-induced GI damage (Rainsford, 1987). Studies have demonstrated that oral administration of mixtures of amino acids reduces ulcerogenicity of aspirin in starved rats (Balint, 1991). Orally administered cholestyramine reduced indomethacin lesion index (Brodie *et al.*, 1970). Interleukin administration has been shown to reduce the severity of gastroduodenal ulceration of indomethacin (Wallace *et al.*, 1992). Simultaneous oral administration of the anti-oxidant butylhydroxytoluene and a xanthine oxidase inhibitor with indomethacin prevented the damaging effect of

indomethacin on the rat gastric mucosa (Krupińska *et al.*, 1980; Rainsford, 1990). Conversely, atropine sulfate, scopalamine methylbromide, bretylium, cyproheptadine, pentobarbital, chlorpromazine and aluminum hydroxide have not proved to be effective means to reduce indomethacin-induced intestinal ulceration (Brodie *et al.*, 1970).

Intensive efforts are now being made to develop selective inhibitors of COX_2, assuming that these agents will inhibit this isoform when it is induced at sites of inflammation, but will not inhibit prostaglandin synthesis in other tissues where COX_1 is constitutively expressed. Preliminary studies of highly selective COX_2 inhibitors in animal models suggest that these agents may reduce GI toxicity (Mansfeerer *et al.*, 1994; Chan *et al.*, 1994).

Another novel strategy to reduce the gastric ulcerogenicity of NSAIDs that has recently been described is the incorporation of a nitric oxide generating moiety into the NSAID molecule (Wallace *et al.*, 1993). Nitric oxide which is bactericidal and a potent inhibitor of neutrophil function (Kubes *et al.*, 1991) may counteract the suppression of COX in the GI microcirculation, inhibit neutrophil adherence to the vascular endothelium, and scavenge free radicals thereby preventing GI damage (Rubanyi *et al.*, 1991; Wallace *et al.*, 1993; 1994a; 1994b; 1995). NO donors and NO-NSAIDs have been shown to reduce GI damage in various animal models (Reuter *et al.*, 1994; Wallace *et al.*, 1994a; Cuzzolin *et al.*, 1995). Metronidazole is a nitroimidazole antibacterial also used in the treatment of inflammatory bowel disease. The drug undergoes reductive activation giving the nitro-free radical, nitroso, nitroso-free radical and hydroxylamine derivatives (Müller, 1986). Metronidazole has also been

shown to be a free radical scavenger (Akamatsu *et al.*, 1991). It has also been shown to reduce NSAID-intestinal inflammation (Bjarnason *et al.*, 1992b). Tempo is a cell permeable, nitroxide stable free radical scavenger that has also been shown to prevent lipid peroxidation (Nilsson *et al.*, 1989), block DNA scission and protect cells from oxidative damage (Samanuni *et al.*, 1991), and appears to prevent upper GI damage induced by NSAIDs in a rat model (Rachmilewitz *et al.*, 1994).

Morris *et al.* (1994) have retrospectively examined the effect of cytoprotective therapy with the prostaglandin E1 analogue misoprostol on the anemia of patients with enteroscopically proven NSAID-induced small bowel enteropathy. An improvement in the anemia in patients on misoprostol therapy with proven NSAID enteropathy was found. Further, in an attempt to modify the NSAID-induced intestinal inflammation sulphasalazine has also been shown to reduce mucosal inflammation, and GI blood loss has been shown to be reduced by treatment with both metronidazole and sulfasalazine (Bjarnason *et al.*, 1990a; Bjarnason *et al.*, 1992b).

Previous cytoprotective approaches have been largely empirical and in the absence of a clear understanding of the mechanisms involved in NSAID-induced GI toxicity. The apparent GI cytoprotective effects of the agents have not been correlated with their actions on the postulated biochemical pathways by way of measurements of changes in products. Furthermore, differentiation between the gastric and intestinal toxicological manifestations of NSAIDs has been largely ignored. Hence, it is not known if the suggested schemes for gastroduodenal protection alleviate intestinal lesions as well.

NSAID-induced side-effects to the GI mucosa have been suggested to result from three general sources: (Halter, 1988)(1) local effects of direct exposure during drug absorption after oral administration (2) systemic effects after absorption, and (3) the local effects of enterohepatic recirculation, excretion in bile, and reappearance in the GI tract. The relative contribution made by each route of access is unknown, however, it has been previously suggested that NSAID-induced intestinal permeability increases during absorption or after biliary excretion, and that systemic effects may be of relatively minor importance (Bjarnason *et al.*, 1991a). Studies in laboratory animals have shown that indomethacin induces much less damage in germ-free or antibiotic-treated rats than in normal rats (Robert and Asano, 1979; Kent *et al.*, 1969), and the composition of food may also be of importance (Satoh *et al.*, 1982). The enterohepatic recirculation of the glucuronide conjugate of the drug regenerated to active drug by the β-glucuronidase in intestinal flora, is thought to be a major factor in the marked intestinal ulcerogenicity of NSAIDs (Kent *et al.*, 1969; Robert and Asano, 1979). Hence, in the belief that enterohepatic circulation may facilitate repeated exposure of the intestinal mucosa attempts have been made to introduce non-acidic NSAIDs (e.g. choline magnesium trisalicylate) or their pro-drugs (e.g. nabumetone) and NSAIDs with limited biliary excretion (e.g. sulindac)(Rampton 1987; Graham *et al.*, 1985). Similarly, NSAIDs are sometimes enteric-coated, assuming that mucosal lesions are due partly to local toxic effect of the drug. β-Cyclodextrin complexation has also been used to improve the dissolution characteristics of NSAIDs and has been suggested to be suitable as a gastroprotective agent (Rainsford, 1990). However, the validity of

these hypotheses have not been unequivocally proven. In a large data base analysis, sulindac conceptually devised to cause less GI damage, was equally or more toxic than most other NSAIDs which may be attributable to either its enterohepatic circulation (4%) or systemic ability to induce damage (Weber, 1984). Nabumetone is an effective pro-drug NSAID that has so far produced fewer clinically significant GI side-effects but certainly is not devoid of these effects either (Bernhard, 1992; Fleischmann, 1992).

On the assumption that mucosal lesions are partly due to a local toxic effect of NSAIDs, enteric coating has been utilized in the hope that this might reduce gastroduodenal toxicity allowing the drug to bypass the gastroduodenal mucosa before dissolving, delaying exposure of the active drug until the drug has reached the small bowel. Indeed, enteric coating of several NSAIDs has demonstrated a reduction in endoscopic findings in the stomach and duodenal bulb (Lanza *et al.*, 1980; Trondstad *et al.*, 1985). However, this formulation may increase the exposure of active drug to the mucosa distally to the duodenal bulb, and thereby increase the toxicity to a region where the effects are difficult to monitor. It has been suggested that modifying dosage forms of NSAIDs by enteric-coating or sustained release formulations to reduce ulcerogenicity may produce as much or more endoscopically demonstrated gastric damage as the parent compound in conventional formulation or produce risks elsewhere in the GI tract (Collins *et al.*, 1988). This region of the GI tract is less available for controlled evaluation, and such an effect may provide more serious sequelae. The slow releasing of drug at high concentrations at a specific site, may cause lower bowel perforation in patients without mucosal disease (Bjarnason *et al.*,

1987a; Rampton, 1987). Slow release enteric coated potassium supplements are known to cause solitary ulcers in the lower small intestine (Farquharson-Roberts *et al.*, 1975). A controlled release indomethacin preparation Osmosin® has also been suggested to cause perforation of the lower bowel which may have lead to its voluntary removal from the market (Day, 1983; Laidler *et al.*, 1985; Cree *et al.*, 1985). The reported problems of Osmosin® may illustrate the importance of considering the potential of intestinal toxicity of other NSAIDs that are marketed in sustained release or novel formulations. The efficacy of the protective measures of modified release formulations on the GI tract is dependent on the relative importance of local versus systemic effects of NSAIDs. Interestingly, an increasing number of case reports implicate a causal use of enterocoated and sustained release NSAID consumption with the induction of small and large bowel toxicity (Tables 7 and 8).

Table 7. Case Reports of Enterocoated and Sustained Release NSAID-Induced Intestinal Toxicity

Authors	NSAID(s)	#	Age	Gender	Duration of Use	Toxicological Effect
Davies and Brightmore (1970)	sodium salicylate EC & acetylsalicylic acid	1	51	F	NR	perforation of jejuno-ileal junction
Day (1983)	indomethacin SR (Osmosin)	1	70	M	2 weeks	hemorrhagic perforating ileal and ascending colon ulcers, death
Cree et al. (1985)	indomethacin SR (Osmosin)	1	78	F	10 weeks	abdominal pain, perforated, ileal ulcers, death
Kühl et al. (1985)	indomethacin+	1	26	M	8 days	intestinal perforation, septic shock, peritonitis, multiple colonic perforations
Ritschard and Filippini (1986)	diclofenac EC	1	21	F	2 weeks	ileocolitis, abdominal pain, peritonitis
Bem et al. (1988)	indomethacin SR (Osmosin)	1	70	F	2 weeks	intestinal perforation
		1	83	M	7 weeks	intestinal perforation
		1	76	F	2 months	intestinal perforation
		1	78	F	2 weeks	intestinal perforation
		1	78	F	NR	intestinal perforation
		1	65	F	2 months	intestinal ulcer, peritonitis
		1	78	F	NR	intestinal ulcer, peritonitis
		1	70	M	4 months	peritonitis
Deakin (1988)	diclofenac SR	1	70	M	two weeks	perforation, abdominal pain, peritonitis in left iliac fossa
Lang et al. (1988)	indomethacin SR+	1	71	NR	NR	anemia, subacute small bowel obstruction, death
Witham (1991)	diclofenac EC	1	67	F	1.25 years	ileus and leukocytosis, perforated ileum, sepsis
Cutler et al. (1993)	acetylsalicylic acid EC	1	42	M	NR	fatigue, dyspnea, melena, anemia, abdominal pain, web-like stricture, ulcer, sub mucosal fibrosis

Hudson et al. (1993)	diclofenac SR	1	38	M	5 years	right-sided lower abdominal pain, diarrhea, dyspepsia, ileal and cecal ulcers, lymphocyte infiltrate
Keating and McIlwaine (1993)	diclofenac SR	1	85	M	10 days	abdominal pain, peritonitis, perforation of mid-ileum, stercoral ulcers in ascending colon
Pucius et al. (1993)	indomethacin SR	1	61	F	9 years	colic, weight loss, diarrhea, ileocecal ulceration
Speed et al. (1994)	diclofenac with indomethacin SR	1	52	F	10 years	anemia, intestinal obstruction, fecal occult blood, ulcers, diaphragms
Bielecki et al (1995)	mefenamic acid and diclofenac SR	1	51	M	7 months	colicky pain, ileal ulceration, thicked wall and irregular wall surface of the jejunum, submucosal fibrosis
Davies et al. (1996)	indomethacin SR and naproxen	1	72	F	14 years	iron deficiency anemia, chronic blood loss, hypoalbuminemia, vitamin b12 deficiency

NR= not reported, SR=sustained release, EC=enteric coated, + death

Table 8. Case Reports of Enterocoated and Sustained Release NSAID-Induced Large intestine Toxicity

Authors	NSAID(s)	#	Age	Gender	Duration of Use	Toxicological Effect
Coutrot et al. (1978)	indomethacin SR	1	82	F	4 days	pneumoperitineum, peritonitis, free pus, perforated sigmoid diverticulae, colonic ulcerations
	indomethacin SR	1	74	F	18 days	pneumoperitoneum, peritonitis, perforated sigmoid diverticulae
Schwartz (1981)	naproxen & buffered ASA	1	60	M	1.5 months	rectal bleeding
Day (1983)	indomethacin SR (Osmosin)	1	70	M	2 weeks	diarrhea, hemorrhagic perforating ulcers ileum and ascending colon, death
	indomethacin SR (Osmosin)	1	79	F	2 weeks	peritonitis, perforation
Ritschard and Filippini (1986)	diclofenac EC	1	21	F	2 weeks	ileocolitis, abdominal pain, peritonitis
Güller (1987)	diclofenac EC	1	43	M	12 years	diffuse colitis
	diclofenac EC and naproxen	1	55	M	days	rectosigmoid ulcerations
Carson et al. (1989)	diclofenac SR & acetylsalicylic acid	1	60	F	4 months	colonic ulceration, anemia
	diclofenac SR	1	67	F	2 months	cecal ulceration, anemia
Perreard et al. (1989)	diclofenac EC	1	53	F	3 weeks	right iliac fossa and colitis, abdominal pain, colonic ulcers with bleeding

Sheer and Williams (1989)	diclofenac SR	1	73	F	several years	stricturing scars
	diclofenac SR	1	66	F	2 years	colitis
Hollingworth and Alexander-Williams (1991)	ketoprofen SR	1	76	F	1 year	abdominal pain, fecal perforation and peritonitis
Witham (1991)	diclofenac EC	1	81	F	7 days	rectal bleeding, ulceration of ileal colic anastomosis
	diclofenac EC & acetylsalicylic acid EC	1	55	F	18 days	rectal bleeding, ulceration of the ascending colon
Huber et al. (1991)	diclofenac SR	1	61	M	6 years	colonic ulcers & strictures, iron deficiency, submucosal fibrosis, anemia, bloody mucous, diarrhea
Stamm et al. (1991)	diclofenac EC	1	NR	NR	NR	colonic ulcerations, iron deficiency anemia, hematochezia, abdominal pain
Fellows et al. (1992)	diclofenac SR	1	77	F	5 years	microcytic anemia, colonic stricture, weight loss
Gibson et al. (1992)	diclofenac EC	1	82	F	3 months	anemia, weight loss, diarrhea, weakness
	diclofenac EC	1	87	F	10 weeks	cecal ulceration, abdominal pain, colitis, anemia,
	acetylsalicylic acid EC	1	71	M	5 years	weight loss, fever, night sweats, ascending colon ulcers, anemia
Haque et al. (1992)	diclofenac SR and various NSAIDs	1	49	F	5 years	cecal diaphragm, iron deficiency anemia, melena, ulcers, submucosal fibrosis

Ridell et al. (1992)	acetylsalicylic acid EC diclofenac EC	8 1	NR NR	NR NR	2-15 years 2 years	collagenous colitis, diarrhea, mucosal inflammation
Whitcomb et al. (1992)	diclofenac EC	1	63	F	2.5 years	colonic strictures, iron deficiency anemia, abdominal pain
Carratù et al. (1993)	ketoprofen SR	1	49	M	17 days	diarrhea, rectal bleeding, colitis
Cutler et al. (1993)	acetylsalicylic acid EC	1	42	M	NR	fatigue, dyspnea, melena, anemia, abdominal pain, web-like stricture, ulcer, sub mucosal fibrosis
Halter et al. (1993)	diclofenac SR	1	59	F	1 year	diarrhea, semilunar colonic ulcers and "diaphragm-like" stenosis, colicky RUQ abdominal pain,
	diclofenac SR	1	79	F	2 years	anemia, "diaphragm-like" stenosis
	diclofenac SR	1	72	M	1 year	anemia, diarrhea, weight-loss "diaphragm-like" strictures and ulcers
	diclofenac SR	1	51	F	2 years	"diaphragm-like" strictures and stenosis, RLQ colicky pain, diarrhea
	diclofenac SR	1	71	F	3 years	anemia, ulcers
Hudson et al. (1993)	diclofenac SR	1	38	M	5.5 years	abdominal pain, diarrhea and anemia, right iliac fossa mass, ileum and cecal ulceration

Keating and McIlwaine (1993)	diclofenac SR	1	85	M	10 days	abdominal pain, peritonitis, perforation of mid-ileum, stercoral ulcers in ascending colon
Pucius et al. (1993)	indomethacin SR	1	61	F	9 years	colic, weight loss, diarrhea, ileocecal ulceration, stictures of ascending colon
D'alteroche et al. (1994)	diclofenac SR	1	66	F	2 years	colonic diaphragms and cecal ulcers, anemia, stenosis
Hayllar et al. (1994)	various NSAIDs	1	21	NR	>1 year	intestinal inflammation and blood loss
Baert et al. (1995)	diclofenac SR	1	68	F	2 years	transverse colon ulcerations, erythema, fibrosis, granuloma
Gargot et al. (1995)	aspirin and diclofenac SR	1	81	M	2 years	iron deficiency anemia, diarrhea, multiple thin circumferential mucosal membranes, ulcers of the ascending colon
Robinson et al. (1995)	diclofenac EC & acetylsalicylic acid	1	53	F	5 years	abdominal pain, perforated cecum, stricture

NR= not reported, SR=sustained release, EC=enteric coated

1.1.10 Stereoselective Pharmacodynamic and Toxicological Considerations of Chiral NSAIDs

The fundamental basis for studying the concentrations of a drug in biological matrices, and the subsequent establishment of its pharmacokinetic indices is that there is a measurable relationship between drug concentration and the degree(s) of pharmaco-dynamic activities. Whereas NSAIDs are used extensively in the treatment of rheumatic diseases their clinical use remains largely empirical as there is little evidence in support of a correlation between dose or plasma concentration and therapeutic efficacy or toxicity.

Generally, a relationship between pharmacodynamic effect and plasma concentrations of NSAIDs has been difficult to achieve with wide ranging explanations (Day *et al.*, 1988). NSAIDs are a structurally heterogeneous and diverse group of compounds (Table 1) Furthermore, some members are unique in that they are chiral and marketed as racemates (i.e. equivalent proportions of enantiomers) (Table 9). Naproxen was initially the only NSAID to be marketed and used clinically as the pure S-(+)-enantiomer, however, S-ibuprofen has been recently introduced onto the market in Austria (Chlud, 1995).

The most common and simplistic example of chirality is that due to a sp^3-hybridized tetrahedral carbon atom to which 4 different substituents are attached. The word chirality comes from the Greek 'cheir' meaning hand. Just as we have a right hand that mirrors the left hand, our hands are non-superimposable "enantio" or opposite. A drug such as ibuprofen has one chiral carbon and therefore exists as a pair

of enantiomers. The enantiomers like our hands are nonsuperimposable on its mirror image. Most chiral NSAIDs are currently marketed as a racemate or a 50:50 composite of both of its enantiomers.

Additionally, sulfur, nitrogen, silicon, and phosphorus, can all act as chiral centers. Although the chiral carbon atom predominates in pharmaceuticals, sulindac is an example of a racemic NSAID with a chiral sulfur heteroatom.

Table 9. Chiral Nonsteroidal Anti-inflammatory drugs

Arylpropionic	**Acids**	**Arylalkanoic Acids**	**Non Acidic Agents**
Carprofen	Ketoprofen	Butibufen	Azapropazone
Cicloprofen	Loxoprofen	Clindanac	Bumadizone
Fenoprofen	Miroprofen	Etodolac	Oxyphenbutazone
Flunoxaprofen	Naproxen	Flobufen	Talnifumate
Flurbiprofen	Piroprofen	Indobufen	
Furprofen	Pranoprofen	Ketorolac	
Indoprofen	Suprofen	Metbufen	
Ibuproxam	Tiaprofenic Acid	Sulindac	
Ketoprofen	Thioxaprofen		
Loxoprofen	Ximoprofen		

Particularly with the 2-APA class of NSAIDs which constitute some of the most commonly prescribed medications throughout the world, possession of a chiral carbon centre and general clinical administration as a 50:50 composition of S and R enantiomers contributes to this difficulty in establishing concentration-effect relationships. It has been a general belief that for all members of the 2-APAs, it is the S

or (+) enantiomer which possesses the anti-inflammatory activity of the racemate, with the R or (-) enantiomers essentially devoid of activity (Hutt and Caldwell, 1984). However, the R enantiomer of some 2-APAs may undergo a unique *in vivo* chiral inversion to the S enantiomer. This inversion process is dependent on both the drug and the species. Therefore, depending on whether an agent undergoes inversion, the R enantiomer cannot be presumed inactive and perhaps may be considered a pro-drug. Furthermore, the R enantiomer may contribute to the toxicity as will be discussed later. Each enantiomer can exhibit distinctive pharmacokinetics from its antipode and the nature of the chiral carbon allows the unidirectional R to S inversion of antipodes although among the commercially available 2-APA's, this is probably only significant for ibuprofen and fenoprofen in humans (Berry and Jamali, 1988; Jamali *et al.*, 1988a).

The apparent lack of dose-effect or concentration-response of chiral NSAIDs administered as racemates may in part be due to their consumption as racemates. In an attempt to delineate concentration-effect relationships of 2-APA NSAIDs novel stereospecific HPLC assays have been developed for many chiral NSAIDs which are able to separately quantitate concentrations of each enantiomer (Wright *et al.*, 1992; Berry and Jamali, 1988; Palylyk and Jamali, 1991). The measurement of the active component(s) with pharmacodynamic effect(s) may provide the means for delineation of concentration-effect, dose-effect relationships. A rational decision regarding the therapeutic use of either racemate or single enantiomer(s) must involve the consideration of pharmacodynamic, toxicological and pharmacokinetics of each chiral NSAID on an individual basis.

1.10.1 Pharmacodynamic Aspects

In vitro studies have shown the anti-inflammatory activity of the 2-APA's is ascribed mainly to the S enantiomer (S/R activity 30-100) (Hutt and Caldwell, 1983; Hutt and Caldwell, 1984). Therefore, it has been suggested that the R enantiomer of NSAIDs is an unnecessary impurity or 'isomeric ballast' and that a stereochemically pure enantiomer is superior to its respective racemate (Ariens, 1984).

It is likely that a relevant correlation does exist between the active isomer concentration and therapeutic effect. For a drug with stereoselective pharmacokinetics such as ibuprofen the correlation between effect and total drug concentration is positive but weak (Laska *et al.*, 1986). However, the correlation for naproxen, a sterochemically pure NSAID, is substantially greater than that reported for ibuprofen (Day *et al.*, 1982). More recently, Evans *et al.* (1991) have found that inhibition of thromboxane production derived from COX_1 by blood platelets was closely related to the unbound fraction of S-ibuprofen in plasma.

There are few clinical reports examining whether the single S enantiomer of a NSAID offers advantages over its respective racemate with respect to efficacy. Administration of the single S flurbiprofen in post-episiotomy pain provided analgesia superior to its racemate (Sunshine *et al.*, 1987). Further, clinical studies comparing racemic NSAIDs to each of the individual enantiomer(s) in terms of pharmacodynamic response are warranted.

1.10.2 Toxicological Aspects

In the case of 2-APA NSAIDs it has been suggested that by marketing the S enantiomer it may be possible to circumvent the toxic effects ascribed to the "inactive" R enantiomer (Caldwell *et al.*, 1988). Alternatively, it has also been suggested that as a prodrug via bio-inversion, the R enantiomer may be a safer alternative to the available racemate or a product that contains the active S antipode (Williams and Day, 1988). Consequently, the rationale behind the marketing of chiral drugs and the therapeutic advantages of administering chiral drugs as racemates or as single enantiomers has been questioned (Ariens, 1984.)

However, several recent publications suggest activities for the R enantiomers of NSAIDs. Some analgesic activity has been ascribed to R-flurbiprofen (Brune *et al.*, 1991; Malmberg and Yaksh, 1994), and it has been suggested that reduced renal function in isolated perfused rat kidney is also caused by R-ibuprofen (Cox *et al.*, 1991). The R enantiomers of ibuprofen and flurbiprofen also have effects on β-oxidation of fatty acids and mitochondrial respiration (Williams *et al.*, 1995). Additionally, the R enantiomer may also be a substrate for chiral inversion and incorporation into natural fatty acids and form hybrid triglycerides (Williams *et al.*, 1986). The formation of hybrid triacylglycerols has been speculated to contribute to central nervous system side-effects of these drugs (Hutt and Caldwell, 1983). It has been proposed that the R enantiomer is stereoselectively converted to its coenzyme A thioester which is then hydrolyzed by a non-stereoselective racemase to release the S enantiomer (Wechter *et al.*, 1974). As the S enantiomer is not a substrate for this enzymatic reaction, the inversion proceeds unidirectionally. Coenzyme A thioester of

carboxylic acids can replace the natural fatty acid in triacylglycerols to form "hybrid" triglycerides (Caldwell and Marsh, 1983). It has been hypothesized that commonly used NSAIDs which are substrates for chiral bioinversion, may selectively deposit in adipose tissue. Thus the R enantiomers of these drugs may also possess intrinsic pharmacological activity. It must be recognized that, in the absence of inversion, the R enantiomer which represents 50 % of the dose is essentially an impurity and, as such, may have a toxicity profile independent of the S enantiomer.

There are few clinical reports examining whether the single S enantiomer of a NSAID offers toxicological advantages over its respective racemate. In a limited clinical study after administration of single short term S-ibuprofen in patients with RA none of the 4 patients studied experienced any GI distress postulated to be due to a reduced metabolic load (Geisslinger *et al.*, 1990). In addition there were no significant differences in the number of patients experiencing GI or CNS disturbances in fifty RA patients given S- ibuprofen or the racemate (Stock *et al.*, 1991)

1.10.3 Pharmacokinetic Considerations

Most of the enantiomeric pharmacokinetic data of 2-APA NSAIDs have shown an AUC of the S enantiomer exceeding the R enantiomer (Evans, 1992). However, for at least two chiral NSAIDs, (etodolac and ketorolac) the AUC(R) exceeds that of the AUC(S) by several fold (Jamali, 1988; Vakily et al, 1995). Thus, it may be misleading to relate pharmacodynamic or toxicological data to total drug concentration of some chiral NSAIDs.

The relative concentration of the pharmacologically active S enantiomer of ibuprofen (S:R ratio) increases with the prolongation of the GI transit time of racemic formulations due to a corresponding increase in chiral inversion of the R to S enantiomer in the intestine (Jamali *et al*, 1991a). Administration of S-ibuprofen, therefore, reduces the formulation dependent variability in the concentration of the active enantiomer in the body.

In addition, when comparing the pharmacokinetics of a single enantiomer versus the racemate the possibility of an enantiomeric interaction must be examined as is evident for flurbiprofen (Berry and Jamali, 1988). Furthermore, the possibility of different dosage forms of the same chiral drug giving rise to differences in AUC has also been observed with flurbiprofen (Jamali *et al.*, 1991b). Differences in physio-chemical parameters of the racemate such as solubility compared to the individual enantiomers may also affect dissolution rate (Dwivedi *et al.*, 1992) and lead to faster absorption of stereochemically pure NSAIDs. If NSAID-induced GI toxicity is indeed absorption rate dependent then the decision of whether to market a stereochemically pure NSAID as well as the type of formulation may impact on the extent of NSAID-induced GI toxicity.

II. Rationale for Study and Research Objectives

While the NSAIDs are therapeutically equipotent, epidemiological data suggests that they may vary in their GI toxicity profiles (Fries *et al.*, 1991). Available data, however, have concentrated mainly on the upper GI tract side-effects. However, recent reports demonstrate the increased incidence of lower GI toxicity (Allison *et al.*,

1992; Bjarnason *et al.*, 1993a). Hence, the entire GI tract must be studied. There is, however, a lack of a suitable, reproducible animal model. The initial objective of this work was to develop and validate a suitable animal model for the examination of NSAID-GI damage. As NSAIDs induce increased GI permeability at the level of the mucosal tight junction and these changes correlate with GI toxicity we chose to measure GI permeability as a measure of NSAID-induced GI damage (Bjarnason *et al.*, 1984a; Meddings *et al.*, 1993). Urinary excretion of ^{51}Cr-EDTA and sucrose have proved to be reliable, sensitive, simple, and non-invasive methods for measuring intestinal and gastroduodenal permeability, respectively (Meddings *et al.*, 1993; Bjarnason *et al.*, 1986a).

The rat is proposed to be a suitable model for the proposed experiments. It is prone to NSAIDs-induced GI abnormalities; the animal is routinely used as a model for experimentally induced arthropathies as well as for gross toxicological NSAID-induced GI abnormalities. Animal models of NSAID-induced gastric and small intestinal ulceration have been widely used for studies of the pathogenesis of these disorders and for evaluation of potential therapies. The gastric ulceration associated with NSAID treatment in the rat has demonstrated a good correlation with the studies performed in humans (Rainsford, 1988; Shriver *et al.*, 1975; Beck *et al.*, 1990). The interest in NSAID-induced toxicity in the intestine is in part due to animal studies conducted as early as 20 years ago on inflammation and ulcers (Brodie *et al.*, 1970; Kent *et al.*, 1969). It has previously been suggested that indomethacin-induced intestinal damage in rats resembles NSAID-induced enteropathy of the human intestine

(Trevethick *et al.*, 1991). GI blood loss and ulceration are end points of a complex damage process and are relatively insensitive markers of NSAID-induced GI damage. However, intestinal permeability has recently been shown to correlate with intestinal ulceration and is an easily detectable and sensitive index of NSAID-induced intestinal damage (Ford *et al.*, 1995).

There is considerable knowledge regarding the pharmacokinetics of various NSAIDs in the rat model (e.g., Berry and Jamali, 1988; Satterwhite and Boudinot, 1990). In contrast to the gross toxicological manifestations (ulceration and inflammation) of NSAID use where there has been extensive use of the rat as an animal model, there does not appear to have been detailed examination of the effects of NSAIDs on gastroduodenal or intestinal permeability in this species. Several investigators have done preliminary studies of the assessment of intestinal permeability in the rat (Lökken and Sögnen, 1967; Ramage *et al.*, 1988; Bjarnason *et al.*, 1985b) but these studies have not been extended to the detailed examination of NSAID-induced alterations. Given the apparent parallels between the occurrence of GI tract adverse effects between rats and humans it is of interest to examine whether the permeability changes between these species throughout the GI tract also parallel one another. The development of a rat model would allow for the detailed study of the toxicokinetics of GI permeability.

At the genesis of this project, a review of the literature revealed the absence of a suitable animal model for non-invasively evaluating NSAID-induced GI permeability

throughout the entire GI tract. Initially, therefore, the development of a suitable animal model sensitive to changes in NSAID dose and protective agents was necessary.

As a consequence of the lack of a suitable animal model, that provides reproducible results, is relatively inexpensive, responsive to NSAIDs, and protective agents in a manner similar to humans there had not been extensive examination of the toxicokinetic effects of NSAIDs on induction of GI permeability. The first step was to establish a suitable animal model in order to address several important issues related to the toxicokinetics of NSAID-induced GI damage.

Hypotheses

1. The rat is a suitable animal model for studying NSAID-induced GI permeability changes

1.1. The rat model responds to pharmacological protection of NSAID-induced permeability (e.g. glucose/citrate, misoprostol, sulfasalazine, metronidazole, tempo, allopurinol).

Administration of gastroduodenal protective agents for prevention of GD toxicity induced by NSAIDs has been investigated using a wide variety of agents. However, it is not known if the suggested schemes for GD protection alleviate NSAID- induced intestinal lesions as well. Regulation of intestinal permeability may be, at least in part, regulated by prostaglandins, and misoprostol has previously been shown to partially protect the intestinal mucosa from the effects of indomethacin in clinical studies (Bjarnason *et al.*, 1989a). In humans, NSAID-induced permeability changes were not reduced by H_2-antagonists (Table 6). H_2-antagonists may reduce

the incidence of gastric ulceration by elevating the gastric pH whereas intestinal damage appears to be pH independent. Furthermore, in humans NSAID-induced permeability was not reduced by sucralfate (Aabakken *et al.*, 1989a). Sucralfate has little or no effect at reducing NSAID-induced gastric ulceration and it appears to afford no protection in the intestine. Results also found in humans suggest indomethacin effects on permeability were reduced by sulfasalazine (Banerjee *et al.*, 1986). It has been speculated that sulfasalazine may limit neutrophil recruitment to sites of inflammation, scavenge free radicals, and thereby ameliorate the inflammatory process. In addition, it has been suggested that the presence of glucose and citrate in the intestinal lumen may modify the reaction to indomethacin or that citrate may protect against free radical damage caused by NSAIDs (Bjarnason *et al.*, 1992a). In humans, a formulation containing 15 mg glucose and 15 mg citrate to each mg of indomethacin prevented the expected effect of the NSAID on intestinal permeability (Bjarnason *et al.*, 1992a). Several compounds with nitric oxide moieties have been recently shown to have GI protective effects in models of GI toxicity (Rachmilewitz *et al.*, 1994; Wallace *et al.*, 1994a). These compounds may inhibit neutrophil adherence to the vascular endothelium, scavenge free radicals or protect the GI tract through undetermined mechanisms. In addition, it has been suggested that the xanthine oxidase inhibitor allopurinol can also protect against NSAID-induced GI damage by scavenging superoxide radicals (Rainsford, 1990).

In order to elucidate the pathogenic mechanisms involved in NSAID-induced GI complications and to validate the rat model of NSAID-GI permeability various

protective agents (glucose/citrate, sucralfate, sulfasalazine, H_2-antagonists, and misoprostol) were evaluated. Two structurally dissimilar compounds that have a nitric oxide moiety (tempo) or can be metabolized to radical nitric oxide intermediate(s) (metronidazole) were also examined.

2. To elicit GI cytotoxicity, NSAIDs must be present at the site, i.e., GI epithelium, in sufficient concentration.

A high site concentration can be achieved 1) during GI absorption, 2) *via* the systemic circulation when the drug is distributed into GI epithelial cells and 3) during enterohepatic recirculation. It is possible that an NSAID may present itself in a small concentration in the GI epithelial cells, or in contrast, an NSAID may be found in high concentrations at the site of the side-effect. For example, sulindac, an inert prodrug, with limited biliary excretion of the active moiety, was thought to be a safer alternative to other NSAIDs due to the lack of local exposure of the GI tract to the active metabolite (Dobrinska *et al.*, 1983). It is now known that sulindac is indeed more toxic than many other NSAIDs. This may be due to a high uptake of the active metabolite by the epithelial cells *via* systemic distribution.

The distribution of racemic ibuprofen into various segments of the intestinal epithelium (i.e. duodenum, jejunum, and ileum) and the drug concentrations were assessed and correlated with the established index for intestinal damage (^{51}Cr-EDTA excretion) after administration of a range of doses.

3. Stereochemically pure NSAIDs are not necessarily safer than the respective racemate.

It has previously been suggested that by marketing the S enantiomer of NSAIDs it may be possible to circumvent potential toxic effects ascribed to the "inactive" R enantiomer (Caldwell *et al.*, 1988). Indeed, the rationale behind the marketing of chiral drugs and the therapeutic advantages of administering chiral drugs as racemates or as single enantiomers has been questioned (Ariens, 1984). This issue of administration of racemic versus stereochemically pure enantiomers of NSAIDS was addressed through the administration of etodolac, flurbiprofen, ketoprofen, and ibuprofen and by measuring intestinal permeability changes in the developed rat model.

4. The site of GI toxicity of NSAIDs may be influenced by the site of drug delivery

Considering the fact that NSAIDs are the most frequently prescribed class of therapeutic agents, their side-effects present a relatively major problem for the public. To overcome this problem, various approaches have been taken, including preparation of enteric coated or slow release formulations, development of prodrugs, once-daily dosing (long $t_{1/2}$ NSAIDs) and coadministration with cytoprotective drugs. None of these approaches, however, have offered a completely satisfactory solution to the problem of GI toxicity. The rat model for studies of GI permeability may help to elucidate the relative safety profile of NSAIDs with different pharmacokinetic characteristics and formulations with different release patterns. A present trend in NSAID development is to improve therapeutic efficacy and reduce the severity of upper GI side-effects through modification of dosage forms of NSAIDs by enteric-coating or through sustained release formulations. Indeed, enteric coating of several

NSAIDs has demonstrated a reduction in endoscopic findings in the stomach and duodenal bulb (e.g. Lanza *et al.*, 1980). The therapeutic rationale behind these new formulations has not been unequivocally proven, as it has been suggested that these formulations may increase the exposure of active drug to the mucosa distally to the duodenal bulb, and thereby increase toxicity to this region where the effects are difficult to monitor (Collins *et al.*, 1988). The slow release of an NSAID at high concentrations at a specific site, may cause lower bowel perforation in patients without mucosal disease. Therefore, the kinetics of NSAID release in the GI tract may be important in the site of GI toxicity. SR products of NSAIDs are expected to be released mainly in the intestine and these changes in the release rate and its effect on prolonged intestinal presence may affect the overall GI toxicity. The establishment of an animal model for studies of the entire GI tract permeability subsequently enabled assessment in the gastroduodenal and distal intestine of the relative safety profile of a commercially available regular and sustained release formulation of a NSAID (flurbiprofen) which has different pharmacokinetic and release characteristics.

Given the apparent parallels between the occurrence of GI tract adverse effects of NSAIDs between rats and humans it is of interest to examine whether the permeability changes between these species in the gastroduodenum and intestine also parallel one another. Integration of human results with appropriate animal models may considerably increase our understanding of the toxicokinetics of NSAID-induced GI permeability.

3. EXPERIMENTAL

3.1 Materials

Racemic ibuprofen powder was kindly provided by the Upjohn Co. of Canada, (Don Mills, Canada). The R and S enantiomers of ibuprofen were supplied by Sepracor, (Marlborough, MA, USA), and Ethyl Chemicals Group, (Baton Rouge, LA, USA) and were 98 and >99 % pure respectively, as determined by a stereospecific HPLC method (Wright and Jamali, 1993). The enantiomers of ketoprofen were R (99 % pure) and S (98.9 % pure) as determined by HPLC (Wright and Jamali, 1993), and obtained as gifts from Rhone Poulenc-Pharma (Montreal, Canada). Pure enantiomers of flurbiprofen (FL) were kindly supplied by Organon Canada Ltd. (Westhill, Canada). The purity of the S and R enantiomers was 98.6 % and 99 %, respectively as determined by HPLC (Wright and Jamali, 1993). Racemic etodolac and the R and S enantiomers, 97 % and 99 % stereochemically pure as determined by HPLC (Wright and Jamali, 1993), were supplied by Wyeth-Ayerst (Montreal, Canada). Tempo was purchased from Aldrich Chemical Co. (Milwaukee WI, USA). Indomethacin, sulindac, nabumetone, R-(+)-α-phenylethylamine (PEA)(>99% pure), neomycin sulfate, erythromycin, corticosterone, sucrose, Trinder's Reagent, mefenamic acid, naproxen, diclofenac, and carrageenan Type 1 powder were purchased from Sigma (St. Louis., MO, USA). Misoprostol (Cytotec, Searle, Canada), sulfasalazine (Salazopyrin, Pharmacia, Canada), sucralfate (Sulcrate, Nordic, Canada), allopurinol (Novopharm, Canada), cimetidine (Tagamet, Smith-Kline-Beecham, Canada), famotidine (Pepcid, Merk-Sharp-Dome, Canada), and flurbiprofen sustained release capsules (Organon Canada Ltd., Westhill, Canada) were purchased from a local pharmacy. Aspirin was

purchased from Mallinckrodt Chemical Works (St. Louis, MO. USA). ^{51}Cr-EDTA (specific activity 570 MC/mg) was purchased from Dupont NEN (Wilmington, DE, USA). Fenoprofen was kindly provided by Eli Lilly (Toronto, Canada). Plate Count Agar was obtained from (Becton, Dickson and Company, Cockeysville, MD, USA). ELISA assay plates were purchased from Fisher Scientific (Edmonton, Canada). Chloroform and triethylamine were purchased from Fisher Scientific (Fair Lawn, NJ., USA). Isopropanol, acetonitrile, (both assurance grade), ethyl chloroformate, hexane, methyl cellulose, citric acid, D-glucose and isooctane were obtained from BDH Chemicals Canada Ltd. (Edmonton, Canada). HPLC-grade acetonitrile (UV cut-off 210 nm) was purchased from Caledon Laboratories Ltd. (Georgetown, ON, Canada). Mobile phase water was distilled and filtered (Corning Mega-Pure SA-70 System, Corning, NY, USA).

3.2 Instrumentation and Equipment

High-Performance Liquid Chromatography:

The HPLC apparatus (Waters, Mississauga, Canada) consisted of a Model 590 pump, a Model 481 variable-wavelength ultraviolet detector, a 710B Wisp autosampler, a 10 cm x 4.6 mm I.D. C_{18} analytical column packed with 5-μm diameter reversed-phase material. Both the analytical column and precolumn were operated at ambient temperature. The recorder-integrator was a Model 3390 A (Hewlett-Packard, Palo Alto, CA, USA).

Gamma Counter: Minaxi γ Auto-Gamma 5000 counter (Packard, Meriden, CT, USA)

General Experimental: vortex-type mixer (Vortex-Genie^{R}, Fisher Scientific Industries,

Springfield, MA). ELISA plate reader (Cayman Chemical, Ann Arbor, MI, USA) with a 492 nm lens.

3.3 Ethics Approval

All animal experimentation protocols were reviewed and approved by the University of Alberta Animal Care Ethics Committee.

3.4 Animals

Male rats (wt = 300 - 500 g) were of the Sprague-Dawley strain of *Rattus norvegicus* (Lindsey, 1979) and raised in house by the University of Alberta Animal Colony Facility.

3.4.1 Standard Procedures for Animal Experiments

3.4.2 Animal Handling

Animals were housed at ambient temperature and humidity in individual metabolic cages with wire mesh floors allowing for separate quantitative collection of urine and feces. Animals were fed a standard rat chow and allowed free access to food and water for the duration of the experiment unless otherwise stated.

3.5 Intestinal Permeability Experimentation

To test intestinal permeability, 0.5 mL of a solution containing 10 μCi/mL of ^{51}Cr complexed with EDTA was administered orally following the dose of NSAID. The solution was delivered through an 18 gauge 5 cm curved feeding needle attached to a 1 mL syringe. Urine was collected 0 to 8, and 8 to 24 hours following the administration of the ^{51}Cr-EDTA solution. The urine was collected in cups containing 1 mL of 1 M H_2SO_4 to inhibit microbial growth. After each collection period 10 mL

of tap water was used to rinse the urine collection trays and was transferred to scintillation vials.

3.6 Gastroduodenal Permeability Experimentation

To test baseline gastroduodenal permeability, 1.0 mL of a solution containing 1.0 g/mL of sucrose was administered to each individual rat orally through an 18 gauge 5 cm curved feeding needle attached to a 1 mL syringe. Urine was collected 0 to 8 h following the administration of the sucrose solution.

To examine the effect of NSAIDs, each drug (indomethacin, aspirin, flurbiprofen, ibuprofen, and aspirin with 0.5 mL of 50% ethanol) was suspended in 1% methylcellulose and administered orally to each group of rats at the same time of day (9 a.m.) 1 hour prior to the sucrose solution. The above NSAIDs doses are equal to those used previously by us to examine intestinal permeability changes. Sucrose dosing and urine collection were performed as for the baseline study.

Relative permeability was determined by calculating the sucrose present in each urine sample as a percent of the administered dose after correcting for baseline levels of glucose and sucrose present in urine for each individual rat.

3.7 Time Course of NSAID-Induced Intestinal Permeability

Considering the maximum recommended daily dose of racemic ibuprofen, 2500 mg/day in clinical practice, the dosages of ibuprofen administered were chosen to reflect an equivalent range based on a proportion of the daily dose to body weight in humans.

Randomly assigned groups of rats received ibuprofen either i.p., p.o. or s.c. or indomethacin or flurbiprofen orally. At 9:00 a.m. of the morning of the study, each rat, received a single dose of each NSAID as a bolus. All doses were based on body weight and adjusted to a 0.5 mL volume. To test the time course of intestinal permeability, ^{51}Cr-EDTA was administered orally 0, 1, 3, 6, 12, 24, 36, 48, 72, or 91 h following the NSAID administration.

3.8 Dose-Response Relationship

Randomly assigned groups of rats received ibuprofen either i.p., p.o. or s.c. or flurbiprofen or indomethacin at the same time of day (9 a.m.) 3 h or 12 h prior to the administration of the ^{51}Cr-EDTA. Groups of rats were dosed with racemic ibuprofen at doses of 10, 25, 50, 75 or 100 mg/kg. Dosing of ibuprofen rats followed a standard five by five latin square design with a one week wash out period between drug administration. Groups of rats were also dosed with indomethacin in doses ranging from 0, 1, 3, 5, 10 and 20 mg/kg. Intestinal permeability was assessed as described previously at 12 h after the drug administration. Rats were also dosed with racemic flurbiprofen doses ranging from 0, 1.5, 3, 6.7, 10, 15 and 20 mg/kg. Intestinal permeability was assessed as described previously 1 h after the drug administration.

3.9 NSAID Enantiomer Drug Dosage Regimens

A 25 mg/kg ibuprofen oral dose induced measurable and significant permeability changes in the rat. Other NSAIDs therefore, were administered in doses therapeutically equivalent to that of ibuprofen given that the recommended daily human dose of racemic ibuprofen is 2500 mg/day/70 kg. For the individual

enantiomers of etodolac, flurbiprofen and ketoprofen doses equivalent to the amount of the enantiomer present in the racemate (i.e. 1/2 of the racemic dose) were orally administered. Each drug was administered to groups of rats (n = 4) at the same time of day (9 a.m.) on separate occasions prior to the administration of the ^{51}Cr-EDTA.

3.10 Dosing to Account for R to S bioinversion of Ketoprofen and Ibuprofen

Based on previous studies in rats it has been estimated that R-ketoprofen and R- ibuprofen are bioinverted 80 % (Foster and Jamali, 1987) and 60 %, (Jamali and Berry, 1990) respectively. Hence, in order to examine the potential effect of R enantiomer various doses of ketoprofen (3.3 mg/kg racemate, 3 mg/kg S-ketoprofen, and 3.75 mg/kg R-ketoprofen) and ibuprofen (25 mg/kg racemate, 20 mg/kg S-ibuprofen and 33 mg/kg R- ibuprofen) were administered (n = 3-6). Considering the extent of inversion, administered doses were chosen to yield constant systemic body loads of 3 mg/kg S-ketoprofen and 20 mg/kg S-ibuprofen while containing varying amounts of the R enantiomer (0.33, 0 and 0.75 mg/kg R-ketoprofen, and 5, 0 and 13 mg/kg R-ibuprofen, respectively).

3.11 Pharmacological Protection Studies

Sixteen rats were randomly assigned to 4 groups. Group 1 (Control). Group 2 received 0.5 mL oral allopurinol suspended in a 1% methylcellulose solution 10 mg/kg daily for 7 days (allopurinol control group). Group 3 received 0.5 mL of oral allopurinol 10 mg/kg daily for 7 days followed by a single oral dose of indomethacin 20 mg/kg suspended in a 1% methylcellulose solution. Group 4 received a single oral

dose of indomethacin 20 mg/kg. ^{51}Cr-EDTA intestinal permeability tests were performed as outlined above for all groups.

Single doses of misoprostol (prostaglandin E1), sulfasalazine, cimetidine, tempo, sucralfate, famotidine and glucose / citrate were suspended in 1% methylcellulose and 0.5 mL administered 1 h prior to each NSAID. Metronidazole was given as two divided doses 12 hours apart and 30 minutes prior to each NSAID.

3.12 Jugular Vein Cannulation

Male Sprague-Dawley rats were anesthetized initially with ether and then maintained on methoxyflurane (Metofane®, Pitman-Moore LTD, Mississauga, ON, Canada) during the procedure. The jugular vein was cannulated with polyethylene tubing (PE-50, Clay-Adams, Parsipanny, NJ, USA) tipped with Silastic Tubing (Dow Corning, Omaha, NE, USA). Animals recovered overnight before drug administration.

3.13 Drug Administration and Sample Collection

The sustained release and regular release flurbiprofen products were administered orally 10 mg/kg under light ether anesthesia. Rats were also dosed orally with either indomethacin 10 mg/kg in 1% methylcellulose and indomethacin 10 mg/kg in 1 % methylcellulose and glucose and citrate (1:15:15). Whole blood samples (0.25 mL) were withdrawn from the cannula at 0, 0.25, 0.5, 1, 2, 4, 6, 12, 24, 27, and 30, 48 h after drug administration. The whole blood samples were immediately centrifuged on a Fisher Model 235A microcentrifuge for 3 min and the serum separated, transferred to new 1 mL polypropylene vials (Fisher Scientific, Edmonton, AB, Canada). All plasma samples were stored and frozen at -20° C until assayed.

3.14 Histological Assessment

Upon subsequent death of the rats histological assessment was made by a pathologist blinded to the previous drug treatment. Because of the diffuse nature of intestinal injury with indomethacin, no attempt was made to quantify the extent of injury.

3.15 Intestinal Tissue Distribution

On the morning of the initial tissue distribution study, 3 groups of rats (n = 3 each), received single p.o., i.p., and s.c. doses of 100 mg/kg racemic ibuprofen. On the morning of the second study, 3 groups of rats (n = 4), received single i.p. doses of 25, 50 or 100 mg/kg racemic ibuprofen. Following induction of anesthesia, a vertical midline incision was made and each animal sacrificed by cardiac puncture 3 h after dosing. Intestinal segments were excised and immediately placed at -20° C until analyzed.

The duodenal segments were removed immediately distal to the pyloric sphincter. The jejunum was removed starting 30 cm distal from the pyloric sphincter, the ileum was excised immediately proximal to the cecum. Blood was collected via cardiac puncture and plasma separated by centrifugation at 1800 g for 5 min. and stored at -20 °C until analysis. Immediately after isolation intestinal segments were weighed and homogenized. Briefly, Sorensen's buffer 0.067 M pH 6.0 was added to weighed tissue samples (wet weight = 0.15-2.0 g) which were homogenized into 5-10 mL samples using Potter-Elvehjem tissue grinders driven by a T-line laboratory stirrer (Talboys Engineering, Montose, PA, USA).

Concentrations of R and S-ibuprofen were measured using a stereospecific reverse phase HPLC assay. Standard curves were prepared (0-200 μg/mL) in 100 μL of all tissue homogenates and were linear r^2 >0.99. Ibuprofen concentrations in each homogenate were determined by interpolation from the calibration curves.

3.16 Bowel Sterilization Study

Sixteen rats were randomly assigned into 4 groups. Group 1 (control) received 0.5 mL of saline twice daily for 3 days. Group 2 received 500 mg/kg of both erythromycin and neomycin sulfate twice daily orally for 3 days (antibiotic control group). Group 3 received 0.5 mL of saline twice daily for 3 days followed by a single i.p. dose of racemic ibuprofen 50 mg/kg. Group 4 received 500 mg/kg of both erythromycin and neomycin sulfate twice daily orally for 3 days followed by a single i.p. dose of racemic ibuprofen 50 mg/kg. On the morning of the fourth day of the study Groups 3 and 4 received a single i.p. bolus dose of racemic ibuprofen. ^{51}Cr-EDTA intestinal permeability tests were performed as outlined above for all groups. After completion of the permeability tests the upper jejunum and ileum of all rats were excised. Sterility tests, were performed for jejunal and ileal contents obtained from all antibiotic treated and control rats.

3.17 Media Preparation and Sterility Tests

Twenty three g of plate count agar medium was suspended in 1 L of cold fresh distilled water and then was sterilized by autoclaving at 121°C for 15 min. After cooling, the medium was dispensed into disposable petri dishes under aseptic conditions. Using a sterile swab under aseptic conditions, blank media (control), and

samples of the intestinal contents were taken from the excised tissues, transferred onto media and incubated at 37°C for 24 h.

3.18 Carrageenan Footpad Edema

0.05 mL of 1 % (w/v) carrageenan in 0.9% (w/v) aqueous NaCl isotonic with body fluid was injected through a 26 gauge needle into the plantar region of the right hind paw. one hour after carrageenan challenge animals were dosed orally with indomethacin and indomethacin and glucose/citrate (1:15:15). Swelling of the paw reached a peak in 3 to 5 h, then retained about the same degree of edema for about 2 h before returning to baseline. Paw volume was measured by submerging each hind paw, in water up to an ink mark on the skin over the lateral malleous and the volume displaced measured initially after injection and finally 3 and 5 h after injection. Results are expressed as an increase in paw volume in mL above initial volume. Paw diameter was measured as the ventral-dorsal footpad thickness using calipers (Mitutoyo Mtg. Co., Japan No. 2412-08 calipers). Results are expressed as the difference in thickness [= Δ pt. (mm)] between baseline and the carageenan-injected paw.

3.19 Pharmacodynamic Fitting

Pharmacodynamic modeling was performed using the extended least squares regression program PCNONLIN (Lexington, KY, USA). For observed effects of a plot of ^{51}Cr-EDTA permeability versus dose the pharmacological model employed was the E_{max} with the existence of a baseline effect and was of the mathematical form:

$$E = \frac{E\max Dose}{ED50 + Dose} - E0 \quad \text{or} \quad E = \frac{E\max C}{EC50 + C} - E0$$

where E is the measured effect, E_{max} is the maximal observed effect, E_0 is the baseline measured effect, ED_{50} and EC_{50} are the dose or NSAID plasma concentration required to produce 50% of maximal response, respectively, D and C are the NSAID dose or concentration. The appropriateness of the pharmacodynamic fitting was determined by analysis of the distribution of the residuals and maximum log-likelihood.

3.19.1 Pharmacokinetic / Pharmacodynamic Analysis

The indomethacin plasma concentrations versus time data was fit to a one-compartment open model with first order input using PCNONLIN version 4.1 (Metzler and Weiner, 1992) and the first order rate constants describing absorption (K_a) and elimination (β) estimated. The appropriateness of the fitting was determined by the Schwartz and Akaike information criteria determined by PCNONLIN as well as by analysis of the distribution of the residuals. The area under the plasma concentration versus time curve ($AUC_{0-\infty}$) was estimated by linear trapezoidal approximation from 0 to 30 h. The AUC from 30 h to ∞ was estimated by dividing the plasma concentration at 30 h by β. The apparent total body clearance (Cl/F) was obtained by dividing the dose by the $AUC_{0-\infty}$. The apparent volume of distribution (Vd_β/F) was estimated by dividing the apparent total body clearance by β.

Analysis of the dose ranging studies was performed using the E_{max} model available with PCNONLIN version 4.1 (Metzler and Weiner, 1992). The equation used is of the form:

$$E = \frac{E_{max} Dose}{ED_{50} + Dose}$$

where E is the measured effect, D is the dose, and ED_{50} is the dose that produces a 50 % of maximal response. Appropriateness of the fitting was determined by the Schwartz and Akaike information criteria determined by PCNONLIN as well as by analysis of the distribution of the residuals.

Combined pharmacokinetic / pharmacodynamic analysis of the plasma concentration versus time data and the time course of effect data was performed using effect compartment modeling (Holford and Sheiner, 1981). For a one compartment model with first-order input the concentration of drug in the effect compartment (Ce(t)) can be calculated as (Holford and Sheiner, 1981):

$$Ce(t) = A\, e^{-\beta t} + B\, e^{-K_A t} + C\, e^{-k_{e0} t}$$

where:

$$A = F\ Dose\ K_A\ k_{e0} / Vd(K_A - \beta)(k_{e0} - \beta)$$

$$B = F\ Dose\ K_A\ k_{e0} / Vd(\beta - K_A)(k_{e0} - K_A)$$

$$C = F\ Dose\ K_A\ k_{e0} / Vd(\beta - k_{e0})(K_A - k_{e0})$$

and k_{e0} is the first order rate equilibration constant between the central and effect compartments and F/Vd is the reciprocal of the apparent volume of distribution.

The time course of pharmacological effect can then be rewritten as:

$$E(t) = \frac{E_{max} Ce(t)}{EC_{50} + Ce(t)}$$

This model was written as a user defined model under PCNONLIN version 4.1 and used to estimate the maximal effect (E_{max}), plasma concentration producing a 50 % of maximal response (EC_{50}) and the equilibration rate constant (k_{e0}). The kinetic parameter values used were the means of those determined for the 10 and 20 mg/kg dose groups respectively. The effect measurements used were the mean values of the time course studies for the 10 and 20 mg/kg dose groups. Appropriateness of the fitting was determined by the Schwartz and Akaike information criteria determined by PCNONLIN as well as by analysis of the distribution of the residuals. In addition the appropriateness of fitting was evidenced by the collapse of any counter-clockwise hysteresis in the effect concentration relationship when the points were joined in temporal order (Holford and Sheiner, 1981).

3.2.0 Dissolution Studies

A Erweka Dt-6 dissolution apparatus was set-up for the U.S.P. type II paddle method dissolution. Suspensions were sealed in a polyethylene screen (Spectra / Mesh, 100 μm, Fisher Scientific, Edmonton, Canada) filled with 0.5 mL of a suspension of indomethacin or indomethacin and glucose/citrate (1:15:15) then dispersed in wells containing 500 mL of simulated gastric (U.S.P.) or intestinal fluid (U.S.P.) which was previously equilibrated to 37±0.5 °C. The release medium was agitated with a paddle rotation of 50 r.p.m. during the period of the experiment. 5 mL samples were withdrawn from the dissolution media at 0, 0.25, 0.5, 1, 2, 4, and 6 h. Samples volumes were replaced with fresh dissolution media. The samples were stored at -20°C until HPLC analysis. Experiments were conducted in triplicate.

3.21 Statistical Analysis

Statistical evaluations were performed on all pharmacokinetic, and pharmacodynamic parameters using ANOVA and 2-way ANOVA with replication. The Student t-test for unpaired data and Duncan's test were used to determine differences between groups and for comparison among means. The level of significance chosen for all statistical analyses was always $\alpha = 0.05$. The results are expressed as mean ± standard deviation (s.d.) unless otherwise stated.

3.22 Standard Solutions

Ethyl chloroformate (6-60 mM) was prepared in acetonitrile. Indomethacin and mefenamic acid (100 μg/mL) were prepared in acetonitrile. The derivatization reagent, R-(+)-α-pheylethylamine (PEA) (0.5 M), was prepared in triethylamine (TEA)-acetonitrile (2:8). Stock solutions of racemic ibuprofen, flurbiprofen, ketoprofen (100 μg/mL) and fenoprofen (25 μg/mL) were prepared in 10 mL of methanol and sufficient 0.01 M NaOH to make 100 mL of each solution. Stock solutions of sucrose (1.9 mg/mL), glucose (1.0 mg/mL), and Trinder's Reagent were prepared in Sorensons phosphate buffer (pH = 7.0). All solutions were stored at 5°C.

3.23 Assay for ^{51}Cr-EDTA

Rat urine samples were counted by a gamma counter (Beckman Gamma 8000) for 1 minute in a counting window scanning within a range of 0-2 Mev. At least two standards of 100 μL of the administered ^{51}Cr-EDTA solution were counted with every set of urine samples. Relative permeability was determined by calculating the activity present in each urine sample as a percent of the administered dose after correcting for

background radiation. The apparent permeability is reported as the % excretion in urine from 0 to 8 h.

3.24 Assay for Sucrose

To a 100 µl aliquot of rat urine were added volumes of either glucose or sucrose for calibration curves between 0-100 µg/mL. The constituents were then Vortex-mixed for 30 s. Since sucrose is not reactive with the Trinder's Reagent, it was completely cleaved to glucose and fructose using 25 µl of 2M H_2SO_4 per 100 µl urine sample followed by a brief Vortex-mix and 10 min in a boiling water bath. 40 µl of 2M NaOH was then added followed by Sorensons phosphate buffer q.s. 0.5 mL. In preliminary work, glucose standard curves done with acid hydrolysis and heating showed no significant change in absorbance from those prepared with acid hydrolysis. 1 mL Trinder's Reagent was then added and the entire mixture was gently vortex-mixed and allowed to stand for 18 min before reading absorbance. Following preparation of the samples 100 µl of sample were plated into well bottom ELISA plates and absorbance was determined on an ELISA plate reader (Cayman Chemical, Ann Arbor, MI, U.S.A.) with a 492 nm lens.

3.25 Assay for Ibuprofen

Concentrations of R and S-ibuprofen, were measured using a stereospecific reverse phase HPLC assay (Wright *et al.*, 1992). Briefly, 100 µL samples were transferred into test tubes, 50 µL fenoprofen (internal standard) (25 µg/mL) added, and acidified with 200 µL of 0.6 M H_2SO_4. Ibuprofen was extracted with 3 mL of isooctane: isopropyl alcohol (95:5). After mixing for 30 sec and centrifugation at 1800

g for 5 min the organic layer was transferred to clean test tubes and evaporated to dryness. Derivatization was performed as for plasma samples using ethylchloroformate and R-(+)-α-PEA. The formed diastereomers were extracted into 3 mL chloroform, which was evaporated to dryness. The residue was reconstituted in 200 µL of mobile phase and aliquots of 10-150 µL were injected into the HPLC. Standard curves were prepared in plasma and tissue homogenates and were linear r^2 >0.99.

3.26 Assay for Flurbiprofen

Quantification of the enantiomers of flurbiprofen used the methods of Berry and Jamali (1988) with minor modifications. The method involved extraction of the drug from 100 µL of plasma with 5 mL isopropanol / isooctane (5/95% v/v), evaporation of the organic layer and addition of 1)TEA/acetonitrile 2) ethyl chloroformate/acetonitrile and 3) L-leucinamide/methanol:TEA. After addition of water, the solution was injected into an HPLC equipped with a UV detector at 250 nm for ketoprofen (internal standard) and for flurbiprofen. The mobile phase consisted of acetonitrile: 0.067M KH_2PO4:TEA (35:65:0.02 v/v) pumped at a flow of 1 mL/min through a stainless steel (100 mm x 4.6 mm I.D.) end-capped column packed with 5-µm size octadecyl-bonded silica (Partisil 5 ODS-3, Whatman Inc. Clifton, NJ, USA) preceded by a 5-cm guard column containing 10-µm particle size octadecyl-bonded silica.

3.27 Assay for Indomethacin

The plasma samples were analyzed for indomethacin using a reverse-phase HPLC method similar to that developed by Dusci and Hackett (1979). Briefly, to a

100 μL aliquot of plasma was added 25 μL of mefenamic acid (internal standard) (100 μg/mL in acetonitrile) and 1 mL of ice-cold acetonitrile. The mixture was vortex-mixed for 30 s and then centrifuged for 5 min at 1800 *g*. The supernatant was then transferred to clean borosilicate tubes and evaporated to dryness (Savant Speed Vac concentrator-evaporator, Emerston Instruments, Scarborough, Canada). The residue was reconstituted in 200 μL mobile phase and aliquots of 25 μL were injected into the HPLC system.

The HPLC system consisted of a Model 590 pump, a Model 481 variable wavelength detector, a 710B Wisp autosampler and a 25 cm x 4.6 mm I.D. C_{18} analytical column (Phenomenex, Torrance, CA, USA). The mobile phase consisted of acetonitrile-45 mM potassium dihydrogen phosphate (55:45 v/v, pH 3). All analyses were performed under ambient temperature. The detection wavelength used was 225 nm.

A calibration curve was prepared over a range of 1 - 100 μg/mL in rat plasma for each analysis. Indomethacin concentrations in the collected samples were determined by interpolation from the calibration curves. A plot of the area ratio of the peaks corresponding to indomethacin and mefenamic acid was linear ($r > 0.9999$) from 1-100

μg/ mL indomethacin and the inter-day variation of the calibration curve slope and intercept were less than 2 %.

4. Results and Discussions

4.1 The Rat as a Model of NSAID-Induced Intestinal Permeability

4.1.1 Results

The urinary excretion of the ^{51}Cr-EDTA was approximately evenly divided between the 0 to 4 and 4 to 8 h collection intervals although some rats produced little urine in one or the other collection interval. An excellent correlation between the urinary excretion from 0 to 8 h and that of 0 to 24 h was found for both male (r^2=0.95) and female (r^2=0.98) control rats (Fig. 1). This correlation was maintained after rats were treated with NSAIDs with or without protective agents. Therefore, unless otherwise stated, the combined 0 to 8 h interval was used as a measure of intestinal permeability. The mean baseline permeability in untreated male rats (n = 100) after oral administration was determined to be 1.78±0.44% of the ^{51}Cr-EDTA dose administered. There were no differences found between the two sexes of male and female (n = 7) in baseline permeability values (Fig. 1).

The baseline permeability values in male rats from 0-24 h are positively skewed with a mean of 2.36±1.13%, a median of 2.13% (95% CI = 2.11-2.86), and a range of 0.70-7.68 (Fig. 2). Uniform fecal distribution of radioactivity was seen in rat feces (n = 5) and greater than 99% of the administered dose of ^{51}Cr-EDTA was recovered during the first 24 h post-test.

Fig. 3 summarizes the NSAID-induced permeability data in male Sprague-Dawley rats using ^{51}Cr-EDTA. The studies in rats demonstrate an increased intestinal permeability after oral NSAID administration except for aspirin. The ^{51}Cr-EDTA excretion in urine also appears to be dose-dependent for ibuprofen, naproxen, and indomethacin (Fig. 4). Indomethacin induces the highest permeability changes (Fig. 4).

Further, the oral administration of 10 mg/kg corticosterone also appeared to induce significant permeability changes in the rat with 4.13±0.7% (n=4) excreted into urine (Fig. 3).

The indomethacin-induced permeability changes in the rat model appear to be reduced by co-administration of a stable PGE_1 analog (misoprostol), glucose/citrate, and sulfasalazine but not by famotidine (Fig. 5).

Naproxen-induced changes in intestinal permeability are not antagonized by H_2-antagonists or sucralfate but are significantly reduced by misoprostol administration (Fig. 5).

4.1.2 Discussion

The intestinal permeability of ^{51}Cr-EDTA in 12 normal rats was previously determined to be 2.06±0.44% over 5 h following intragastric administration (Bjarnason *et al.*, 1985b). Additionally, the mean % of a ^{51}Cr-EDTA dose by gavage from 0-24 h has previously been reported to be 1.6% (Ramage *et al.*, 1988). Thus our findings (Figs. 1 and 2) are consistent with previously reported results.

Similar to our observations in the rat (Fig. 2), the frequency distribution of baseline permeability values in 65 healthy male volunteer humans from 0-24 h are also positively skewed with a median of 2.45% (95% CI = 2.11-2.86) (Aabakken, 1989). In humans there is a good linear relationship between the 0-6 and 6-24 h cumulative excretion of ^{51}Cr-EDTA (Bjarnason *et al.*, 1984a) which parallels the results in the rat model (Fig. 1). Furthermore, there does not appear to be any sex-related differences in

baseline permeability values in human studies (Mielants *et al.*, 1991a,b) which parallels the results seen in male and female Sprague-Dawley rats (Fig. 1).

It is believed that anti-inflammatory drugs may modulate the intestinal tight junction thereby inducing increases in paracellular permeability (Bjarnason *et al.*, 1984a). Increased intestinal permeability is suggested to be a prerequisite to NSAID-induced ulceration (Bjarnason *et al.*, 1986a). Similar to our observations in the rat (Fig. 3), NSAIDs enhance human intestinal permeability as has been reported for indomethacin (Bjarnason *et al.*, 1984a; Bjarnason *et al.*, 1986a; Bjarnason *et al.*, 1989a; Banerjee *et al.*, 1986; Bjarnason *et al.*, 1991a; Davies and Rampton 1991; Bjarnason *et al.*, 1992a; Davies *et al.*, 1993), naproxen (Jenkins *et al.*, 1988; Aabakken, 1989; Aabakken *et al.*, 1989a; Aabakken *et al.*, 1989b; Aabakken and Osnes, 1990; Aabakken *et al.*, 1990), flurbiprofen (Jenkins *et al.*, 1987), and ibuprofen (Bjarnason *et al.*, 1984a; Bjarnason *et al.*, 1986a). After therapeutically equivalent doses, the highest potency was observed with indomethacin and flurbiprofen. Interestingly, aspirin, a very potent inhibitor of cyclooxygenase has little or no effect on the intestinal permeability in both humans (Bjarnason *et al.*, 1986a; Jenkins *et al.*, 1987) and rats (Fig. 3). This may be due to the rapid absorption of aspirin from the upper part of the GI tract and its efficient hydrolysis to salicylic acid (Rowland and Riegelman, 1968) which limit both direct exposure of the more distal intestine to the drug and the availability of aspirin for systemic distribution into the intestinal mucosa. Salicylic acid is a very weak inhibitor of cyclooxygenase. The concentrations required for 50% inhibition of COX_1, have been determined to be 0.3 and 35 mg/L for aspirin

and salicylic acid, respectively (Mitchell *et al.*, 1994). Nevertheless, the lack of effect of aspirin on the intestinal permeability, despite the well documented ulcerogenic effect of the drug on the upper GI is a common observation in humans and rats.

The prodrugs, sulindac and nabumetone increased intestinal permeability significantly but to a limited extent (Fig. 3). In human studies, the increasing effect of these two drugs did not reach statistical significance (Bjarnason *et al.*, 1991b; Davies *et al.*, 1993). This may suggest a higher sensitivity for the rat model to detect increased intestinal permeability as compared with humans. Indeed, a comparison of human and rat data indicates less variability in response for the latter species.

The effect of indomethacin (Bjarnason *et al.*, 1984a), naproxen (Bjarnason *et al.*, 1984a; Aabakken, 1989a,b,c), and ibuprofen (Bjarnason *et al.*, 1984a) on human intestinal permeability has been shown to be dose-dependent. Similarly in the rat, elevation of the dose of these drugs resulted in further increases in the urinary excretion of ^{51}Cr-EDTA (Fig. 4).

The regulation of intestinal permeability may be, at least in part, regulated by prostaglandins (Powell *et al.*, 1981). However, Bjarnason *et al.* 1986a who examined the influence of concomitant administration of prostin E_2, a naturally occurring prostaglandin, did not notice a reduction in the NSAIDs-induced permeability changes perhaps due to the instability of the preparation. However, it did seem that prostaglandin E_2 itself significantly decreased baseline absorption of ^{51}Cr-EDTA. On the other hand, misoprostol which had no effect on permeation of ^{51}Cr-EDTA when given alone, partially protected the small bowel mucosa from the effects of

indomethacin (Bjarnason *et al.*, 1990a). The protective effect of misoprostol seems to be dose-dependent as 800 μg misoprostol doses did not alter the effect on either indomethacin (Davies *et al.*, 1993) or naproxen (Jenkins *et al.*, 1988) while 1200 μg doses partially blocked the effect of indomethacin (Bjarnason *et al.*, 1990a). The observations in the rat model (Fig 5) are consistent with the findings in humans.

Similar to the observations made in humans, indomethacin (Bjarnason *et al.*, 1984a) and naproxen (Aabakken *et al.*, 1989a; Aabakken *et al.*, 1990) effects on intestinal permeability were not reduced in the rat by H_2-antagonists (Fig. 5). H_2-antagonists may reduce the incidence of gastric ulceration by elevating the gastric pH whereas intestinal damage appears to be pH independent. Furthermore, the lack of protection found in humans with naproxen and sucralfate (Aabakken *et al.*, 1989a) effects on intestinal permeability were parallel in the rat (Fig. 5). Sucralfate shows limited protection against NSAID damage in the stomach and it appears to afford no protection in the distal intestine.

In parallel with the results found in humans, indomethacin (Banerjee *et al.*, 1986) effects on permeability were reduced in the rat by sulfasalazine (Fig. 5). It has been speculated that sulfasalazine may limit neutrophil recruitment to sites of inflammation and thereby ameliorate the inflammatory process (Bjarnason *et al.*, 1993a).

In addition to inhibiting prostaglandin synthesis, NSAIDs may inhibit glycolysis and the tricarboxylic acid cycle resulting in inhibition of oxidative phosphorylation thereby reducing adenosine triphosphate (ATP) production damaging the enterocyte

leading to cell death (Bjarnason *et al.*, 1993a). It has been suggested that the presence of these sugars in the intestinal lumen may modify the reaction to indomethacin or that citrate may be cytoprotective against free radical damage caused by NSAIDs (Bjarnason *et al.*, 1992a). In humans, a formulation containing 15 mg glucose and 15 mg citrate to each mg of indomethacin prevented the expected effect of NSAID-induced intestinal permeability (Bjarnason *et al.*, 1992a). A similar observation was made in the rat (Fig 5). In addition, the protective effects of glucose/citrate have also been previously demonstrated in NSAID-induced intestinal ulceration in the rat (Rainsford, 1980).

Mielants *et al.* (1991a,b) reported that there were no significant difference in altered intestinal permeability between patients taking NSAIDs and patients taking corticosteroids. This further suggests that alteration of intestinal permeability may not only be accounted for by an inhibition of mucosal COX activity but that other pathways in the arachidonic cascade might be implicated. Increased intestinal permeability after administration of corticosteroids to the rat was observed (Fig. 3).

Tests of intestinal permeability have been employed in a wide array of applications in the investigation of intestinal disease (Bjarnason *et al.*, 1986b). These tests are safe, well tolerated, reproducible, and easy to perform and because of their non-invasive nature can be easily applied to diagnostic screening and research, and could possibly replace the need for invasive investigations of intestinal disease such as radiology, biopsy and enteroscopy. An implicit advantage of permeability tests is that they reflect the functional integrity over a major area of the intestinal mucosa, whereas

biopsy may suffer from sampling error if damage is distributed randomly to inaccessible areas. Gastrointestinal blood loss and ulceration are end points of a complex damage process and are relatively insensitive markers whereas intestinal permeability in the rat has subsequently been shown to correlate with intestinal ulceration and is an easily detectable and sensitive index of NSAID-induced intestinal damage (Ford *et al.*, 1995).

The use of ^{51}Cr-EDTA urinary excretion test has previously been questioned in the claim that NSAIDs may increase glomerular filtration and that this could account for the increased urinary excretion of the probe (Simpson, 1985). However, subsequent studies have proven this concern to be unwarranted (Bjarnason *et al.*, 1987a).

Given the well-documented changes in intestinal permeability parallel with the increasing body of knowledge of NSAID related distal GI side-effects in the literature (Bjarnason *et al*, 1993a; Tables 3,7,8); the rat model may help elucidate the mechanisms of action of NSAIDs in producing intestinal abnormalities. The rat appears to be a suitable animal model to study NSAID-associated changes in intestinal permeability since it seems to respond in a similar fashion to the changes described in humans.

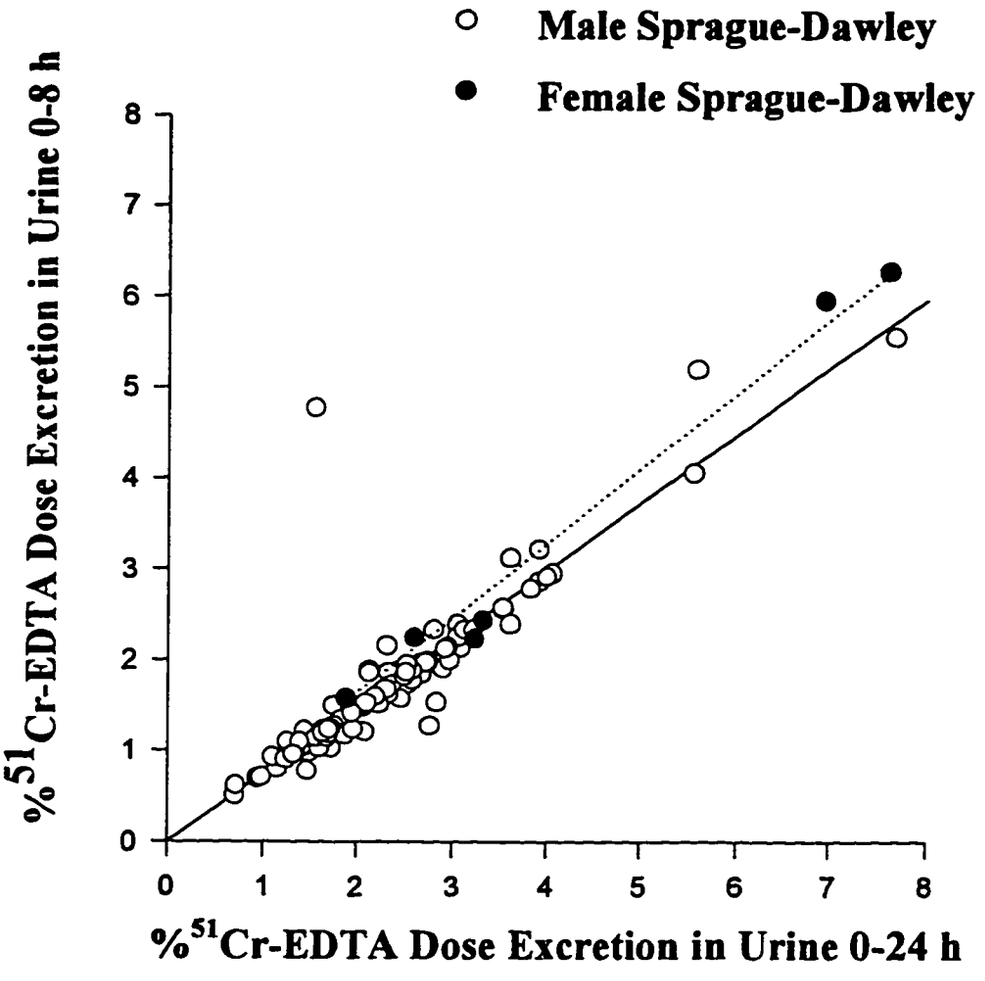


Figure 1. Correlation between 0-8 h and 0- 24 h urinary excretion of ^{51}Cr-EDTA in individual rats after oral administration of 0.5 mL of a solution containing 10 μCi/mL of the probe in male (O) and female (●) Sprague-Dawley rats.

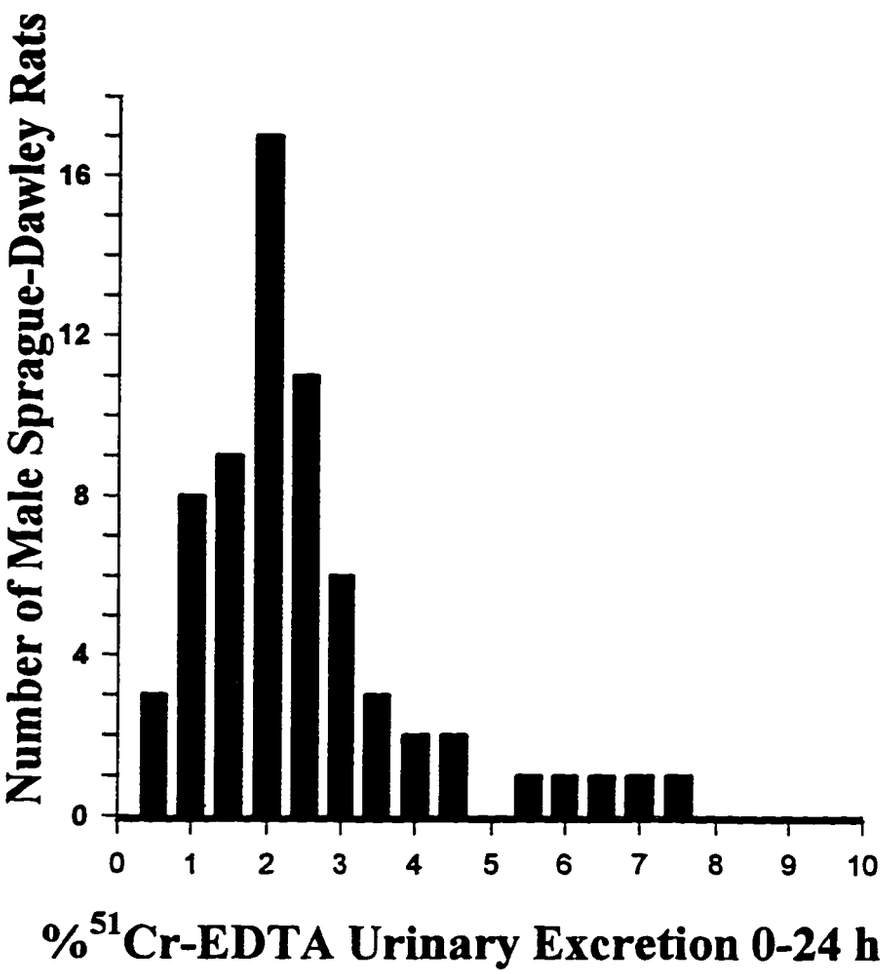


Figure 2. Frequency distribution histogram of baseline ^{51}Cr-EDTA urinary excretion in control male Sprague-Dawley rats after oral administration of 0.5 mL of a solution containing 10 μCi/mL of the probe.

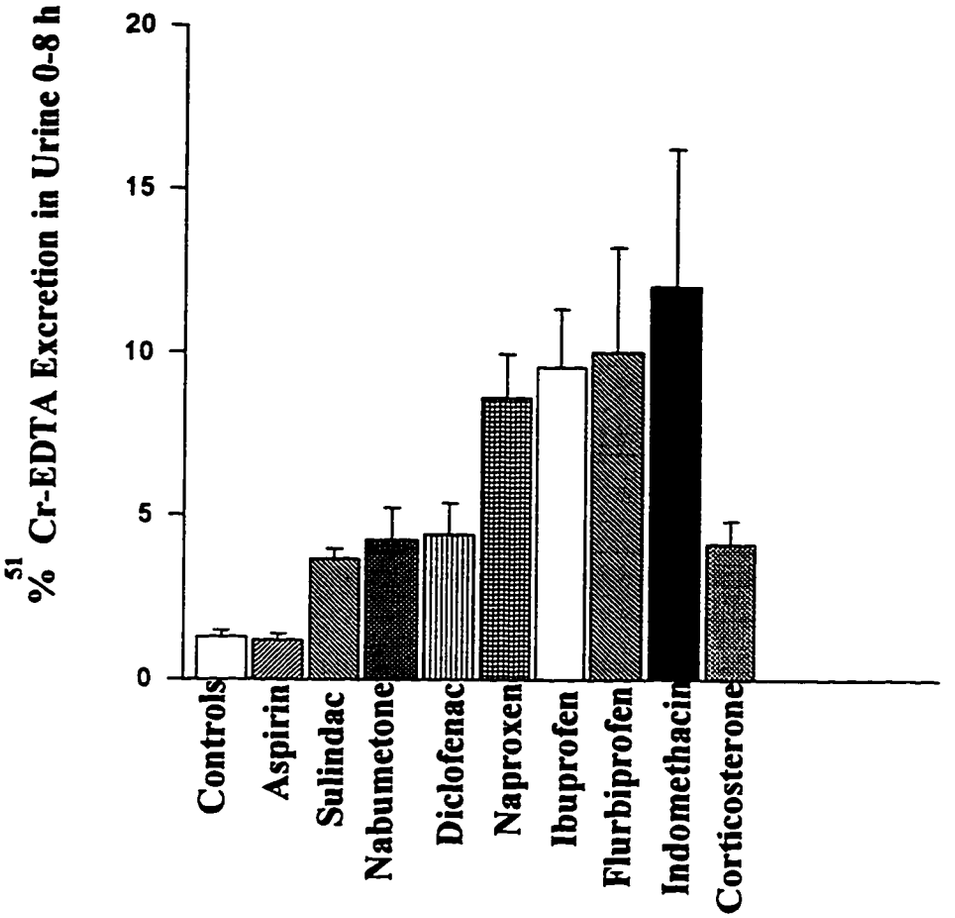


Figure 3. Mean (± s.e.m., n=4) intestinal permeability measured as % urinary excretion of ^{51}Cr-EDTA following oral administration of single doses of various antiinflammatory drugs. All treatments are significantly different from baseline except asprin. Doses in (mg/kg). Aspirin (42), Sulindac (8.3), Nambumetone (42), Diclofenac (4), Naproxen (20), Ibuprofen (50), Flurbiprofen (3), Indomethacin (3), and Corticosterone (10).

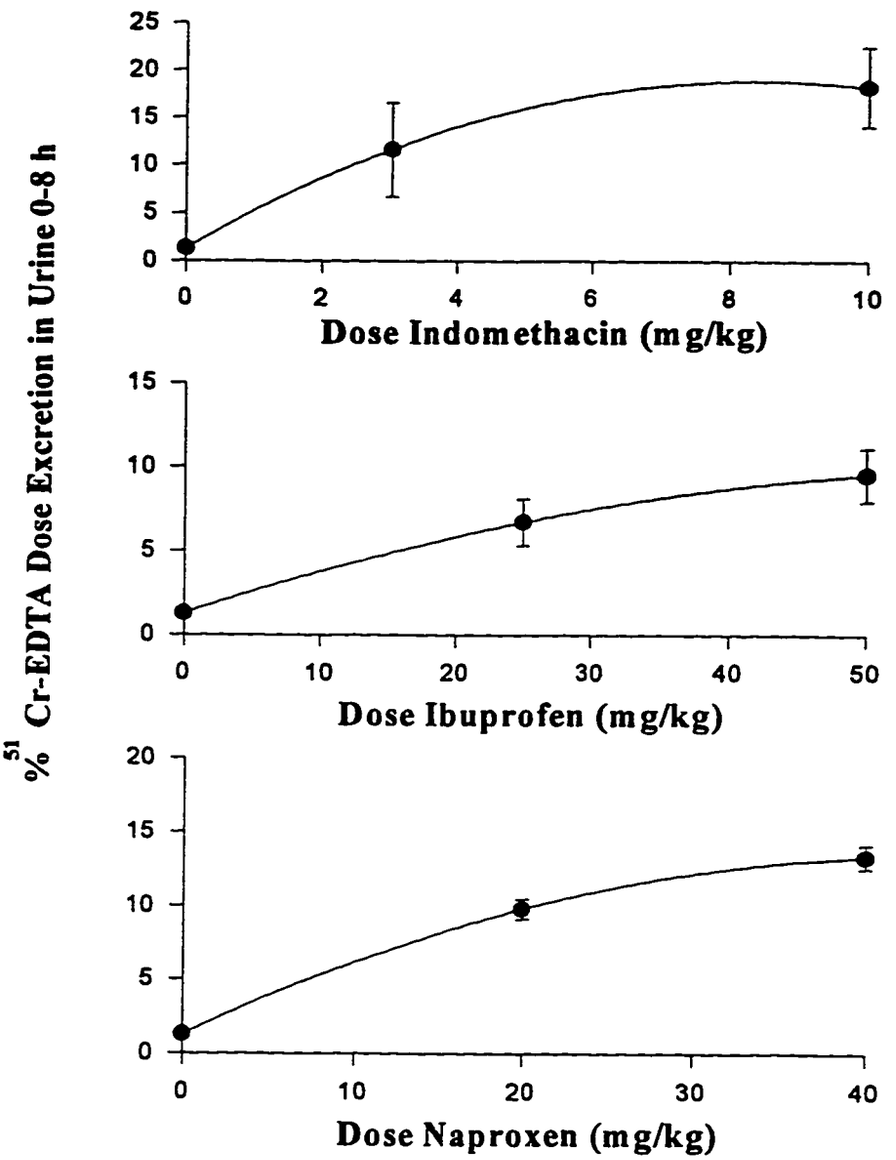


Figure 4. Dose dependency of the mean (± s.e.m., n = 4) intestinal permeability after single oral doses of indomethacin, ibuprofen and naproxen. All treatments are significantly different from baseline.

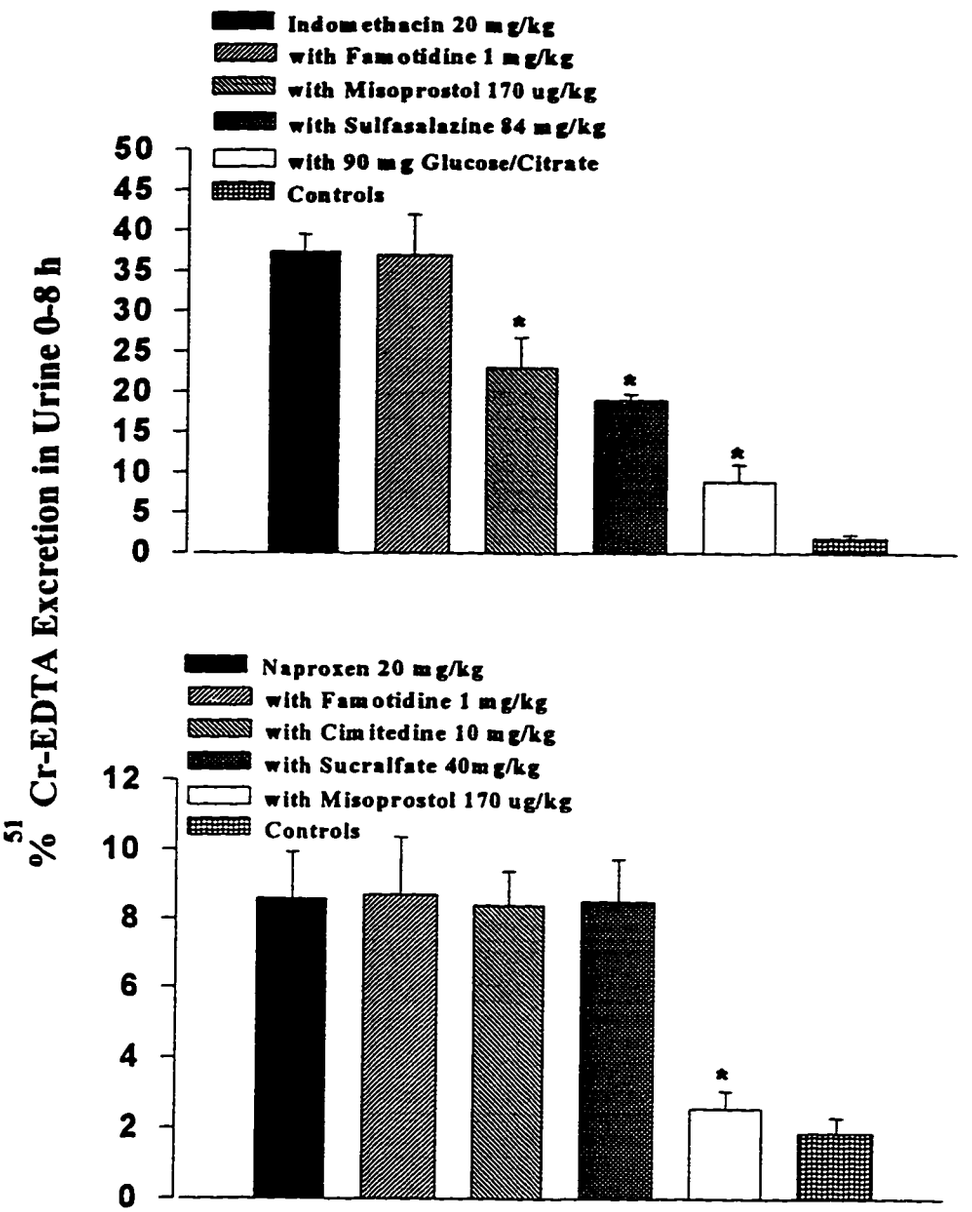


Figure 5. Top: Mean (± s.e.m., n = 4) intestinal permeability after 20 mg/kg single oral doses of indomethacin in rats dosed concomitantly with various protective agents. Bottom: 20 mg/kg oral naproxen in rats dosed with various protective agents. All treatments are signifcantly different from baseline. * Denotes significantly different from indomethacin or naproxen alone. All treatments are significantly different from baseline.

4.2 The Rat as a Model of NSAID-Induced Gastroduodenal Permeability

4.2.1 Results

Linear relationships ($r > 0.99$) were observed for cleaved sucrose and glucose curves (10 -100 μg/ mL) typically described as Absorbancy = 0.004x-0.00024. Sucrose standard curves constructed without acid hydrolysis and heating showed no U.V. absorbancy above baseline at 492 nm. Addition of acid (2M H_2SO_4) without heating did not result in detectable cleavage of sucrose. The interday and intraday coefficients of variance were 7.63 and 6.89%, respectively. The minimal quantifiable concentration was 10 μg/mL.

The mean baseline permeability (percent of the sucrose dose excreted in urine from 0-8 h) in untreated male rats (n = 87) after oral administration was determined to be 0.16 ± 0.14% (Fig. 6). The baseline permeability values in male rats from 0-8 h are positively skewed with a range of 0.00 to 0.77. Negligible amounts of sucrose were found in urine samples collected in a 8-24 h collection period in all treated and untreated animals.

The wide range of values found in the baseline population is indicative of the degree of variability inherent in measurement of gastroduodenal permeability.

Aspirin (42 mg/kg) in the presence and absence of alcohol caused significant increase in the urinary excretion of sucrose by 1.24 ±0.56% and 0.79 ±0.51% of administered dose respectively (Fig. 7 and Fig. 8). The effect of indomethacin appeared to be dose-dependent as 10 mg/kg doses had little effect while a 20 mg/kg dose significantly increased sucrose permeability by 0.59±0.39 % of the dose administered (Fig. 7).

4.2.2 Discussion

Tests of permeability have been employed in the clinical investigation of gastrointestinal disease (Bjarnason *et al.*, 1986a; Meddings *et al.*, 1993). These tests are safe, well tolerated, reproducible, and easy to perform. Their non-invasive nature

allows for easy application to diagnostic screening and research, complementing currently available techniques of GI imaging (Bjarnason *et al.*, 1987a). One advantage of permeability tests is that they can reflect the site-specific functional integrity over an area, whereas biopsy and endoscopy may miss damage caused in inaccessible zones.

The entire GI tract is prone to increased permeability. The effect, however, appears to be NSAID dependent. For example, despite significant upper GI toxicity, regular release aspirin formulations have minimal effect on the distal intestinal permeability in both humans (Bjarnason *et al.*, 1986a) and rats (Fig. 3). In contrast to aspirin, indomethacin permeability enhancing effect appears to be concentrated in the lower GI since the drug increased the sucrose urinary excretion only after 20 mg/kg doses (Fig. 8). At this dose level, indomethacin elicits its maximum effect on intestinal permeability as measured using ^{51}Cr-EDTA (Fig. 4). The possibility of a tissue-selective gut effect highlights the importance of considering the entire GI tract and the usefulness of site- specific probes such as sucrose and ^{51}Cr-EDTA in studies of NSAID-induced GI side-effects. This may be even more important when modified release NSAID formulations are examined for their GI toxicities.

The use of sucrose over other markers offers unique advantages. Sucrose is inexpensive, non-toxic, and is specific due to its cleavage after passing through the stomach into the intestine. The use of a calorimetric assay to quantify sucrose in rat urine does not require extensive sample preparation. The cleavage of sucrose with acid and heat appears to be complete and without degradation. Samples can be processed using economical commercially available reagents without the need for expensive HPLC equipment.

The findings reported here for baseline gastroduodenal permeability of sucrose in rats are similar to that seen in rabbits, dogs and humans. In rabbits, gastroduodenal permeability of sucrose was previously determined to be 0.7 % of the administered sucrose dose over 24 h following intragastric administration (Meddings *et al.*, 1993).

The mean percent of an oral sucrose dose excreted in urine of endoscopically normal mixed-breed male dogs (0-5 hours) has previously been reported to be 0.22±0.03 % (Meddings *et al.*, 1995). The mean percent of an oral sucrose dose excreted in urine of healthy controls (0-5 hours) has previously been reported to be 0.11±0.005 % (Meddings *et al.*, 1993). Thus findings reported here (Fig. 6) are consistent both qualitatively and quantitatively with previously reported results in other species (Meddings *et al*, 1993; Rabassa *et al*, 1994; Irvine *et al*, 1995; Meddings *et al*, 1995).

Similarly, the findings reported here for gastroduodenal permeability after treatment with NSAIDs parallel those seen in other species. Gastroduodenal permeability of sucrose in humans shows almost a four-fold increase with 600 mg of aspirin and 50 mL of vodka or aspirin alone (Meddings *et al.*, 1993; Rabassa *et al.*, 1994; Irvine *et al.*, 1995). Our results in rats (Fig. 7) are similar to these findings. The effect of 20 mg/kg indomethacin on rabbit gastroduodenal permeability has been demonstrated to be up to 5- fold above baseline (Meddings *et al.*, 1993). Similarly, in the rat a 5-10 fold increase in gastroduodenal permeability above baseline was demonstrated at the 20 mg/kg dose of indomethacin (Fig. 8).

The rat appears to be a suitable animal model to study NSAID-associated changes in gastroduodenal permeability. Changes seen in rat gastroduodenal permeability of sucrose after NSAID therapy are similar to changes described in humans. Gastroduodenal permeability of sucrose in all species tends to show some degree of variability creating the need for slightly larger sample sizes as well as individual baselines. This makes the rat ideal for investigational study due to its low cost. The investigation of NSAID-induced GI permeability changes may also help to explain the toxicokinetics of NSAIDs in producing abnormalities throughout the GI tract. There is also the possibility for application to other drugs and disease states that alter GI function. The rat appears to be a suitable and useful investigational animal model of gastroduodenal permeability as detected by urinary excretion of sucrose.

Combined use of sucrose and ^{51}Cr-EDTA may allow for non-invasive examination of abnormalities of the entire GI tract.

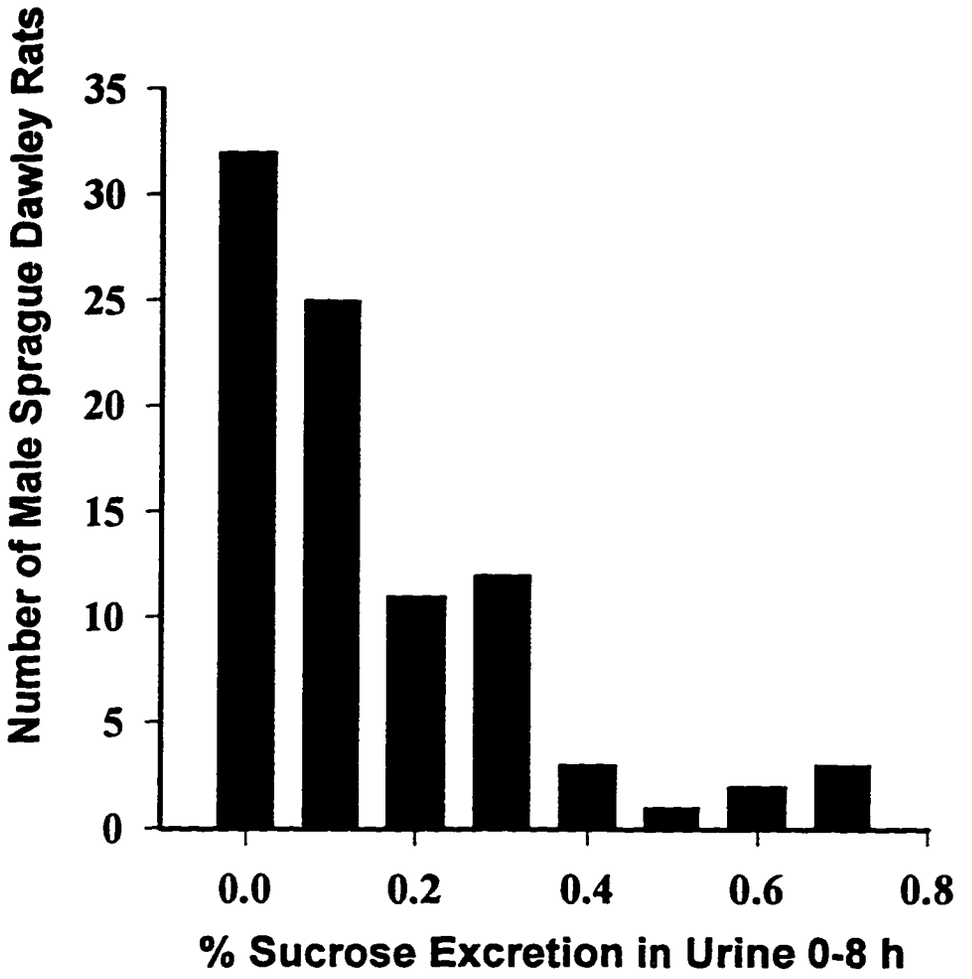


Figure 6. Frequency distribution histogram of baseline sucrose urinary excretion 0-8 h in control male Sprague-Dawley rats after oral administration of 1 mL of a solution containing 1 g/mL of the probe.

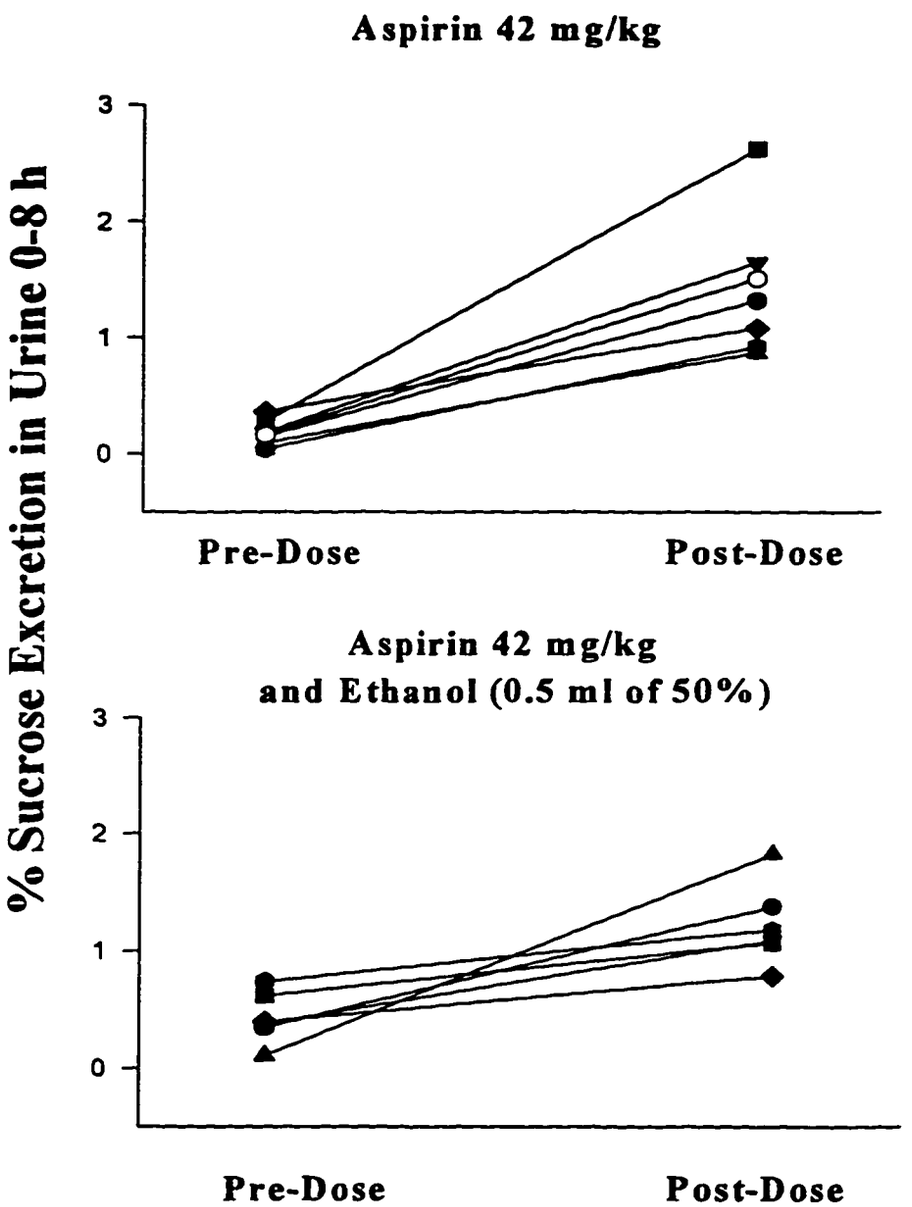


Figure 7. Gastroduodenal permeability measured as % urinary excretion of sucrose excretion in urine 0-8 h following administration of single doses of 42 mg/kg Aspirin without (upper panel) or with ethanol (lower panel) (0.5 mL, 50% ETOH). All treatments are significantly different from baseline.

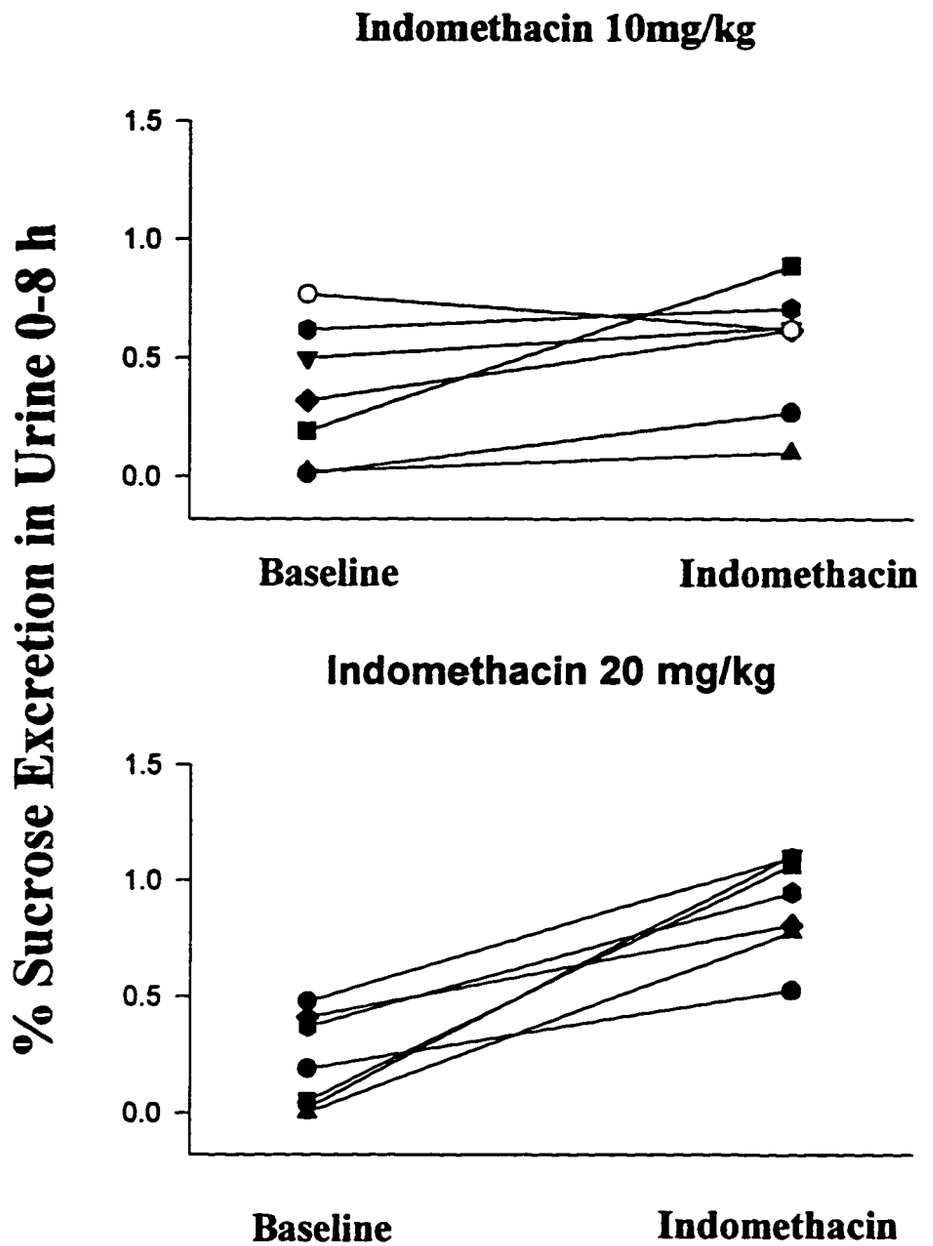


Figure 8. Gastroduodenal permeability measured as % urinary excretion of sucrose 0-8 h following oral administration of a single dose of 10 (upper panel) and 20 mg/kg Indomethacin (lower panel). The 20 mg/kg treatmnet was significantly above baseline (p=0.009).

4.3 Enantiomers and NSAID-Induced Intestinal Permeability

4.3.1 Results

When rats were dosed with either single doses of racemic or the R or S enantiomers of etodolac (ET) or flurbiprofen (FL), statistically significant increase in urinary ^{51}Cr-EDTA were observed for each compound (Figs. 9 and 10). Also, for ET and FL the increase in urinary excretion of ^{51}Cr-EDTA by the S enantiomer appears to be equal to if not greater than that of the racemate. Further, the combined increase in urinary excretion of ^{51}Cr-EDTA of the R and S enantiomers appears to be greater than that of the racemate despite the fact that the enantiomers were given in equivalent proportions to which they were present in the racemate (Figs. 9 and 10).

When rats were dosed with the racemate or the R or S enantiomers of ketoprofen (KT) and ibuprofen (IB) statistically significant increases in urinary ^{51}Cr-EDTA excretion from 0 - 8 h were found for both NSAIDs (Fig. 11). For IB and KT the S enantiomer alone administered at one half of the racemate dose induced approximately half the intestinal permeability as the racemate. Comparatively, FL induced quantitatively higher permeability changes than either IB, KT or ET (Figs. 9-11).

Furthermore, for both IB and KT the correlations between the extent of urinary ^{51}Cr-EDTA excretion and the body load of the R enantiomer in the presence of a constant dose of the S enantiomer were insignificant, $r = 0.56$ and $r = 0.59$ for ibuprofen and ketoprofen respectively (Fig. 12). Similarly, no significant differences

were found between various doses of R+S in the presence of a constant dose of the S enantiomer.

4.3.2 Discussion

IB, KT, FL and ET are available and used clinically as racemates. Recently, S-ibuprofen has been marketed and available in Austria under the trade name Seractil® (Garbo, Fieberbrunn, Austria) (Chlud, 1995). The prostaglandin synthesis inhibition, the antiinflammatory activity and GI toxicity of chiral NSAIDs are mainly attributed to their S enantiomer (Hutt and Caldwell, 1984). Hence, the administration of the racemate and the S enantiomer are expected to result in increased GI permeability. This study clearly demonstrates that oral administration of the racemate and both enantiomers of these chiral NSAIDs induce increases in the permeation of ^{51}Cr-EDTA (Figs. 9-12).

The intrinsic effect of the R enantiomer is difficult to examine for many chiral NSAIDs due to metabolic chiral inversion to the active S enantiomer (Jamali, 1988). In rats and humans, the R enantiomer of ET does not appear to undergo chiral inversion due to the fixed nature of its asymmetric centre (Markey *et al.*, 1987). ET, therefore, provides a unique opportunity for assessing intrinsic effects of an R antipode. In the case of R-etodolac administered as a suspension, this surprising small but significant increase in the extent of urinary excretion of ^{51}Cr-EDTA is direct evidence for the intrinsic activity of the R enantiomer in increasing intestinal permeability. This may suggests that inhibition of prostaglandin synthetase may not be the only mechanism through which ET affects the GI tract. This observation contrasts with the up to 150

fold difference in potency observed *in vitro* of these enantiomers against prostaglandin synthetase (Markey *et al.*, 1987). The activity of the R enantiomers of FL in increasing small intestinal permeability may also suggests that inhibition of prostaglandin synthetase may not be the only mechanism through which these drugs act. However, for FL, the effect of the R enantiomer may also be due to formation of the S enantiomer as 10 % chiral inversion has been reported for FL (Berry and Jamali, 1988). In the present study it also appears that low doses of R-flurbiprofen (<1.5 mg/kg) are only approximately 1.5 to 4 fold less potent than the S enantiomer (Fig. 10) while a 100 fold difference has been reported between the enantiomers with respect to their *in vitro* inhibitory activity of prostaglandin synthetase (Hutt and Caldwell, 1984).

The effect of the R enantiomers of KT and IB are expected due to the fact that 80 and 60 % of the administered enantiomers undergo chiral inversion to the corresponding active antipode (Foster and Jamali, 1987; Jamali and Berry, 1990). In an attempt to further explore the intrinsic effect of R-ibuprofen and R-ketoprofen, we administered varying doses of R and S enantiomers so that after accounting for inversion, a constant body load of the S enantiomer was attained. If the R enantiomer has intrinsic potency, a progressive increase in the activity with an increased dose of R enantiomer should have been evident (Fig. 12). Although such a trend was observed the correlation between increased permeability and the R enantiomer dose did not reach statistical significance. This indicates that the R enantiomers of IB and KT have little or no intrinsic activity in terms of increasing intestinal permeability.

The precise mechanisms which cause an increased intestinal permeability to ^{51}Cr-EDTA after NSAID ingestion are uncertain however there is evidence that permeability changes may be related to the NSAIDs ability to inhibit cyclooxygenase since concomitant prostaglandin administration partially reverses permeability changes (Bjarnason *et al.*, 1989a; Fig. 5). In general most of the therapeutic effects of chiral NSAIDs; the pharmacological activities, and in particular the prostaglandin inhibition, and thereby systemic toxicology have been almost exclusively attributed to the S enantiomer (Hutt and Caldwell, 1983). Several reports, however, have questioned whether inhibition of prostaglandin synthetase is the sole mechanism by which NSAIDs exert their pharmacological and toxicological effects (Kantor and Hampton, 1978, Bjarnason *et al.*, 1992a). Other recent reports suggest intrinsic pharmacological activity for the R enantiomers of 2-APA NSAIDs (Brune *et al.*, 1991; Cox *et al.*, 1991; Brune *et al.*, 1992; Malmberg and Yaksh, 1992; Villaneuva *et al.*, 1993; Zhao *et al.*, 1992).

Interestingly, for FL and ET the increase in urinary excretion of ^{51}Cr-EDTA of S is equal to the racemate although the dose is only half as much as the racemic dose. Furthermore, the sum of the urinary excretion of ^{51}Cr-EDTA for the R and S enantiomers appears to be more than that of the racemate despite the fact that the enantiomers were given in equivalent proportions to which they were present in the racemate (Figs. 9 and 10). This may suggest that given the apparent activity of both FL and ET enantiomers, their presence in the racemate may exert an ameliorating effect on the extent of intestinal permeability observed. The present data also suggest

that mechanisms other than peripheral prostaglandin synthetase inhibition may be responsible for the effects of R-etodolac and R-flurbiprofen on intestinal permeability. The enantiomers and racemate of ET and FL may have enantiospecific or enantioselective effects on one or more of the physiological or biochemical regulators that influence paracellular permeability of the intestine. The apparent differences between the effects of individual enantiomers and the racemate may result, in part, from differences in their physicochemical properties such as solubility, melting point, and partition coefficent which in turn may alter their biodistribution (Dwivedi *et al.*, 1992).

The increase in intestinal permeability of ^{51}Cr-EDTA by S-ibuprofen and S-ketoprofen is half that of the racemate given that the dose was only half that of the racemic dose (Fig. 11). In addition, the sum of the urinary excretions of ^{51}Cr-EDTA for the R and S enantiomers of IB and KT appears to be equal to the urinary excretion of the racemate. If the intestinal permeability changes due to the racemate are equal to the sum of the S enantiomer alone plus S-enantiomer due to inversion of the R enantiomer to S enantiomer alone (i.e. Racemate = S + (R→S), then the predicted sum of the resulting S enantiomer effect should be less than the racemate (Fig. 11). This difference may be attributable to the variability of the inversion process and or a small contribution from the R enantiomers.

From a pharmacokinetic viewpoint it is interesting to note that there are no enantiomer-enantiomer pharmacokinetic interactions when the racemates of IB and KT are administered (Foster and Jamali, 1987; Iwaka *et al.*, 1991; Menzel *et al.*, 1993)

whereas, an enantiomeric interaction has been previously demonstrated to occur with FL (Berry and Jamali, 1989; Knihiniicki *et al.*, 1990). In the rat the administration of R-flurbiprofen alone produced an area under the curve (AUC) of approximately twice that when administered in the racemate, and when S was administered alone there was also a trend for a higher AUC (Berry and Jamali, 1989; Knihiniicki *et al.*, 1990). The increase in AUC as a result of reduced clearance may account for the S enantiomer being almost as active as the racemate in terms of intestinal permeability despite a one fold difference in dose. Whether the beneficial antiinflammatory and analgesic effects of flurbiprofen may also be enhanced when administered as the pure enantiomer has not been elucidated but warrants further investigation.

Our observations present yet another example of the pharmacological complexity of chiral drugs that are administered as the racemate. From a therapeutic perspective, the decision to employ either the racemate or stereochemically pure-enantiomer must be based on examination of the relevant and possibly diverse mechanisms of action rather than on the single most obvious effect. It is necessary to evaluate the toxicological properties of the enantiomers of a racemate to assess if the risk/benefit ratio can be improved by the use of only one of the enantiomers. Stereochemically pure compounds must be evaluated as individual pharmacological entities and general guidelines for all chiral NSAIDs cannot be established. Furthermore, the stereochemically pure-enantiomer is not merely a more refined form of the racemate nor is it necessarily safer or therapeutically superior to its respective racemate.

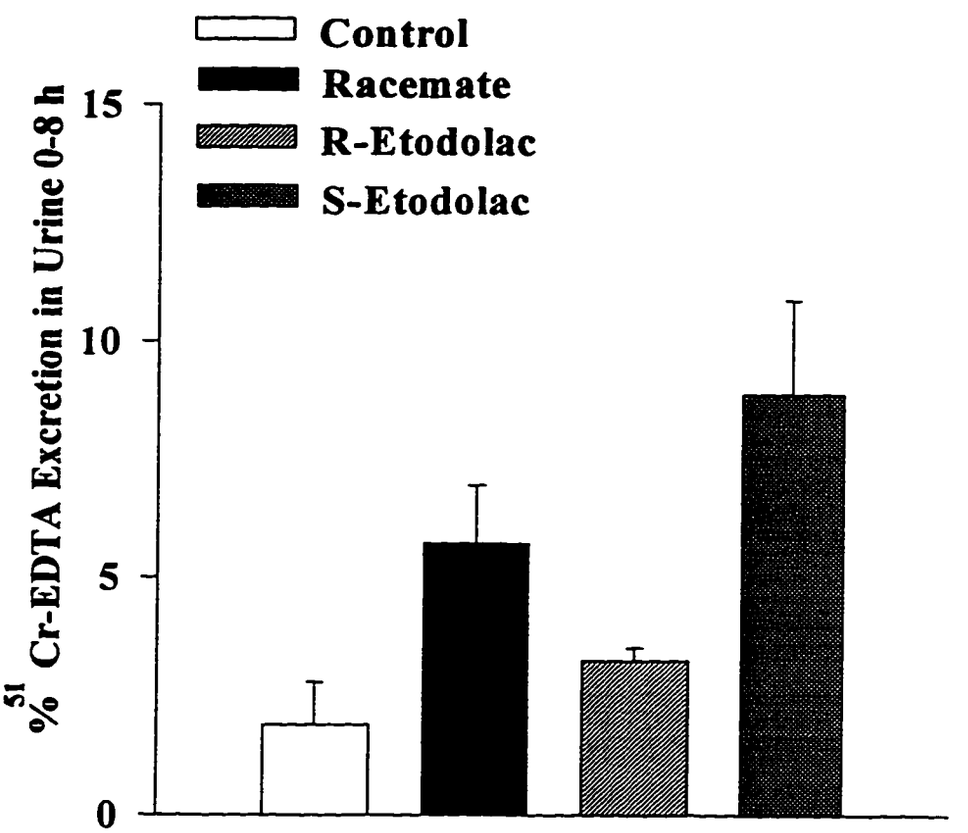


Figure 9. Effects of a single dose of racemic (12 mg/kg) R and S-etodolac (6 mg/kg) on ^{51}Cr-EDTA urinary excretion 0-8 h (n = 6 for each group; mean ± s.e.m.). All treatments are signifcantly different from control. S-etodolac and racemate significantly different from R-etodolac.

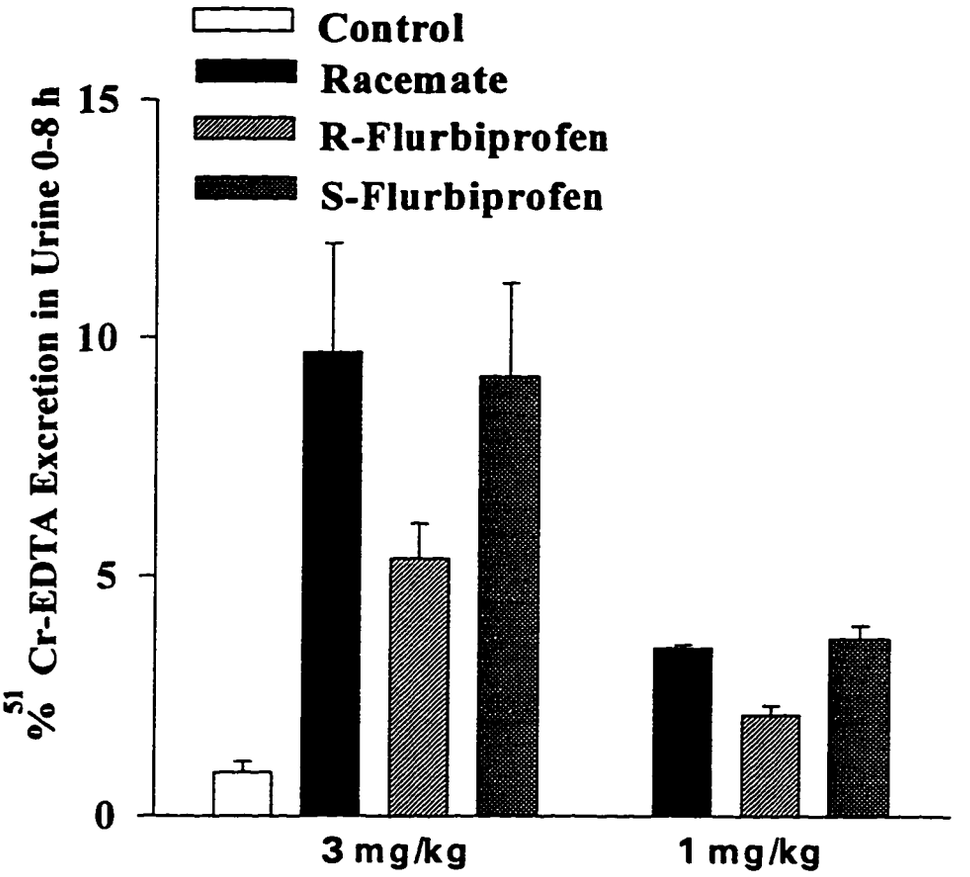


Figure 10. Effects of a single dose of racemic (3 or 1 mg/kg) R and S-flurbiprofen (1.5 or 0.5 mg/kg) on ^{51}Cr-EDTA urinary excretion 0-8 h (n = 6 for each group; mean ± s.e.m.). All treatments are signifcantly different from control. S-flurbiprofen and racemate significantly different from R-flurbiprofen.

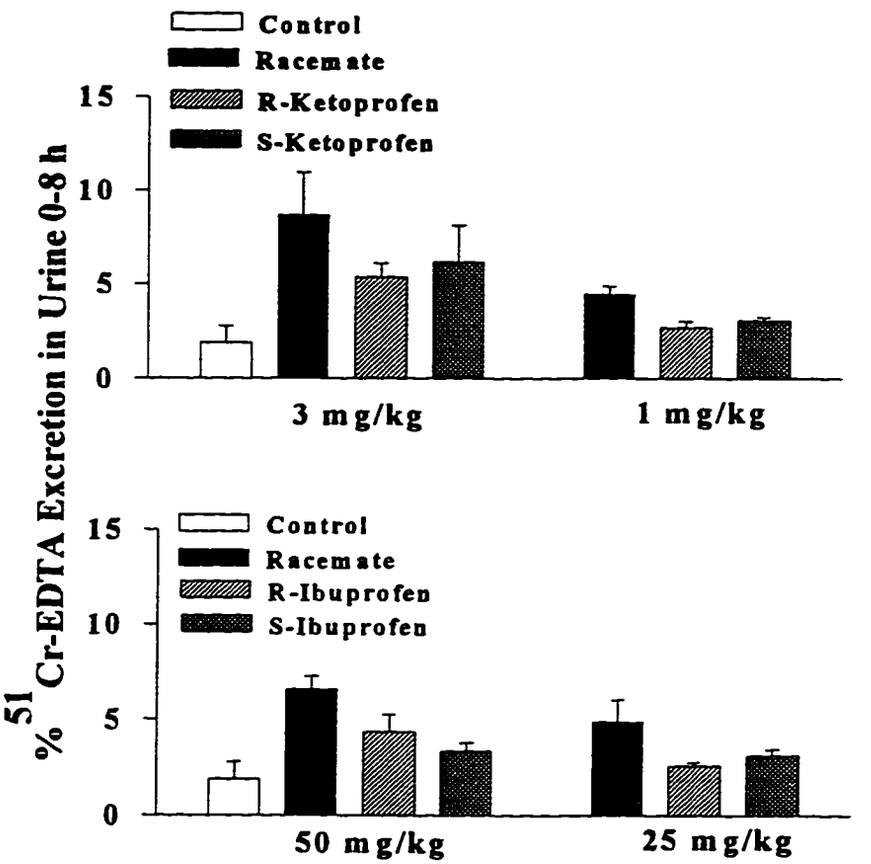


Figure 11. Effects of a single dose of racemic (3 or 1 mg/kg) R and S-ketoprofen (1.5 or 0.5 mg/kg) (upper panel) and racemic (50 or 25 mg/kg) R and S-ibuprofen (25 or 12.5 mg/kg) (lower panel) on ^{51}Cr-EDTA urinary excretion 0-8 h (n = 6 for each group; mean ± s.e.m.). All treatments are signifcantly different from control. S-ketoprofen and racemate significantly different from R-ketoprofen.

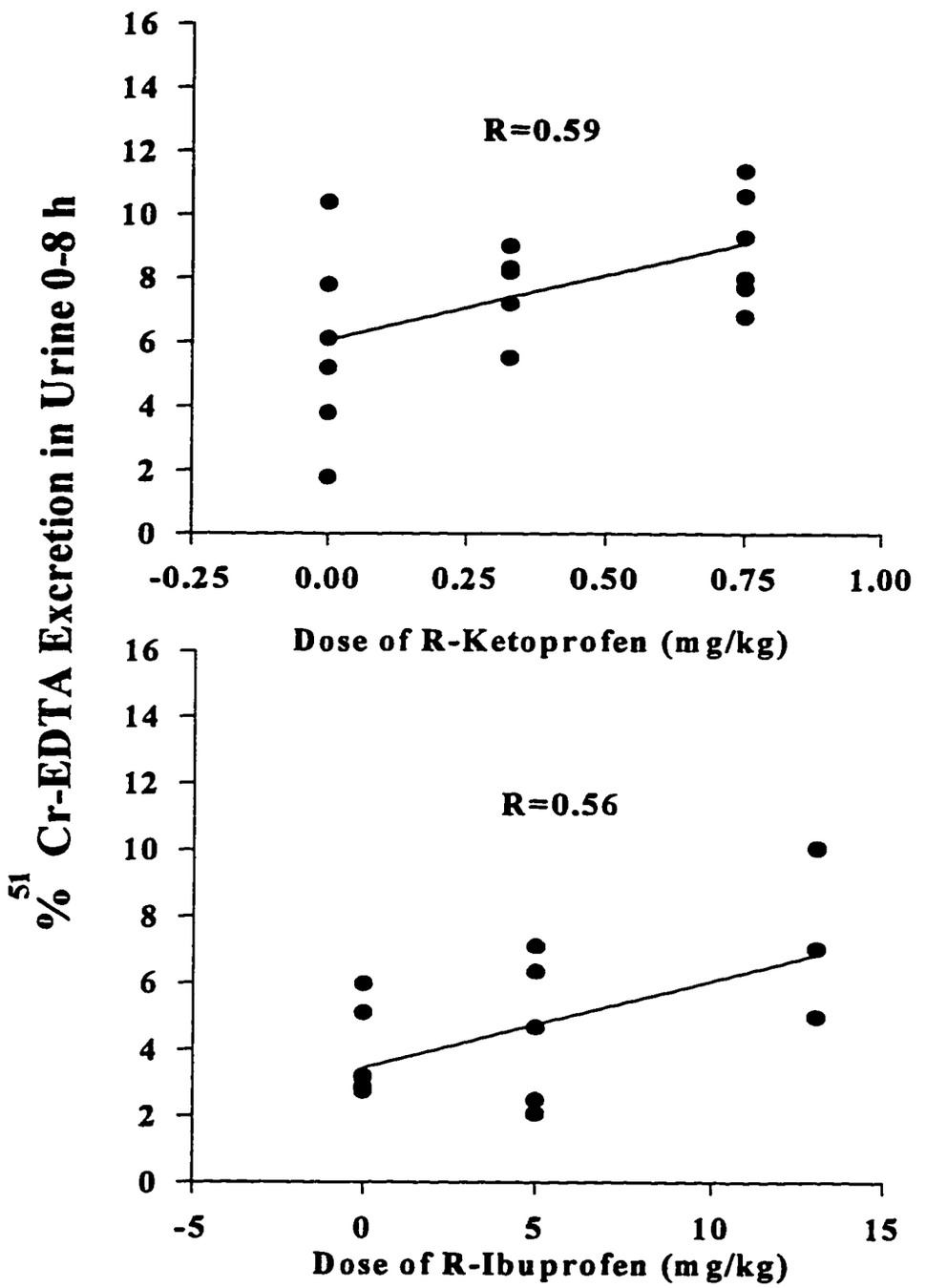


Figure 12. Effects of increasing R-ketoprofen and R-ibuprofen on ^{51}Cr-EDTA urinary excretion in the presence of 3 mg/kg S-ketoprofen and 20 mg/kg S-ibuprofen, respectively (n = 4-6 for each group).

4.4 Toxicokinetics of Indomethacin-Induced Intestinal Permeability

4.4.1 Results

The changes in intestinal permeability as a function of time for rats receiving either 10 or 20 mg/kg of indomethacin are presented in Fig. 13. For both indomethacin doses there was a rapid and significant increase in apparent permeability beginning 1 h post-dose and reaching maximal values 12 h post-dose. Permeability did not return to control values for at least 96 h following the dose in both groups. There appears to be a sustained level of maximal permeability reached which lasted between 12 and 36 h after the 10 and 20 mg/kg doses, respectively.

Since the time of maximal permeability following oral indomethacin treatment occurred at 12 h post-dose, the dose-effect relationship was examined at this time point. As shown in Fig. 14, the dose-effect relationship was sigmoidal and apparently reached plateau values between doses of 16 and 20 mg/kg oral indomethacin. Analysis of this data by PCNONLIN determined an E_{max} value of 34.5 ± 11.8 % (mean ± s.e.m.) and an ED_{50} of 13.66 ± 8.7 mg/kg (mean ± s.e.m.).

The plasma concentration versus time profile of each rat was best described by a one compartment open model with first order absorption (Fig. 15). The pharmacokinetic parameters describing the plasma concentration versus time profiles for the 10 and 20 mg/kg groups are presented in Table 10. There were no significant differences in pharmacokinetic parameters except that the apparent clearance (Cl/F) of the 20 mg/kg group (3.4 ± 0.6 mL/h) was significantly lower than that of the 10 mg/kg group (5.9 ± 0.5 mL/h) (unpaired t-test, $p < 0.05$).

There was a significant time lag between the occurrence of peak plasma concentrations (Fig. 15) and peak effect (Fig. 13). Indeed when the change in permeability was plotted against the plasma concentration for each dose and the points joined in temporal order a significant counterclockwise hysteresis was found (Fig. 16). When combined pharmacokinetic / pharmacodynamic modeling was employed there

was a collapse of the hysteresis (Fig. 17). The pharmacodynamic parameters describing the effect compartment and change in intestinal permeability are presented in Table 10. Again, there were no significant differences between the parameters determined for the 10 and 20 mg/kg groups ($p > 0.05$ unpaired t-tests). Additionally the E_{max} values determined by combined pharmacokinetic/pharmacodynamic fitting and the E_{max} value determined from the dose ranging study are not significantly different (ANOVA, $p > 0.05$).

4.8.2 Discussion

Numerous studies in humans have demonstrated increased intestinal permeability, as estimated by urinary ^{51}Cr-EDTA excretion, in response to a variety of NSAIDs including indomethacin (Bjarnason *et al.*, 1984b, 1987b,d, 1991a,c, 1992a; Table 6). Although these studies uniformly demonstrate significant NSAID-induced increases in intestinal permeability, none have examined the time-course of permeability changes nor the pharmacokinetic / pharmacodynamic relationships of this effect. In humans, however, it would be extremely difficult to examine such pharmacodynamic relationships since this would require repeated exposure to ^{51}Cr-EDTA which may not be ethically feasible.

Similar to the results from the human studies (Bjarnason *et al.*, 1984b, 1987b,d, 1991a,c, and 1992a) the present study demonstrates that the treatment of rats with indomethacin produces significant increases in intestinal permeability. As suggested by preliminary studies by Bjarnason *et al.* in humans (1984b) the magnitude of the change in intestinal permeability is dose related (Figs 13-14), however, these investigators did not examine the possible time-dependency of the increase in permeability. In contrast to the studies in humans, however, studies in rats shown here demonstrated that there are significant time-related changes in the magnitude of intestinal permeability. The time- and dose-dependent patterns of this effect are important particularly when various drugs or doses of the same drug are being

compared. In the present studies the maximum permeability occurs 12 h following dosing, but persists at this level much longer in the 20 mg/kg dose group than in the 10 mg/kg group. Examination of only the time courses of the change in intestinal permeability, however, does not distinguish as to whether this may arise from a dose-dependent pharmacokinetic or pharmacodynamic difference. The observation of time-dependent changes in the magnitude of the increase in intestinal permeability may suggest a methodological problem with the previously reported studies in humans since they were performed without regard to possible time-related changes in permeability and, thus, comparison between various drugs with different pharmacokinetic/ pharmacodynamic profiles may be difficult.

The pharmacokinetic parameters for indomethacin are in good agreement with those found by other investigators (Duggan *et al.*, 1975; Hucker *et al.*, 1966) with the exception of a lower apparent total body clearance (Cl/F) in the 20 mg/kg dose group as compared with the 10 mg/kg dose group. Although there do not appear to be reports of dose-dependent indomethacin clearance (Helleberg, 1981) most pharmacokinetic studies have employed lower doses than those of the present study. It is possible therefore that pharmacokinetic dose-dependency of higher does (i.e. $\approx$ 20 mg/kg) may have gone unnoticed. In addition, Rowe and Carless (1982) have demonstrated that the *in vitro* metabolism of indomethacin is saturable within the range of indomethacin concentrations encountered in these studies. The observed time lag of at least 11 h between the peak plasma concentration and the occurrence of the peak effect was consistent with the counterclockwise hysteresis (Fig. 16) found when the intestinal permeability is plotted against concentration and the points connected in temporal order. Previously such observations have been rationalized through the use of an effect compartment (Holford and Sheiner, 1981). When effect compartment modeling is employed the hysteresis is collapsed (Fig. 17) and both the 10 and 20 mg/kg doses demonstrate similar concentration-effect relationships (Table 10).

Examination of the effect versus effect compartment concentration curves for the 20 mg/kg group (Upper panel Fig. 17) shows that the hysteresis is not as fully collapsed as for the 10 mg/kg group (Lower panel Fig. 17). Further the E_{max} values estimated in this manner (31.28 ± 7.37 and 35.22 ± 9.88 %) are not significantly different from the 34.36 ± 11.79 % estimated from the dose-ranging studies. Alternatively, the apparently greater variability in increased intestinal permeability induced by the 20 mg/kg dose may suggest some augmentation of damage by the 20 mg/kg dose as compared to the 10 mg/kg dose. Thus, the apparent differences in the time courses of the increase in intestinal permeability between the 10 and 20 mg/kg dose groups may be the result of the dose-dependent clearance of the drug rather than any dose-dependent pharmacodynamic differences. It is also worthy to note that the increased intestinal permeability (Fig. 13) persists for much longer than the drug is present in the body (Fig. 15). This may be due to an apparently slow rate of healing or the persistence of drug in a "deep" compartment at extremely low concentrations. It is interesting to note that 15 animals receiving the 20 mg/kg dose of indomethacin in either the dose-ranging or time course studies died approximately 3 days after experimentation. In all cases necropsy revealed jejunal and/or ileal ulceration and perforation. Further there was evidence of widespread inflammation and some indication of areas of ischemia within the intestine. These pathophysiological results may suggest the sequelae of the large increases in intestinal permeability observed in these studies and are consistent with the findings of Ford *et al.* (1995) who have correlated intestinal permeability changes with intestinal ulceration.

In summary, the present study demonstrates the time and dose-dependencies of indomethacin induced changes in intestinal permeability as estimated by urinary ^{51}Cr-EDTA excretion. In addition, the pharmacokinetic / pharmacodynamic relationships between the increase in intestinal permeability and plasma drug concentrations have been shown. The present data suggest that the changes in

intestinal permeability in the rat are qualitatively similar to those previously observed in humans. Further the observation of time-dependent changes in permeability following NSAID administration in the rat suggest that similar considerations should be made when examining the data derived from human studies.

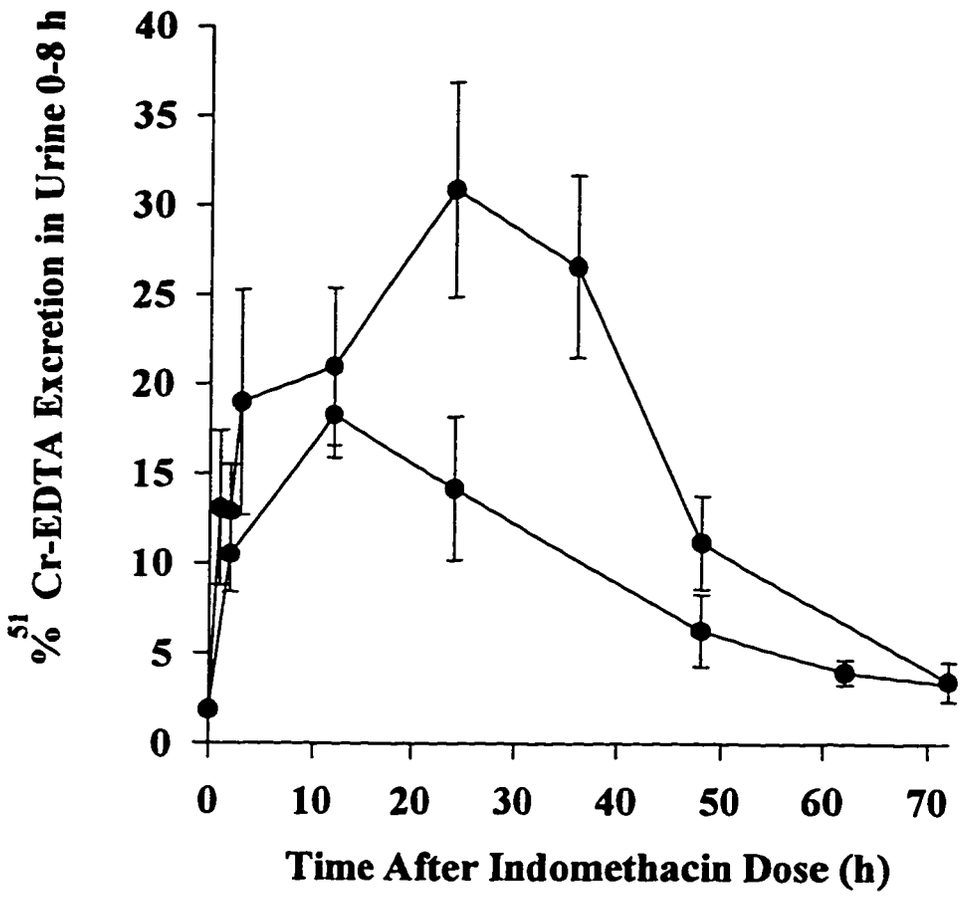


Figure 13. Time courses of intestinal permeability changes (mean ± s.e.m., n = 3 for each point) resulting from the administration of oral indomethacin 20 mg/kg (closed circles) or 10 mg/kg (open circles).

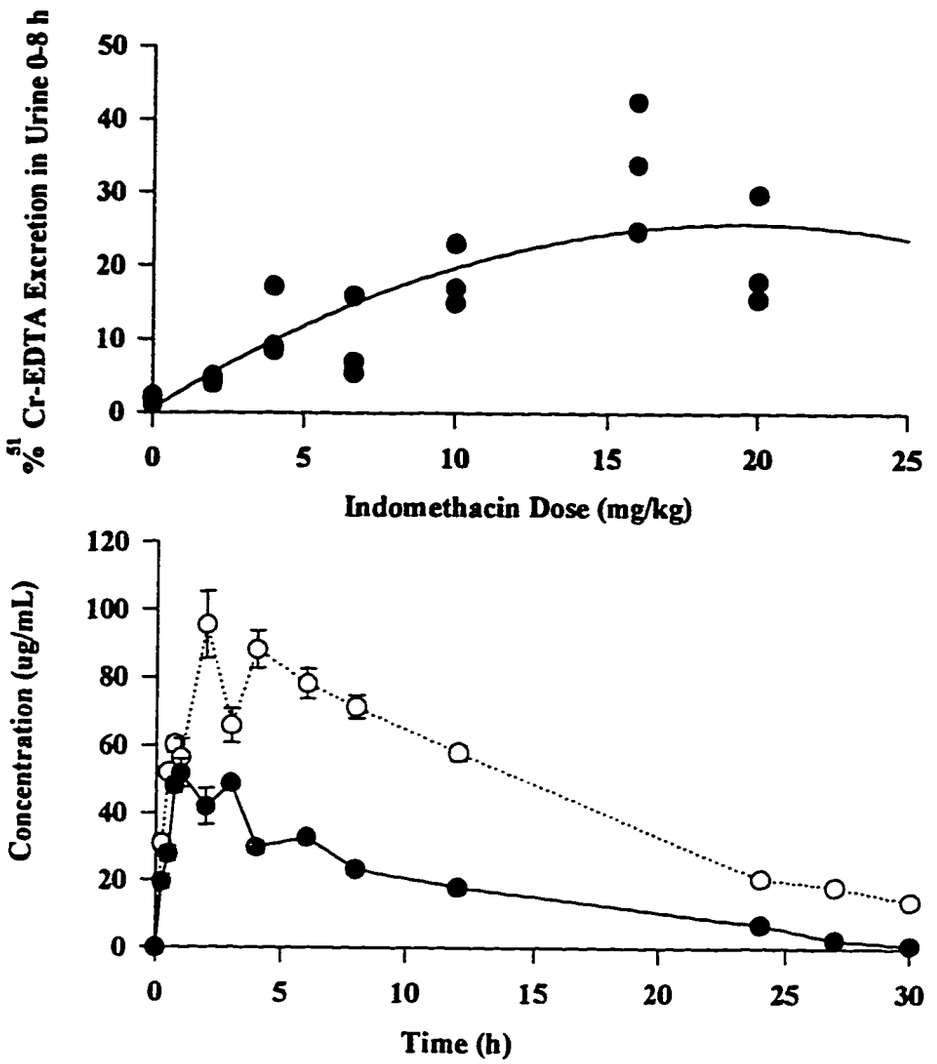


Figure 14. Upper Panel. Dose-response curve for oral indomethacin. Each point represents 1 rat. Parameters describing this curve are given in the results section 4.4.2.

Figure 15. Lower Panel. Mean plasma concentration (± s.d., n = 3) versus time profiles for indomethacin in rats dosed with either 20 mg/kg (closed circles) or 10 mg/kg (open circles) indomethacin orally.

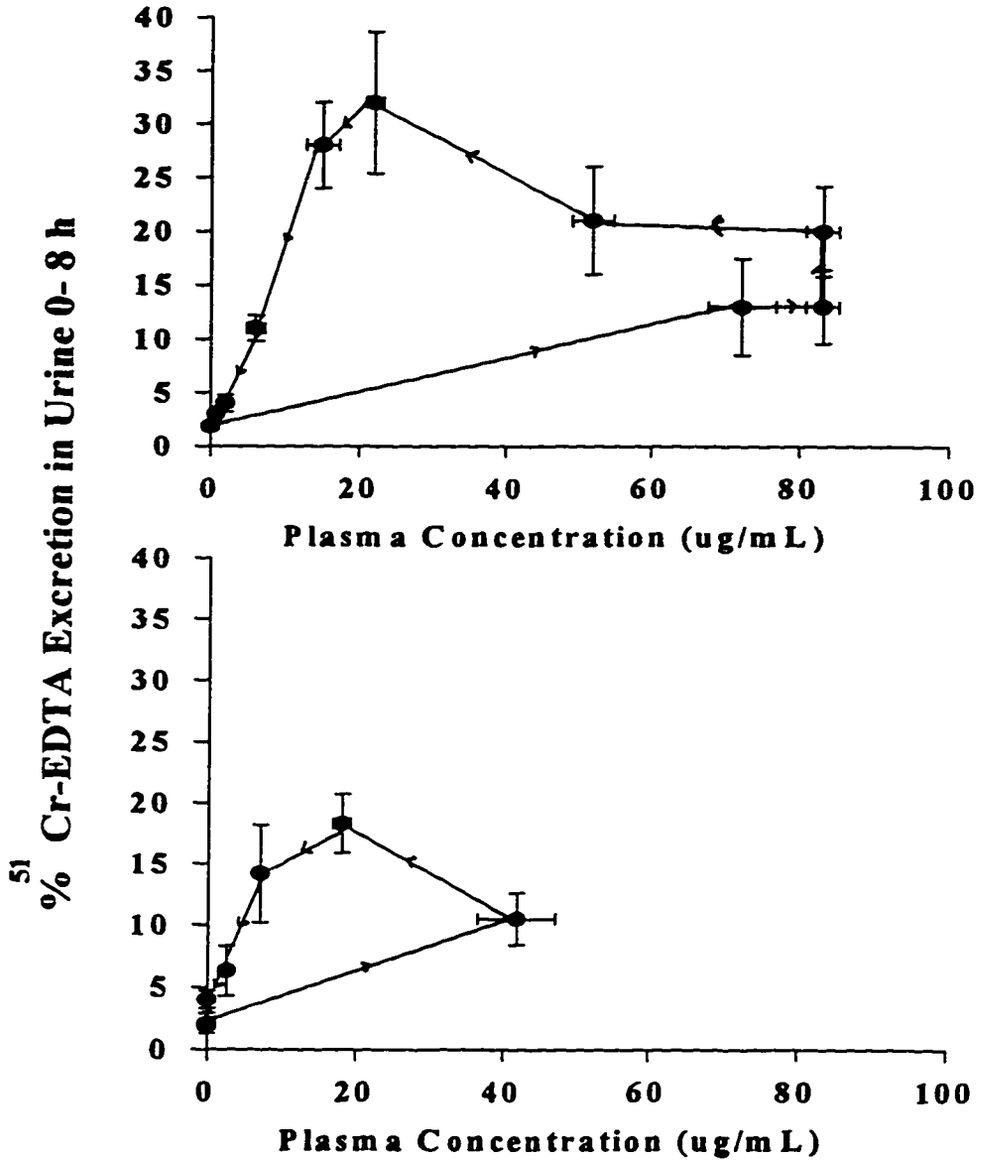


Figure 16. Change in intestinal permeability (mean ± s.e.m., n = 3) versus indomethacin plasma concentration for rats dosed with oral indomethacin 20 mg/kg (upper panel) or 10 mg/kg (lower panel). The points are joined in temporal order. Parameters describing the pharmacokinetic / pharmacodynamic relationships are described in Table 10.

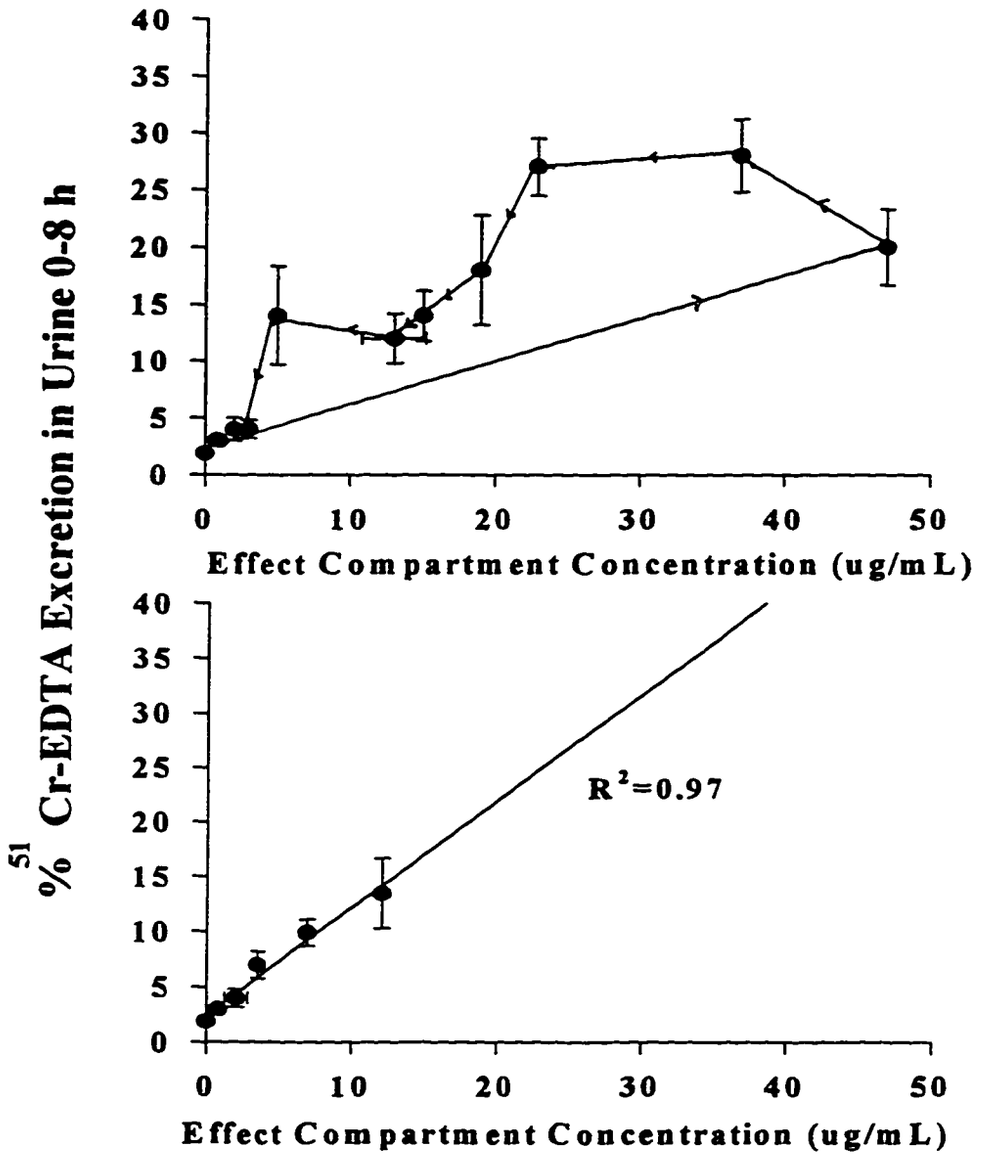


Figure 17. Change in intestinal permeability (mean ± s.e.m., n = 3) versus concentration in the effect compartment for rats dosed with oral indomethacin 20 mg/kg (upper panel) or 10 mg/kg (lower panel). The points are joined in temporal order. Parameters describing the pharmacokinetic / pharmacodynamic relationships are presented in Table 10.

Table 10. Pharmacokinetic and pharmacodynamic parameters describing the change in intestinal permeability following oral doses of 10 and 20 mg/kg indomethacin (mean ± s.e.m., n = 3 for each group).

Parameter	**10 mg/kg**	**20 mg/kg**
$AUC_{0-\infty}$ (μg h/mL)	513 ± 42	1877 ± 330
K_A (h^{-1})	1.97 ± 0.46	1.33 ±0.26
K_E (h^{-1})	0.107 ± 0.016	0.061 ± 0.008
Cl/F (mL/h)	5.9± 0.5	3.4* ± 0.6
Vd/F (mL)	55.1 ± 5.2	61.4 ± 5.0
E_{max} (%)	31.3 ± 7.4	35.2 ± 9.9
EC_{50} (μg/mL)	14.6 ± 5.9	14.4 ± 10.0
k_{e0} (h^{-1})	0.071 ± 0.009	0.106 ± 0.036

* - significantly different from the 10 mg/kg group (unpaired t-test, $p < 0.05$)

4.5 Pharmacological Protection of NSAID-Induced Intestinal Permeability

4.5.1 Results

The excretion values of indomethacin alone were significantly greater than after concomitant administration of misoprostol (Fig. 18). Misoprostol co-administration dose-dependently reduced the ^{51}Cr-EDTA urinary excretion up to 170 μg/kg where reduction appeared to reach an asymptote.

Naproxen showed a linear dose-response relationship over a five-fold dosage range ($r^2 = 0.99$) (Fig. 19). A reduction in permeability was evident upon co-administration of 30 μg/kg of misoprostol. Permeability values did appear to decrease further towards baseline upon co-administration of 170 μg/kg of misoprostol.

The daily administration of the xanthine-oxidase inhibitor allopurinol did not have any preventative effect on indomethacin induced permeability changes at the 20 mg/kg dose (Fig. 20).

The mean ^{51}Cr-EDTA urine excretion values with varying amounts of indomethacin shows that the intestinal permeability of oral indomethacin alone was significantly greater than those administered indomethacin and glucose/citrate given in a mass ratio of (1:15:15) in all the groups (Fig. 21). This reduction in permeability with glucose/citrate appears to be glucose/citrate dose-dependent as a lower glucose/citrate ratio (1:3.75:3.75) did not reduce intestinal permeability whereas a higher ratio of glucose/citrate (1:30:30) significantly reduced indomethacin permeability values (Table 11). However, all treatments resulted in permeability values

above controls indicating a significant difference was evident above baseline for all glucose/citrate and indomethacin formulations ($p<0.05$).

The mean ^{51}Cr-EDTA urine excretion ratios with varying amounts of naproxen were not significantly different from those after naproxen-glucose/citrate (1:15:15) in all the groups (Fig. 22). Table 11 further shows that the urinary excretion values of ^{51}Cr-EDTA were significantly increased above controls by administration of the other NSAIDs tested with or without glucose/citrate

The anti-inflammatory effects of indomethacin in the carrageenan footpad model remain equipotent upon (1:15:15) glucose/citrate challenge as assessed by change in paw diameter and paw volume (Figs. 23 and 24).

The subcutaneous administration of 20 mg/kg indomethacin with glucose/citrate administered orally (1:15:15) did not cytoprotect against these permeability changes (Fig. 25). All the animals in both groups died within 72 hours of indomethacin treatment as a result of peritonitis secondary to enteritis.

The comparative pharmacokinetic profile of the indomethacin glucose/citrate suspension (1:15:15) as compared to indomethacin alone is illustrated in Fig. 26. The concomitant administration of glucose/citrate with indomethacin results in a 50-80 % reduction in bioavailability of indomethacin. The pooled relative bioavailability of indomethacin glucose/citrate suspension was calculated to be 0.38 (Fig. 26).

Individual plots of the (1:15:15) formulation of indomethacin and glucose/citrate shows marked fluctuation and erratic absorption of this formulation

(Fig. 27). Pharmacokinetic studies of indomethacin administered alone showed significantly less variability than the indomethacin and glucose/citrate formulation.

Dissolution profiles of indomethacin alone and indomethacin and glucose/citrate were identical for both formulations.

4.4.2 Discussion

It has been suggested that manipulation of certain metabolic systems within the intestinal mucosa is a therapeutic approach which may overcome some of the adverse GI side-effects induced by NSAIDs (Del Soldato *et al.*, 1985; Rainsford, 1989). As previously demonstrated in humans, despite increases in the dose, misoprostol partially reduces indomethacin induced intestinal permeability (Fig. 18). This may suggest that indomethacin-induced permeability effects are only partially mediated by a reduction in endogenous prostaglandin levels (Davies *et al.*, 1993). Alternatively, misoprostol may change the distribution of indomethacin into the epithelial cell lining of the GI tract. Very large doses of prostaglandins have to be given to reduce the permeability changes induced by single doses of indomethacin and many subjects still have increased intestinal permeability (Davies *et al.*, 1993). Recently, Rafi *et al.* (1995) have demonstrated that mitochondrial oxidative phosphorylation which is an important early event of NSAID-induced GI toxicity was not prevented by misoprostol. This suggests that the uncoupling action of NSAIDs may not be entirely a prostaglandin-dependent process. In addition, *in vitro* evidence also indicates that indomethacin decreases the transepithelial electrical resistance of Caco-2 monolayers indicating an increase in permeability of the tight junction of adjacent enterocytes, however, prostaglandin E_2

does not completely protect against this damage (Davies *et al.*, 1995b). Results in the rat model are consistent with all of these findings.

Interestingly, it has previously been suggested that increased intestinal permeability to ^{51}Cr-EDTA induced by naproxen could not be reversed with co-administration of a prostaglandin E_2 analogue in normal therapeutic doses in a human study (Jenkins *et al.*, 1988). However, the sample size of this study was 6 subjects (Table 6) and there was a trend towards a reduction in intestinal permeability which considering the greater inter- subject variation in humans compared to the rat this might have reached statistical significance with a larger sample size. Nevertheless, that naproxen-induced permeability changes were reduced by concomitant administration of misoprostol is at variance with the results of Jenkins *et al.* (1988). It also appears that the 30 μg/kg dose of misoprostol was close to the maximal protective dose to cover naproxen-induced intestinal damage (Fig. 19). However, indomethacin which quantitatively induces more intestinal damage requires a larger dose of misoprostol to demonstrate a cytoprotective ability of this agent (Fig. 18).

Although, allopurinol has been shown to protect the gastric mucosa from aspirin damage and to reduce ischemia-reperfusion injury in the intestine (Rainsford 1990; Vaughan, 1992), it has lacked a protective effect on indomethacin-induced intestinal permeability. These results suggest that effective protective approaches for the upper GD and ischemia-reperfusion injury may not necessarily protect against NSAID-induced intestinal permeability. These results also suggest that other pathways and pharmacological mediators such as superoxide anions derived from other sources

like neutrophils may be responsible for indomethacin-induced intestinal damage rather than xanthine-oxidase induced free radicals. These data are consistent with the lack of effect of the anti-ulcer agent rebamipide on xanthine-oxidase free radicals, while cytoprotecting against gastric lesions through inhibiting mobilization and activation of neutrophils in association with an attenuation of the decreases in superoxide dismutase (Kim and Hong, 1995). In addition, this data may also suggest that although aspirin and indomethacin are both classified as NSAIDs (Table 1) they may not induce GI damage through entirely the same mechanisms.

NSAIDs may inhibit glycolysis and the tricarboxylic acid cycle in addition to inhibiting prostaglandin synthesis, resulting in inhibition of oxidative phosphorylation thereby reducing adenosine triphosphate (ATP) production and damaging the enterocyte leading to cell death (Somasandarum *et al.*, 1992). It has been suggested that the presence of these sugars in the intestinal lumen may modify the reaction to indomethacin or that citrate may be cytoprotective against free radical damage caused by NSAIDs (Rainsford and Whitehouse, 1987; Walker *et al.*, 1987). In humans, a formulation containing with each mg of indomethacin a (1:15:15) glucose and citrate ratio reduced the expected effect of the NSAID on intestinal permeability and GI microbleeding (Walker *et al.*, 1987; Bjarnason *et al.*, 1992a). However, no improvement in blood loss studies was evident with a 1:3:3 formulation of indomethacin glucose/citrate in other clinical studies (Rainsford and Whitehouse, 1987). In addition, no significant reduction in GI microbleeding induced by the NSAID

azapropazone in a glucose/citrate formulation, of 1:1:1, has been demonstrated (Rainsford et al, 1991).

The protective effects of indomethacin glucose/citrate (1:15:15) formulation have been previously demonstrated in NSAID-induced intestinal ulceration in the rat (Rainsford, 1987). Therefore, it has been previously suggested that this pharmacological effect may not be completely mediated by a reduction in prostaglandins (Rainsford *et al.*, 1987). Similar observations have been made in the rat model an indomethacin and glucose/citrate formulation (1:15:15) which reduced indomethacin-induced intestinal permeability changes (Fig. 21). However, the cytoprotective effect of glucose/citrate could not be demonstrated with a variety of other NSAIDs tested (Table 11) (Fig. 22). Therefore, upon further examination this ameliorative effect of orally administered glucose/citrate on indomethacin-induced intestinal permeability was examined after subcutaneous administration of the NSAID. The lack of protection of glucose/citrate given orally when indomethacin is administered subcutaneously (Fig. 25) suggests a possible presystemic and, perhaps, physiochemical interaction. The reduced intestinal effect of oral doses of indomethacin in the presence of glucose/citrate was accompanied by a significant reduction in bioavailability of the NSAID which could account for the reduced intestinal permeability changes (Fig. 26). The pharmacokinetics of indomethacin in rats following 10 mg/kg oral doses are in good agreement with those found by other investigators (Duggan *et al.*, 1975; Hucker *et al.*, 1966). The observed reduction in bioavailability is postulated to be due to different *in vivo* dissolution kinetics of the

formulations. The pH of the indomethacin glucose/citrate formulation was determined to be ≈ 1.5 whereas the pH of the indomethacin suspension was ≈7. The lower pH of the indomethacin glucose/citrate formulation may decrease the amount of indomethacin in solution and cause precipitation of the drug from solution in the acidic environment of the stomach. The high buffering capacity of the glucose/citrate limits re-dissolution of indomethacin in the distal intestine where some of the drug is being absorbed. A reduced dissolution is consistent with the observed erratic absorption; lower Cmax and longer tmax for the indomethacin glucose/citrate formulation (Figs. 26-27). Indeed to neutralize a 0.5 mL suspension of indomethacin glucose/citrate at least 0.5 mL of 10 N NaOH was needed. Solubility of indomethacin at pH 1.5 in the presence of glucose/citrate was found to be 3.16 μg/mL versus 155 μg/mL at pH 7.0 for indomethacin alone.

Interestingly, the reduction in bioavailability did not show a corresponding decrease in the pharmacodynamic efficacy (Fig. 23-24) as the dose needed to elicit an antiinflammatory effect was exceeded by the administration of either formulation as demonstrated by the equi-effacious pharmacodynmaic response. This is consistent with the adjuvant arthritic rat data of Rainsford and Whitehouse (1987) which demonstrated an equivalent pharmacodynamic efficacy given either formulation.

The observation of a reduced bioavailability caused by the indomethacin and glucose/citrate formulation is not consistent with those of Bjarnason *et al.* (1992a) who found no reduction in the indomethacin bioavailability in humans in the presence of glucose/citrate. This discrepancy between the two studies is postulated to be due to

the fact that in Bjarnason's bioavailability study (1992a) the indomethacin and glucose/citrate formulation was ingested with 100 mL of water, whereas, in their intestinal permeability study the indomethacin and glucose/citrate was administered with only 50 mL of water which might have altered the dissolution of the formulation in the pharmacokinetic study. This is consistent with our dissolution results which showed an apparent identical dissolution of both formulations because of the large volume of dissolution medium used. In Bjarnason's pharmacokinetic study there was a smaller 5 and 17 % reduction in the AUC and Cmax for the indomethacin and glucose/citrate formulation, respectively. This difference was, however, not statistically significant (Bjarnason *et al*, 1992a). In Bjarnason's permeability study the ^{51}Cr-EDTA excretion, although significantly higher for indomethacin alone, the difference between the two formulations was rather small 1.15±0.15 % versus 1.54± 0.19% for indomethacin and glucose/citrate (1:15:15) control and indomethacin-treated subjects, respectively. This is compared to a reduction in the AUC by 62 % and a 70 % reduction in the Cmax (Fig. 27) in the present study, which was accompanied by a 50-70 % reduction in indomethacin-induced intestinal permeability (Fig. 21). In the absence of glucose/citrate a 50-70 % smaller dose than the 10 mg/kg dose administered in this study (Fig. 21) would cause ≈10 % increase in intestinal permeability which is equivalent to that observed after administration of 3-5 mg/kg indomethacin dose (Fig. 14). This clearly explains that the reduced effect of indomethacin on intestinal permeability is indeed due to a reduced bioavailability. Bjarnason *et al.* (1993a) have more recently indicated the protective effect of

glucose/citrate to prevent permeability changes and enteropathy induced by indomethacin is not evident after repetitive administration of the indomethacin glucose/citrate formulation. It is not stated, however, if in this new study, Bjarnason *et al.* (1993a) noticed any protective effect after a single indomethacin and glucose/citrate dose.

The lack of a significant intestinal protection after administration of a variety of NSAIDs with glucose/citrate (1:15:15) (Table 11) is also consistent with a clinical study of azapropazone and glucose/citrate reported by Rainsford (1991). In addition, the lack of intestinal cytoprotection of a (1:3:3) ratio of indomethacin and glucose/citrate is also consistent between the rat and human studies (Rainsford and Whitehouse, 1987).

The precise mechanisms by which indomethacin and other NSAIDs increase intestinal permeability may involve multiple interacting factors and this requires further investigation. The effects of administration of additional cytoprotective agents with NSAIDs will be important to elucidate the pathogenesis of NSAID-induced GI toxicity. Pharmacokinetic studies have significant utility in delineating the toxicological relevance of cytoprotective agents. This study suggests that the reduction in toxicity with a indomethacin and glucose/citrate formulation may be due to a reduction in bioavailability of this formulation due to erratic absorption and a presystemic physicochemical interaction.

Table 11 The Effects of Co-administration of NSAIDs and Glucose/Citrate in (1:15:15) Mass Ratios on Intestinal Permeability

TREATMENT	N	NSAID alone	with Glucose/Citrate
Sulindac 8.3 mg/kg	4	3.67± 0.3	4.00± 2.2
Etodolac 12 mg/kg	4	4.94± 0.36	6.96± 0.42
Nambumetone 42 mg/kg	4	4.24± 0.97	5.11± 2.88
S-flurbiprofen 1.5 mg/kg	3	10.04± 0.97	10.49± 2.15
S-ketoprofen 0.5 mg/kg	3	4.56± 0.79	4.76± 0.78
Mefenamic acid 21 mg/kg	3	9.23± 1.9	5.74± 1.98
Ketorolac 0.5 mg/kg	3	4.24± 0.12	4.19± 1.3
Ibuprofen 50 mg/kg	8	10.57± 0.86	11.23±1.18
Indomethacin 20 mg/kg	8	37.29±4.92	7.22±0.8 (1:30:30)*
Indomethacin 5 mg/kg	8	16.5±2.4	16.3±2.2 (1:3.75:3.75)
Diclofenac 4 mg/kg	4	4.42±1.2	4.78±5.7

* Significantly different from NSAID alone

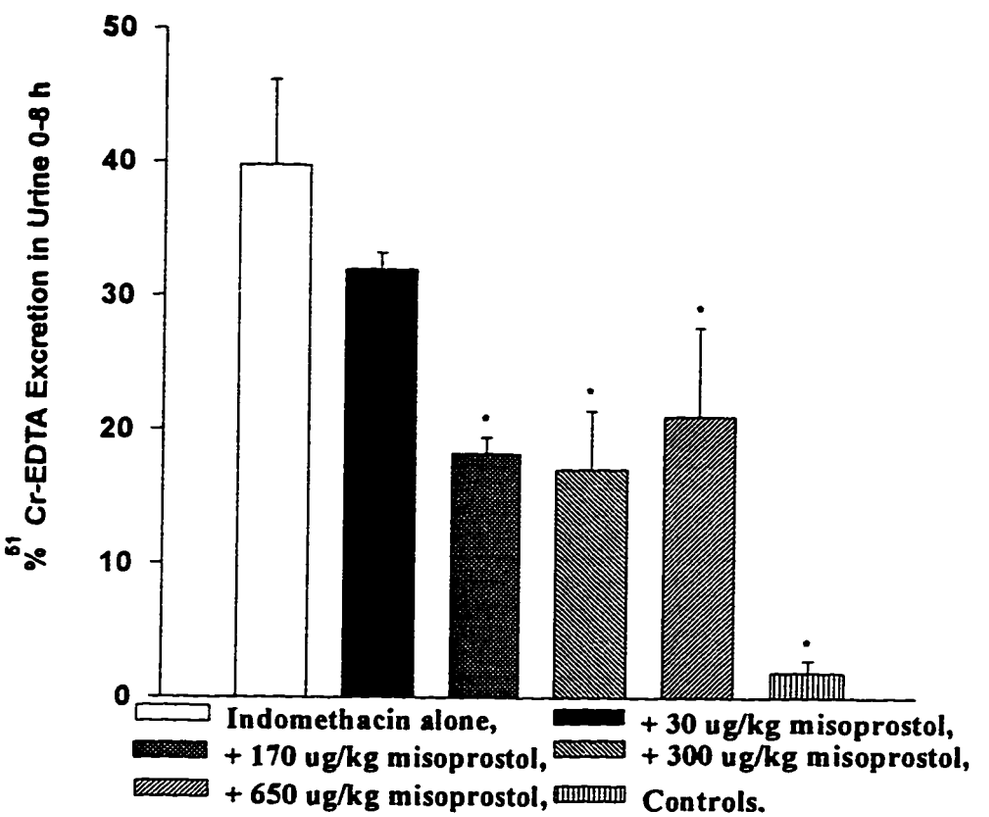


Figure 18. Effects of single doses of indomethacin 20 mg/kg with concomitant misoprostol administration on ^{51}Cr-EDTA urinary excretion (n = 6-10 for each group, mean ± s.e.m.). * Denotes significant difference from indomethacin alone. All treatments are significantly different from controls (ANOVA, $p<0.05$).

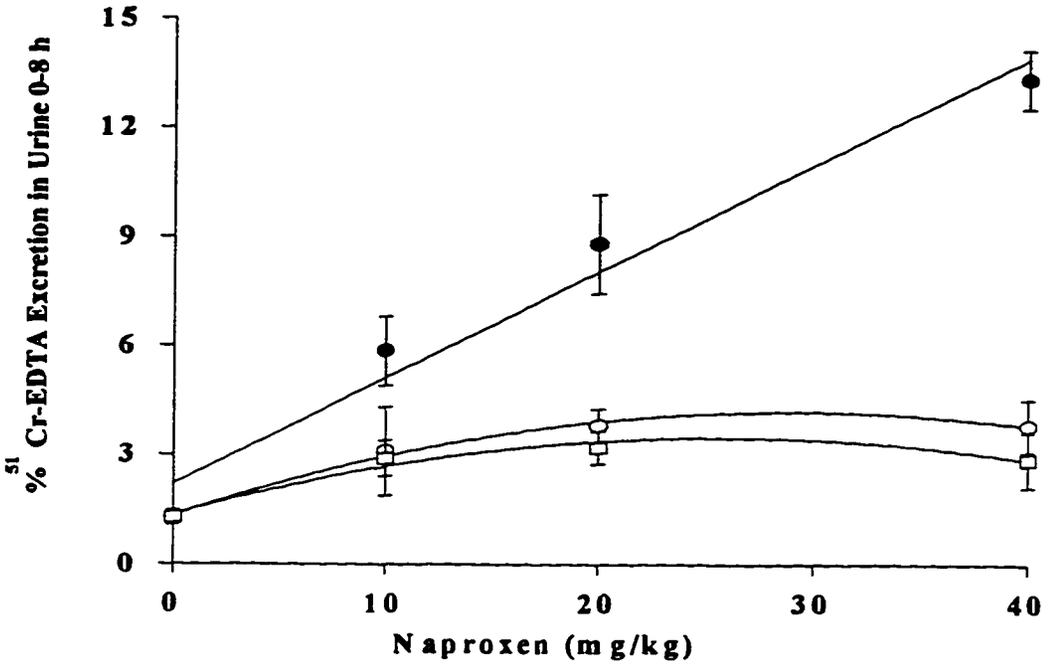


Figure 19. Effects of single oral doses of naproxen with concomitant misoprostol administration on ^{51}Cr-EDTA urinary excretion (n = 4-6 for each group mean ± s.e.m.). ● Naproxen, O Naproxen and 30 μg/kg misoprostol, □ Naproxen and 170 μg/kg, misoprostol. All treatments are significantly different from naproxen alone (ANOVA, $p<0.05$).

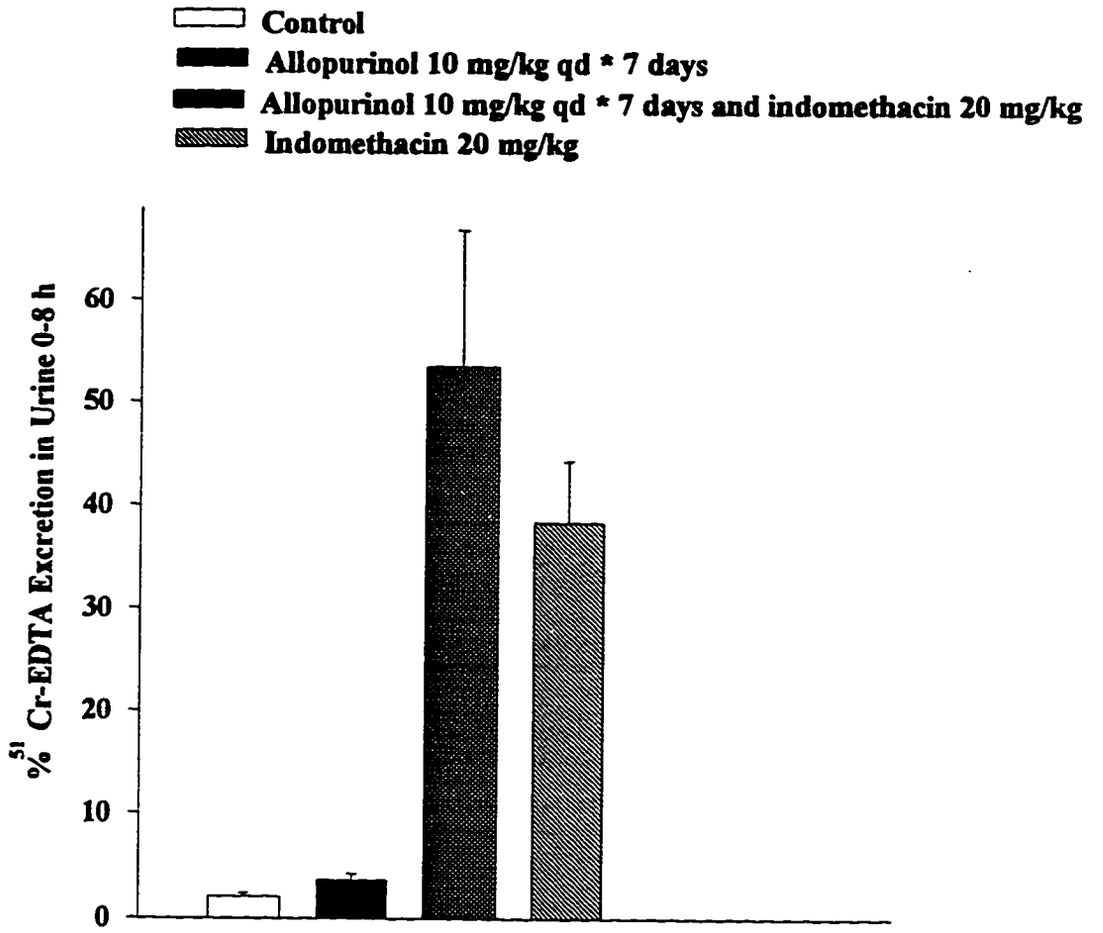


Figure 20. Effects of chronic administration of allopurinol on indomethacin-induced intestinal permeability (n = 4 for each group mean ± s.e.m.). Indomethacin treatments are significantly different from controls.

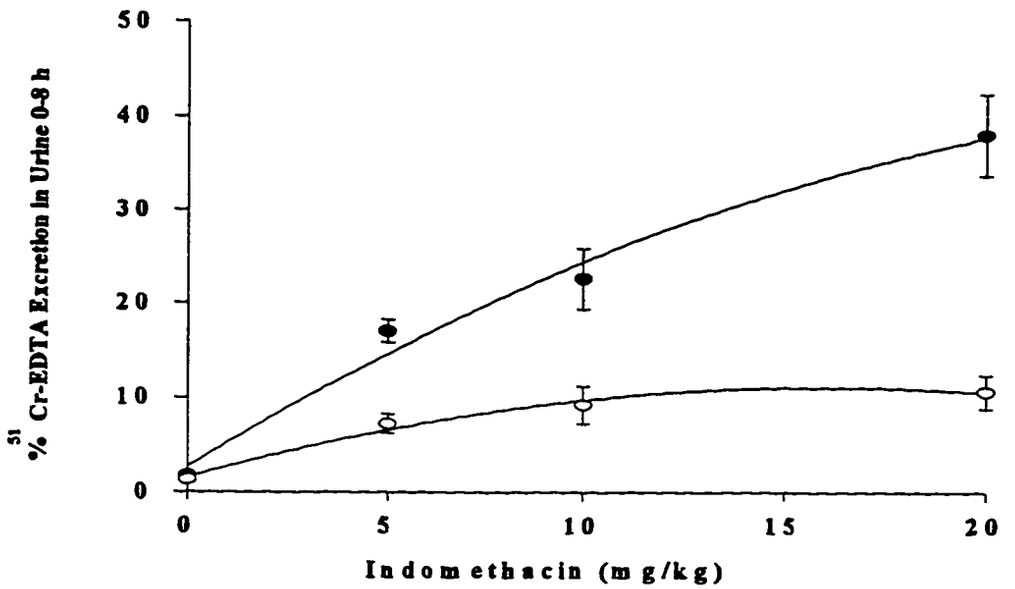


Figure 21. Effects of single oral doses of indomethacin with and without concomitant glucose / citrate administration on ^{51}Cr-EDTA urinary excretion (n = 4-6 for each group mean ± s.e.m.). All treatments are significantly different from controls. All treatments are significantly different from drug alone (ANOVA, $p<0.05$). ● Indomethacin, O Indomethacin and glucose / citrate (1:15:15).

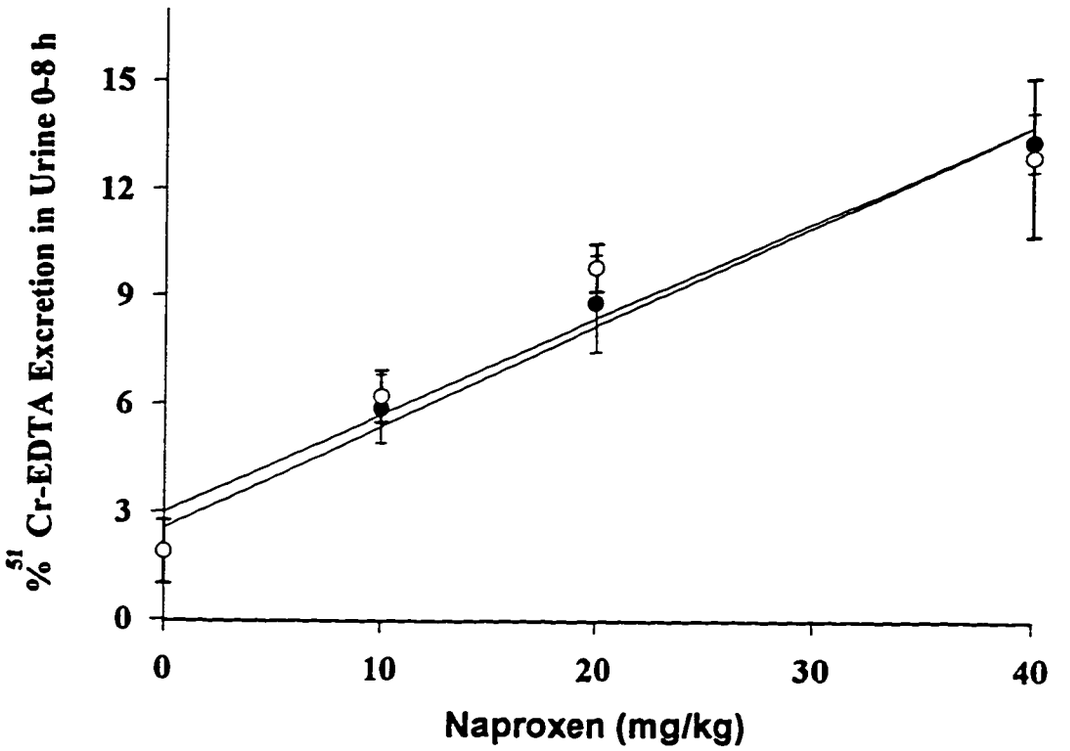


Figure 22. Effects of single doses of naproxen given concomitantly with glucose/citrate (n = 4-6 for each group; mean ± s.e.m.). All treatments are significantly different from controls (ANOVA, $p<0.05$) ● Naproxen, O Naproxen and glucose / citrate (1:15:15).

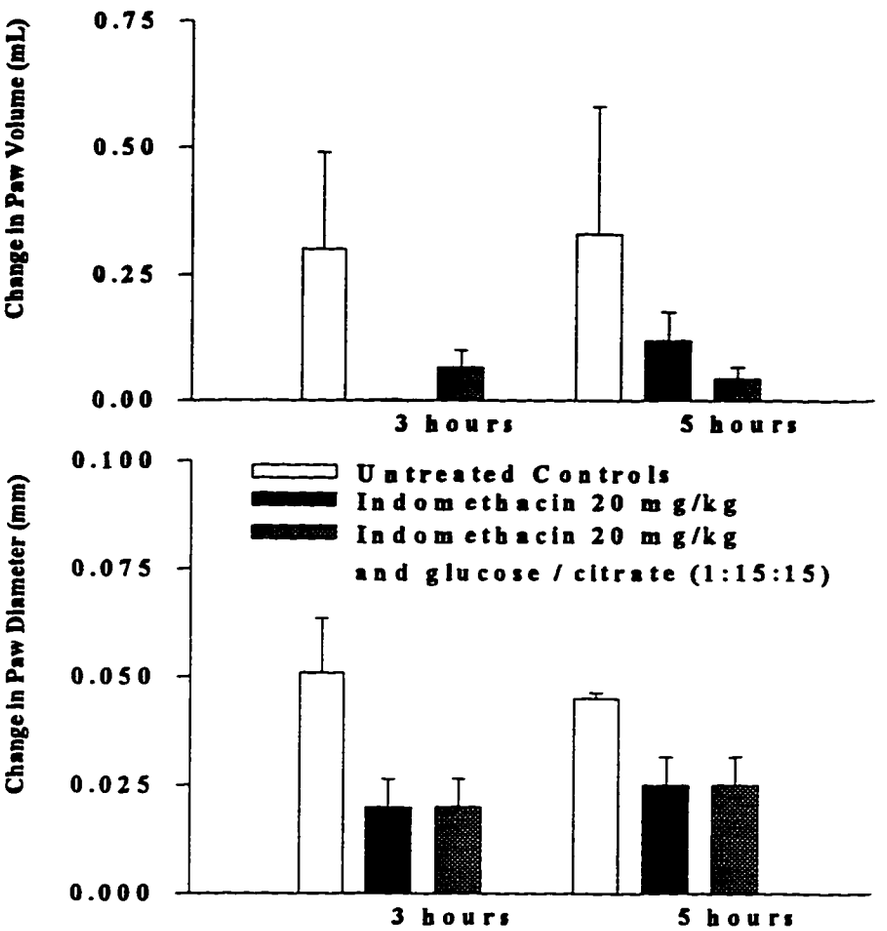


Figure 23. Effects of a single oral 20 mg/kg dose of indomethacin with or without glucose/citrate (1:15:15) on change in rat paw volume (upper panel).

Figure 24. Effects of a single oral 20 mg/kg dose of indomethacin with or without glucose/citrate (1:15:15) on change in paw diameter (lower panel).
(n = 4 for each group; mean ± s.e.m.). No significant differences between treatments (ANOVA, $p<0.05$). All treatments are significantly different from controls (ANOVA, $p<0.05$).

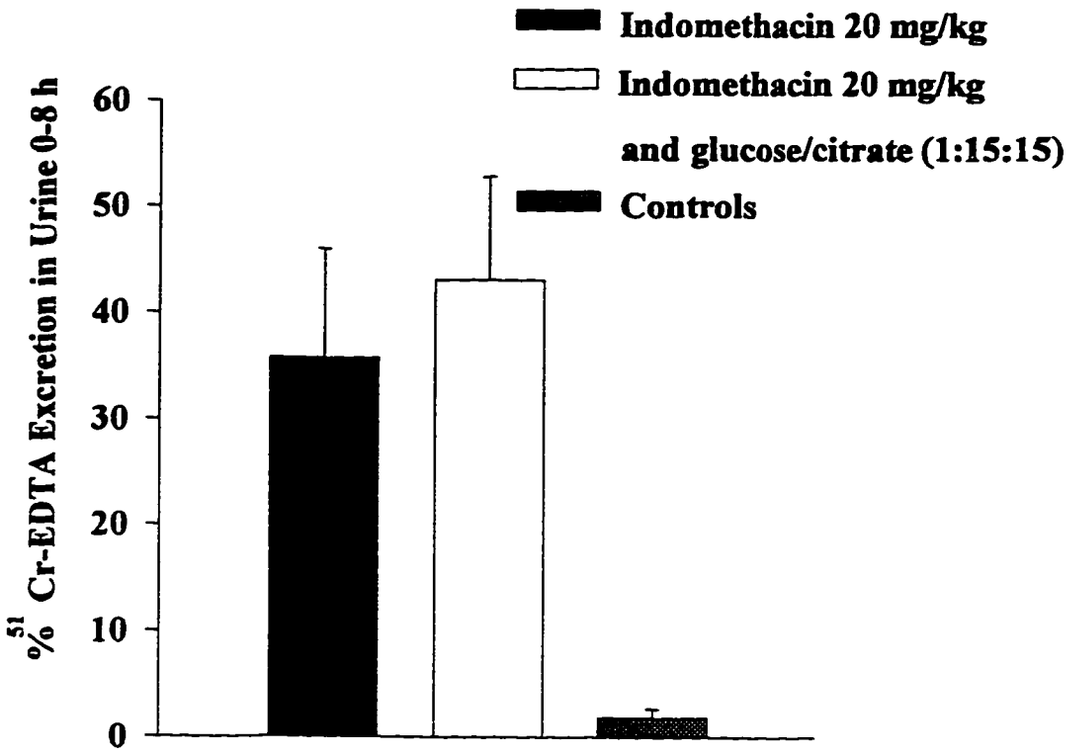


Figure 25. Effects of a single subcutaneous dose of 20 mg/kg indomethacin with and without concomitant oral glucose/citrate (1:15:15) on ^{51}Cr-EDTA urinary excretion (n = 4, for each group, mean ± s.e.m.). All treatments are significantly different from controls. No significant differences between treatments (ANOVA, $p<0.05$).

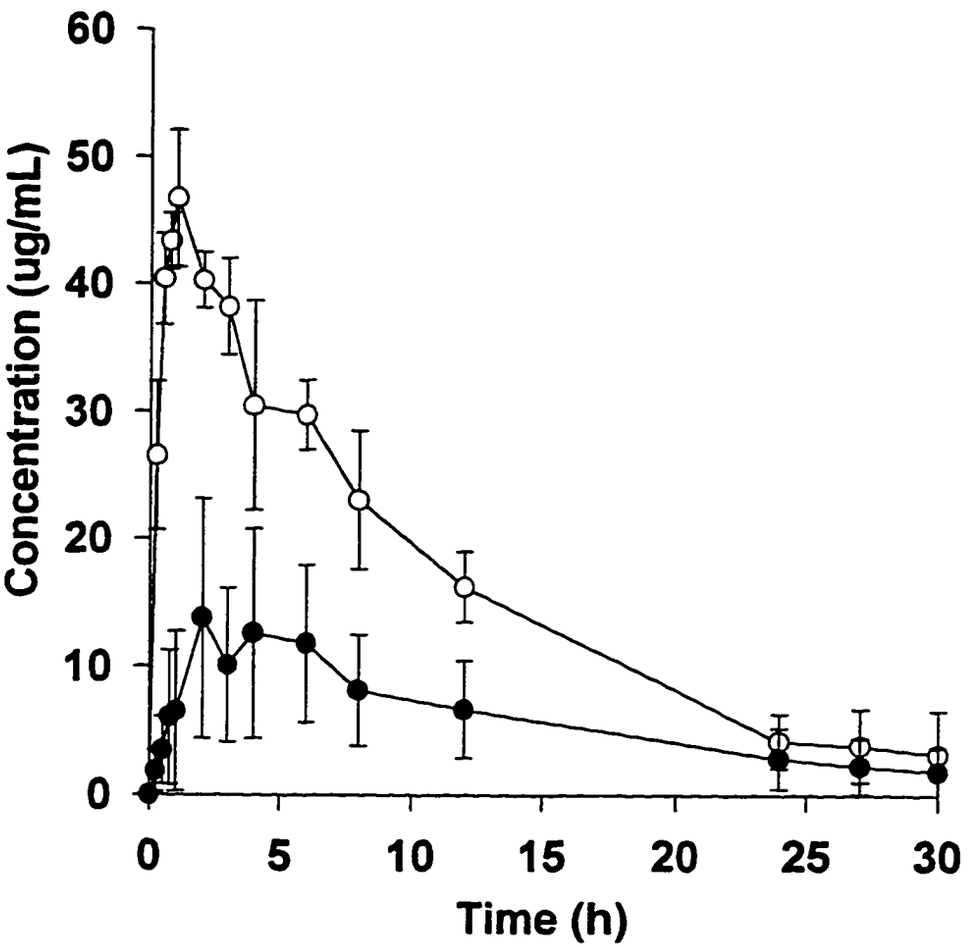


Figure 26. Mean plasma concentrations (± s.d., n = 5) versus time profiles for indomethacin in rats dosed with oral indomethacin 10 mg/kg (open circles) or 10 mg/kg (closed circles) indomethacin and glucose / citrate orally (1:15:15). Significant differences between treatments (ANOVA, $p<0.05$).

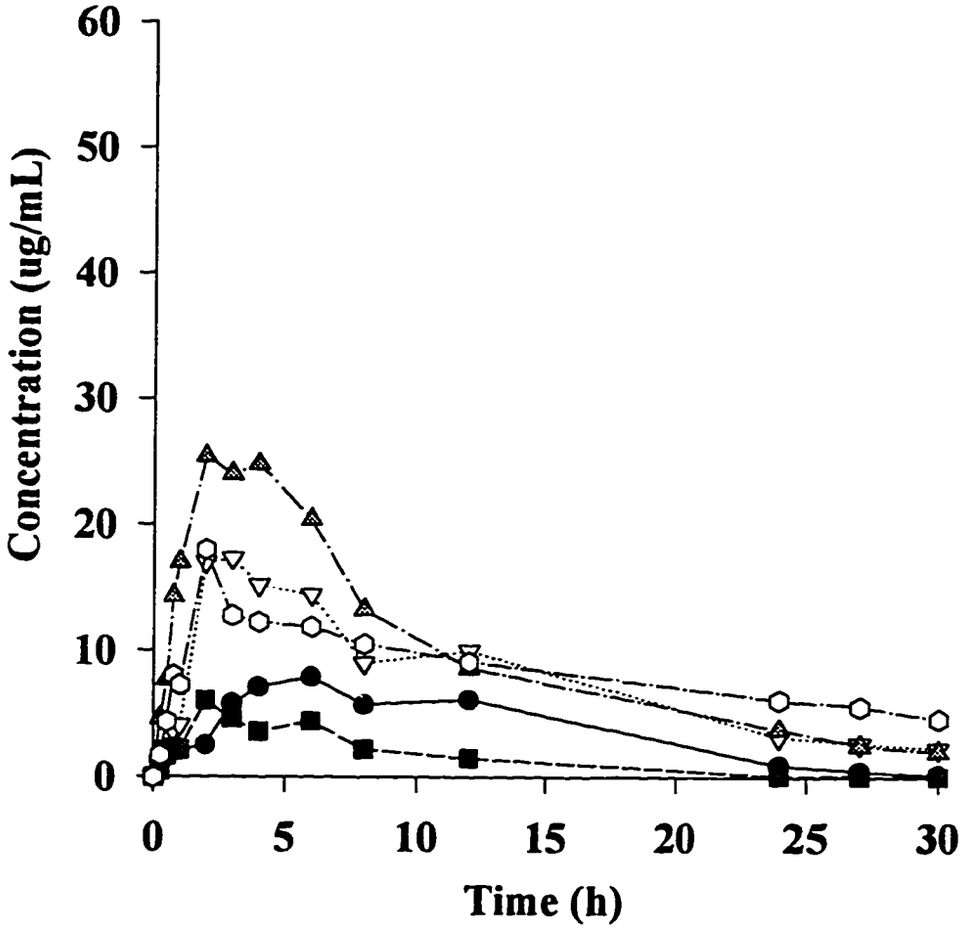


Figure 27. Individual plasma concentration versus time profiles for 5 rats dosed orally with indomethacin and glucose/ citrate (1:15:15).

4.6 Nitric Oxide Moieties and Protection of NSAID-Induced Intestinal Permeability

4.6.1 Results

Both indomethacin and flurbiprofen induced extensive intestinal permeability changes in the rat (Figs. 3-5, 10, 13-14, 18-21, 25, 28-29). Pretreatment with tempo significantly protected both indomethacin and flurbiprofen-induced intestinal permeability in all animals. Conversely, flurbiprofen-induced intestinal permeability observed in rats pre-treated with a single dose of metronidazole showed that it did not antagonize the induced permeability changes (Fig. 29). On the other hand, a two-dose treatment of metronidazole significantly reduced the intestinal permeability changes induced by both indomethacin and flurbiprofen.

All of the animals given indomethacin alone died 48-72 h post-dose, whereas, no rats died in the groups administered a single dose of tempo or two doses of metronidazole prior to indomethacin administration (Fig. 30). Post-mortem findings of all the animals given indomethacin alone indicate the most significant findings were present in the GI tract. Distended intestines were present throughout the entire length with a watery-brown liquid present in the lumen. All animals were in poor condition and slightly to moderately dehydrated. The mid-jejunum had a fibrin pseudo membrane present on the mucosal surface in several animals. The mucosa in this area was completely necrotic and the intestinal wall was congested. There were annular areas of hemorrhage and discoloration of the intestinal wall in several locations distal to this point. The caecum had irregular areas of necrosis of the full thickness of the wall surrounded by a zone of hemorrhage. There were irregular areas of congestion of the intestinal wall which became frequent in the lower jejunum and ileum. Several rats had acquired multiple 1 to 2 mm in diameter pale nodules on the serosal surfaces of the intestine. Clear, brown liquid in the thoracic cavity was present with an intense fibrinous peritonitis which was more severe in the anterior portion of the abdomen.

The inflammatory reaction involved the mesentery which would not be expected if the inflammation was originating from inside the gut and spreading across the gut wall suggesting these effects were predominantly systemic in origin.

Sections made of the GI tract confirm a very severe necrotizing enterocolitis. This was characterized by wide spread ulceration of the mucosa in the affected areas with full thickness necrosis down to and including the serosal surface of the appropriate affected area of gut. The serosal surfaces were inflammed in the immediate adjacent areas and bacterial invasion was occurring directly across into the abdominal cavity. Bacteria were growing on the serosal surfaces and in several areas there was intense neutrophil response with fibrin being exuded onto the surface and diffuse ingestion of bacteria by inflammatory cells. The serosal nodules seen on gross morphology were areas of intense inflammation associated with bacterial implantation extending onto adjacent serosal surfaces. A diagnosis of peritonitis and acute bacterial enteritis was made.

4.6.2 Discussion

Fatal intestinal perforations in all animals within days of indomethacin administration has previously been reported (Kent *et al.*, 1969). The precise mechanisms underlying this sequence of events is unknown but there is substantial data to implicate both reduced synthesis of mucosal prostaglandins and the presence of intestinal bacteria in the pathogenesis of the lesions (Robert and Asano, 1977; Melarange *et al.*, 1992). This damage is characterized sequentially by inflammation, ulceration, perforation, peritonitis and eventually death. These findings in the rat are consistent with several clinical reports of NSAID-induced intestinal damage and mortality in humans (Table 3,7,8).

Although, prostaglandins have been suggested to be involved in maintaining the integrity of the GI tract, it has been reported that no relationship between the degree of mucosal damage and magnitude of inhibition of prostaglandin synthesis

exists in rats (Whittle, 1981). It has also been previously suggested that unlike the damage in the stomach, intestinal injury induced by NSAIDs may not be related to suppression of prostaglandin synthesis in humans and rats (Whittle, 1981; Del Soldato *et al.*, 1985; Davies *et al.*, 1993; Wallace, 1994). Additionally, it has been suggested that inhibition of prostaglandin synthetase may not be the sole mechanism by which NSAIDs exert their pharmacological and toxicological effects (Abramson and Weissmann, 1991) (Chapter 1.1.1.2). Uncoupling of mitochondrial oxidative phosphorylation also appears to be an early event in the pathogenesis of NSAID-induced GI damage. Whereas, indomethacin and flurbiprofen uncoupled rat mitochondria, the nitric oxide NSAID derivative nitroflurbiprofen eliminates uncoupling properties of the NSAID (Mahmud et al, 1995). Interestingly, misoprostol does not prevent the uncoupling action of NSAIDs, demonstrating that it is not a prostaglandin-dependent process (Rafi et al, 1995). Our initial studies in the rat have also suggested that indomethacin-induced intestinal permeability may be only partially mediated by reduced mucosal prostaglandins (Figs. 5,18). Furthermore, there is a report that misoprostol did not reduce indomethacin-induced permeability in humans while metronidazole significantly diminished indomethacin-induced permeability albeit two doses 12 h apart (Davies et al, 1993). These results also parallel clinical studies in which NSAID-induced intestinal inflammation has also been shown to be reduced after administration of metronidazole (Bjarnason et al, 1992b).

The mechanism responsible for reduced NSAID-induced intestinal permeability through protective use of metronidazole and tempo is not yet clear. Metronidazole has been shown to be a free radical scavenger reducing reactive oxygen species generated by neutrophils at sites of inflammation (Akamatsu et al, 1991). Metronidazole does not reduce reactive oxygen species generated by the xanthine-oxidase system (Akamatsu et al, 1991). Tempo is a stable nitroxide free radical which protects cells by selectively detoxifying other free radicals (Rachmilewitz et al, 1994). The beneficial effect of

metronidazole may also imply a role for anaerobic intestinal bacteria in the initial permeability increase as permeability changes did not appear to be modulated with a single dose of metronidazole. A recent report examining NSAID-induced ulcers of the small bowel in the rat has independently demonstrated protective prophylaxis of indomethacin-induced ulceration with a 100 mg/kg tid dose of metronidazole (Collins *et al.*, 1995). There is also some evidence for a contribution of luminal bacteria to the development of intestinal injury (Robert and Asano, 1977; Melarange *et al.*, 1992). Additionally, nitric oxide moieties have been shown to be cytotoxic for invasive micro-organisms (Moncada *et al.*, 1991). Mechanisms other than antibacterial are, of course, not excluded.

In the critical events of the pathogenesis of NSAID-induced GI mucosal injury the adherence of leukocytes to the endothelium of postcapillary venules occurs resulting in partial occlusion of GI microvessels leading to ischemic/hypoxic cell injury and free radical mediated damage to the endothelium and epithelium (Wallace, 1993). The possibility of inhibition of leukocyte adherence attributable to a direct inhibitory effect of the nitro groups exists (Kubes et al, 1991). Neutrophil migration has also been shown to increase permeability of epithelial cells (Milks et al, 1986). NO (or endothelium derived relaxation factor), is a potent inhibitor of leukocyte activation and adherence, and has also been shown to play an important role in modulation of permeability across the epithelial barrier (May et al, 1991; Kubes, 1992).

As NSAIDs induce GI damage they create superoxide anions (Rainsford, 1987). Therefore, NSAIDs and other drugs that generate nitric oxide and metabolic nitroxide intermediates that are formed from one electron donations have previously been shown to reduce both the severity of gastric and intestinal injury in experimental models (Davies *et al.*, 1993; Reuter *et al.*, 1994; Rachmilewitz *et al.*, 1994; Wallace *et al.*, 1994). NO or NO donors also have been found to modulate prostaglandin synthesis in several cell types (Salvemini *et al.*, 1993). Thus, a cooperation between

the two major classes of cytoprotective mediators exists. The regulation of nitric-oxide synthase by NO-NSAIDs shows modulation of the expression of COX_2 (Wallace and Tigley, 1995). Therefore, nitric oxide may be an important endogenous modulator of the sequelae associated with inflammation of the GI tract.

Interestingly, it appears that a single dose of metronidazole was not effective in reducing intestinal permeability changes whereas, two doses of metronidazole significantly cytoprotected against intestinal damage (Fig. 29). This may suggest that higher doses of metronidazole are required or repeated therapy is necessary for effective intestinal protection. The number of deaths in the indomethacin-treated group versus the groups receiving indomethacin and tempo or metronidazole is a novel observation as compared to the study of Collins *et al.* (1995) who terminated their experiments before mortality occurred. The mortality and histological observations also suggest the important toxicological effects of these intestinal permeability changes, and suggest two effective protective agents which can prevent this from occurring.

The GI damage-induced by NSAIDs is the major limitation to their use as anti-inflammatory drugs. The mechanisms responsible for the deleterious effects of NSAIDs on the intestine are unknown, but the central dogma still remains their ability to inhibit prostaglandin synthesis. Attempts to develop NSAIDs with reduced GI side-effects have been numerous. In the present study, a novel nitroxide containing free radical compound (tempo) and metronidazole have been tested, which may represent additional strategies to reduce the intestinal damaging properties of NSAIDs.

These results suggest that the intestinal permeability which is a prerequisite to enteropathy and death may be modulated by pre-administration of metronidazole or tempo. Clearly, further characterization of the mechanism of action of these compounds as well as the minimum effective dose and frequency of dosing to achieve GI protection is warranted. In addition, these compounds may be valuable tools for

gaining a better understanding of the pathogenesis of GI injury induced by existing NSAIDs. Delineating the precise sequence of events in the pathogenesis of NSAID-induced GI damage will also require carefully-designed molecular biological, immunological, and biochemical studies.

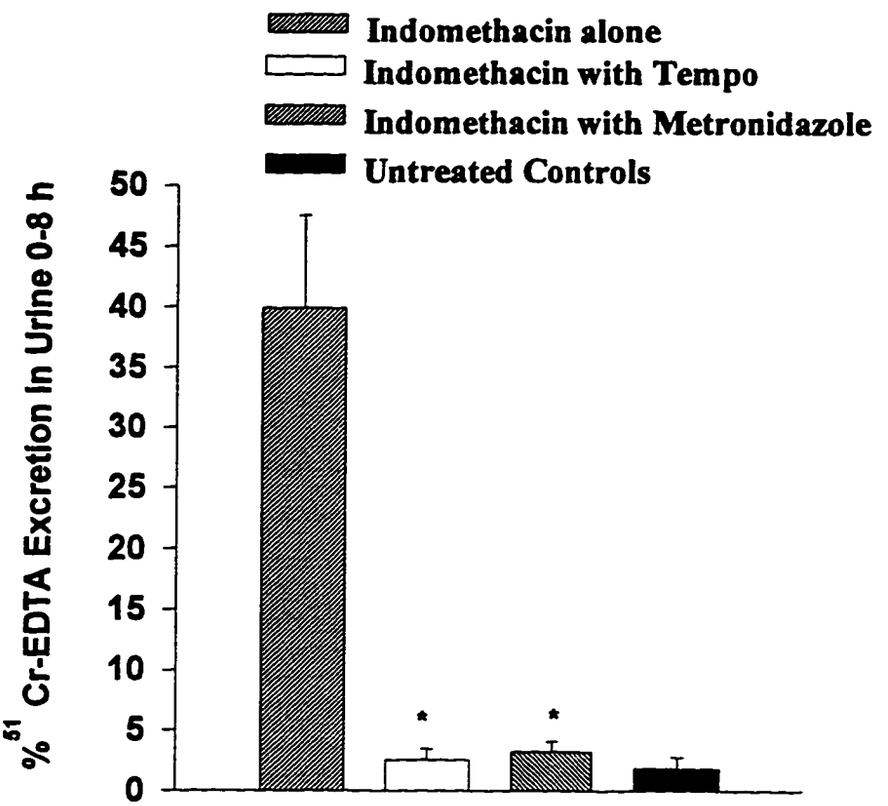


Figure 28. Mean (± s.e.m., n = 10) intestinal permeability measured as % urinary excretion of ^{51}Cr-EDTA following oral administration of a single oral 20 mg/kg dose of indomethacin in rats dosed with metronidazole in two 50 mg/kg doses 12 and 1 h prior to indomethacin or a single dose of 100 mg/kg tempo 1 h prior to indomethacin. * Denotes significantly different from drug alone. All animals treated with indomethacin alone but none from the other groups died of peritonitis subsequent to enteritis within 48-96 h.

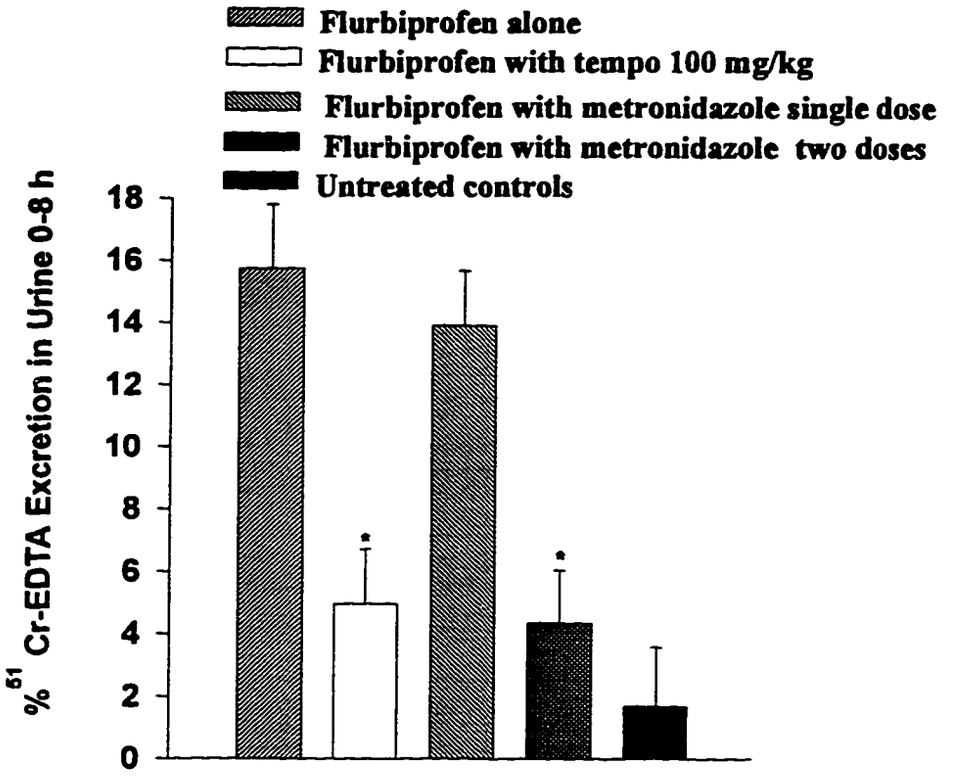


Figure 29. Mean (± s.e.m. n = 6) intestinal permeability measured as % urinary excretion of ^{51}Cr-EDTA following oral administration of a single oral dose of flurbiprofen 10 mg/kg in rats pre-dosed 12 h and 1 h before metronidazole 50 mg/kg and 100 mg/kg tempo prior to flurbiprofen. * Denotes significantly different from drug alone.

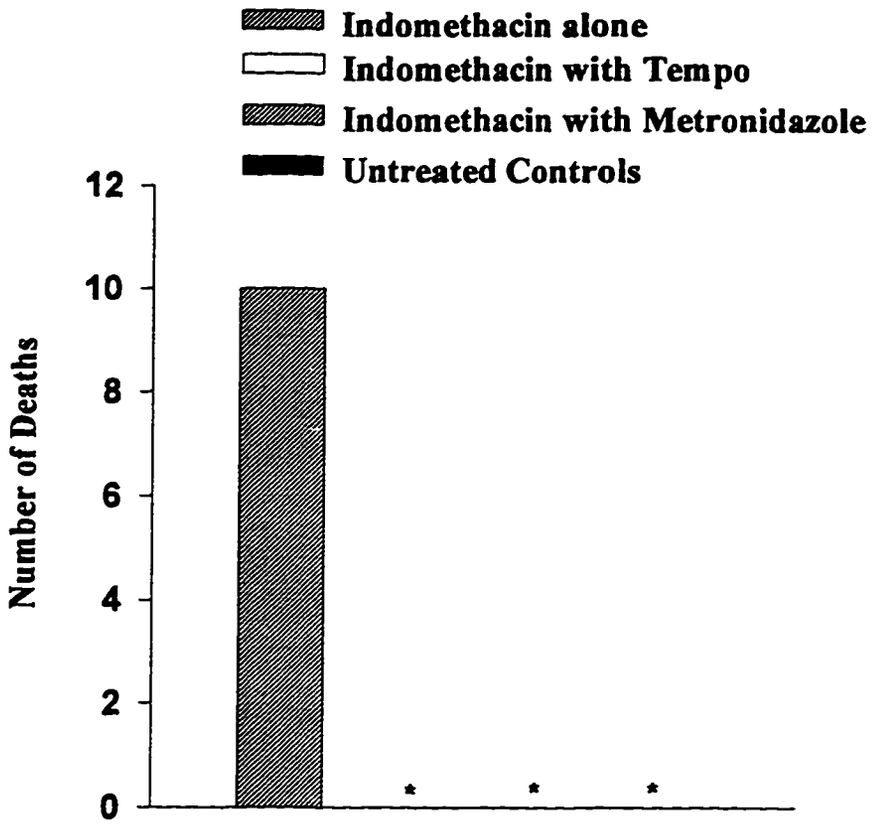


Figure 30. Number of deaths following oral administration of a single oral 20 mg/kg dose of indomethacin to 10 rats dosed with metronidazole in two doses 12 and 1 h prior to indomethacin and 100 mg/kg temp 1 h prior to indomethacin. * Denotes no deaths.

4.7 Toxicokinetics of Ibuprofen-Induced GI Permeability

4.7.1 Results

When rats were dosed with ibuprofen, a statistically significant increase in urinary ^{51}Cr-EDTA excretion occurred for all the dosages and routes of administration tested (Figs. 31 and 32). After single i.p., s.c., and p.o. doses of racemic ibuprofen (Fig. 31), there was a rapid, and significant permeability increase at the first sampling time (1 h). There was no significant difference between 1 and 3 h post-dose, however, the variability at 3 h was less than at 1 h. Therefore, the permeability assessed 3 h post-dose was used as the marker of maximal intestinal permeation of ^{51}Cr-EDTA. Permeability returned to baseline values between 48 to 91 h post-dose. The time-course of the pharmacological effect appeared to be equivalent by all routes of administration (Fig. 31). The intestinal permeability increased with dose until it reached a plateau at a dose of 50 mg/kg racemic ibuprofen irrespective of route of administration (Fig. 32). The dose-effect relationship could be described using the E_{max} model. The E_{max} values expressed as % ^{51}Cr-EDTA dose excreted in urine from 0-8 h were determined to be 16.69±2.02, 17.81±6.62, 14.51± 0.9 after i.p., s.c., and p.o. routes of administration, respectively. The ED_{50} values expressed as mg/kg were determined to be 55.7±41.7, 73.6±44.7, 35.7±5.5 after i.p., s.c., and p.o. routes of administration, respectively. No significant route-dependency was found with respect to either E_{max} or ED_{50}. Following p.o. and i.p. dosing of 100 mg/kg ibuprofen there was a rapid and significant increase in gastroduodenal permeability 30 minutes post-dose as assessed by urinary excretion of sucrose (Fig. 33).

Intraperitoneal serum concentrations of R and S-ibuprofen at 3 h post-dose were stereoselective S>R (Fig. 34). The relationship between 3 h plasma S-ibuprofen intraperitoneal plasma concentrations and ^{51}Cr-EDTA dose excretion in urine 0-8 h could also be described using the E_{max} model with EC_{50} and E_{max} values 7.87±5.33 and 13.06±2.45, respectively (Fig. 35).

Microbiological examination of intestinal contents revealed that the antibiotic treatment of 500 mg/kg erthromycin / neomycin twice daily for three days resulted in eradication of intestinal micro flora. There was no significant microbial colony formation on media plates from all antibiotic pre-treated rat intestinal segments compared to normal healthy intestinal segments which showed significant and appreciable microbial colony formation. The treatment, however, did not significantly affect ibuprofen-induced intestinal permeability changes (Fig. 36). Further, antibiotic treatment by itself did not alter permeability from the baseline.

The analysis of gastrointestinal segments from rats dosed with intraperitoneal ibuprofen 0-100 mg/kg revealed S-ibuprofen concentrations greater than R-ibuprofen concentrations in all intestinal homogenates (Figs. 37-39). All the tissue homogenate concentrations appeared to increase with dose. The highest concentrations were found in the ileal homogenates (Fig. 39).

The relationship between 3 h plasma S-ibuprofen intraperitoneal tissue concentrations and ^{51}Cr-EDTA dose excretion in urine 0-8 h appeared to be sigmoidal for all the intestinal tissue homogenates (Figs. 40-42). The correlation between S-ibuprofen concentrations in plasma and tissue homogenates was jejunal > duodenal >

ileal (Figs. 33, 41-42). The route of administration did not appear to significantly effect the small intestinal distribution of ibuprofen (Figs. 43-45).

4.7.2 Discussion

The dose-dependent effect of ibuprofen on the ^{51}Cr-EDTA permeability (Fig. 32) is independent of the route of administration, which suggests that the systemically mediated effect is the main contribution to damage sustained on the intestine. There was great variability in ibuprofen tissue concentrations, however, no significant differences were evident between the routes of administration in terms of S-ibuprofen concentrations which is consistent with the route of administration-independent intestinal effect data (Figs. 43-45). Recently, Ford *et al.* (1995) also found a significant increase in ^{51}Cr-EDTA permeability after s.c. administration of S-ibuprofen in the rat. Interestingly, the wide variability in our data with ibuprofen is also consistent with that observed by Ford *et al.* (1995) for both intestinal permeability and intestinal ulceration. After absorption and metabolism, NSAID metabolites may reappear in the GI tract via biliary excretion. These biliary excreted metabolites, however, may be biotransformed back to the pharmacologically active parent drug likely by intestinal microflora (Kent *et al.*, 1969), and cause toxicity which may contribute to the overall intestinal damage of these drugs (Duggan *et al.*, 1975). For ibuprofen, however, the magnitude of this contribution is expected to be much less than the toxicity afflicted by systemic exposure of the intestine to the NSAID as the entire dose does not undergo biliary excretion and not the entire biliary excreted metabolite is biotransformed to ibuprofen. In addition, the plasma concentration-time course of ibuprofen doses not exhibit

secondary peaks which are usually attributed to enterohepatic recirculation (Jamali and Berry, 1990). Indeed, only 20-30 % of ibuprofen has been previously shown to be excreted in the bile mostly as inactive glucuronide conjugates (Dietzel *et al.*, 1990). The effect of bowel sterilization (Fig. 36) minimizes deconjugation of conjugated ibuprofen by intestinal microflora and interrupts enterohepatic recirculation of ibuprofen. Although there was a trend towards reducing intestinal permeability changes in antibiotic pre-treated rats compared to rats with normal intestinal flora, our results (Fig. 29) suggest insignificant contribution of biliary recycling to ibuprofen induced intestinal permeability changes. Our results, are consistent with that reported in the literature for ibuprofen-induced intestinal damage: Using the rat as an animal model, germ-free rats dosed with racemic ibuprofen still had measurable intestinal damage as compared with controls (Melerange *et al*, 1992). In contrast, with indomethacin Melerange *et al.* (1992) detected significantly less intestinal damage in germ-free rats as compared with normal rats which is consistent with our previous observations (Fig. 28). The effect of antibiotic treatment on ibuprofen as compared with indomethacin may also support the suggestion of Melerange *et al.* (1992) that there may be differences between NSAIDs with respect to the importance of intestinal bacteria and its role in NSAID-induced intestinal damage. In addition, similar to metronidazole (Chapter 4.6.2) there may also be other pharmacological mechanisms in addition to antibacterial such as prostaglandin suppression, inhibition of neutrophil migration, and free radical scavenging through which cytoprotective agents may reduce intestinal permeability.

The combination of erythromycin / neomycin would not be effective at eliminating anaerobic bacteria. The demonstrated protective actions of metronidazole (Figs 28-30) may suggest the pathogenic importance of anaerobic bacteria contributing to NSAID-induced distal intestinal damage.

It should also be recognized that erthromycin is a pro motility agent which could markedly alter GI transit times and thus have substantial effects on the final outcome of intestinal permeability measurements that are performed using a single probe. However, correlation between the urinary excretion of ^{51}Cr-EDTA in the 0-8 h and 0-24 h samples in this study did not change from that of controls. (Fig. 1). It should be recognized that administration of any protective agent which can effect pre- or post-mucosal factors involved in intestinal permeability changes should be accompanied with the use of an internal standard such as mannitol or rhamnose in addition to ^{51}Cr-EDTA.

In addition to the systemic intestinal effect, ibuprofen appears to exert local effects on the stomach and duodenum as measured by gastroduodenal permeability (Fig. 33). However, the i.p. dose also induced appreciable changes in gastroduodenal permeability which indicates that a major systemic component is also involved in NSAID-induced gastroduodenal permeability.

After a single dose, the effect of ibuprofen on intestinal integrity persists appreciably longer (48-96 h, Fig. 31) than its concentration in plasma ($t_{1/2}$, 2 h) (Dietzel et al, 1990). The time period required for permeability to return to baseline values may represent the duration of effect after a single dose, and/or the healing time

of the intestinal epithelial tight junction after the initial exposure to the drug. It has been postulated that NSAIDs, due to their lipid solubility, are able to diffuse through biological membranes and dissociate in the comparatively neutral intracellular environment of mucosal cells. NSAIDs may thereby be able to preferentially accumulate in mucosal epithelial cells by ion trapping mechanisms thus followed by possible disruption of the intestinal epithelium and cause GI side-effects (Glavin and Szabo, 1992). These data indicate measurable and significant concentrations of ibuprofen throughout the examined segments of the GI tract (Figs. 37-39,43-45). Indeed, a demonstrable relationship appears to exist between measurable S-ibuprofen plasma concentrations and permeability at 3 h post-dose (Fig. 35). It is plausible therefore, that the observed lingering effect may be caused by a small S-ibuprofen concentration below quantifiable limits that returns to the systemic circulation from a small and deep effect compartment. A comparison of the concentration effect relationship between plasma (Fig. 35) and intestinal tissues (Fig. 39-41), i.e. the site of action, however, indicates that NSAID intestinal tissue concentrations did not provide a better prediction of the effect than plasma concentrations. This may be due to the fact that plasma concentrations are quantitatively higher and hence are technically easier to assay using our HPLC method as compared with tissue homogenates.

There appears to be an evident sigmoidal relationship between the concentration of S-ibuprofen in intestinal epithelial cells and intestinal permeability (Fig. 40-42). Despite considerable concentrations of S-ibuprofen, appreciably lower concentrations of R-ibuprofen in the GI tissues were detected. This is expected

because concentrations of S-ibuprofen are considerably higher than of R-ibuprofen in plasma (Fig. 34). Stereoselectivity in the pharmacokinetics of ibuprofen is attributed to a weaker affinity to plasma protein binding of R-ibuprofen leading to its more rapid clearance as compared to S-ibuprofen (Knihiniicki *et al.*, 1990), and an extensive unidirectional chiral inversion of R to S-ibuprofen which also increases the clearance of R-ibuprofen (Jamali and Berry, 1990). In addition, ibuprofen undergoes concentration-dependent protein binding in the rat between 50-100 mg/kg leading to an increase in free fraction in plasma and increased clearance (Satterwhite and Boudinot, 1990; Shah and Jung, 1987). The saturation of ibuprofen binding does not appear to have any apparent consequence on intestinal permeability changes (Fig 32). There is no data on the possibility of stereoselectivity in the observed saturation of protein binding in the rat. However, in humans, Jamali *et al.* (1991) noticed that non-linearity in ibuprofen dose-concentration which resulted from saturation of protein binding was not stereoselective.

It is interesting that the S:R ratio in the ileum and jejunum is greater than in plasma after s.c. (100 mg/kg) and i.p.(100 mg/kg) administration and that among the two, the pharmacologically active enantiomer appears to be preferentially distributed. However, this does not appear to further increase intestinal permeability changes as the ceiling of intestinal permeability changes are attained at a dose of ~ 50 mg/kg (Fig. 35).

The GI distribution of ibuprofen following three routes of administration demonstrates appreciable S-ibuprofen levels were extracted from all intestinal

homogenates with the highest detectable S-ibuprofen levels in the ileum (Figs. 37-39, 43-45). This may suggest a tissue-dependent distribution.

In summary, increased intestinal permeability in male Sprague-Dawley rats induced by ibuprofen was both time and dose-dependent but not route-dependent. There appears to be a major systemic component involved in ibuprofen induced GI permeability changes and little or no contribution of biliary recycling. Further work should try and delineate the importance of the epithelial concentrations of various NSAIDs in the GI tract and its relationship to NSAID-induced GI damage. Future studies should also examine the changes in pharmacological mediators such as cyclooxygenase and nitric oxide synthase isozymes and their relationship to epithelial NSAID concentrations.

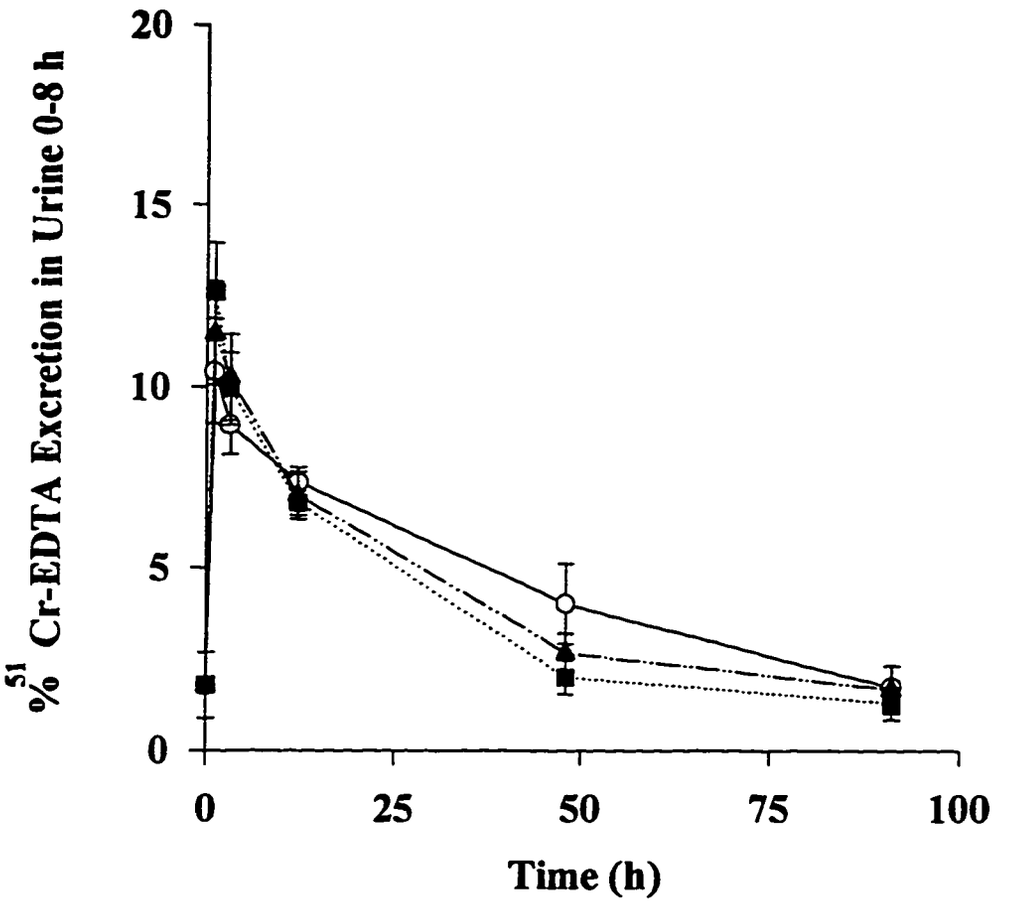


Figure 31. Time-course of racemic ibuprofen on ^{51}Cr-EDTA urinary excretion (n = 6 for each group; mean ± s.e.m.; s.c. ■, p.o. ▲, i.p. O). No significant differences between treatment groups (ANOVA, $p<0.05$).

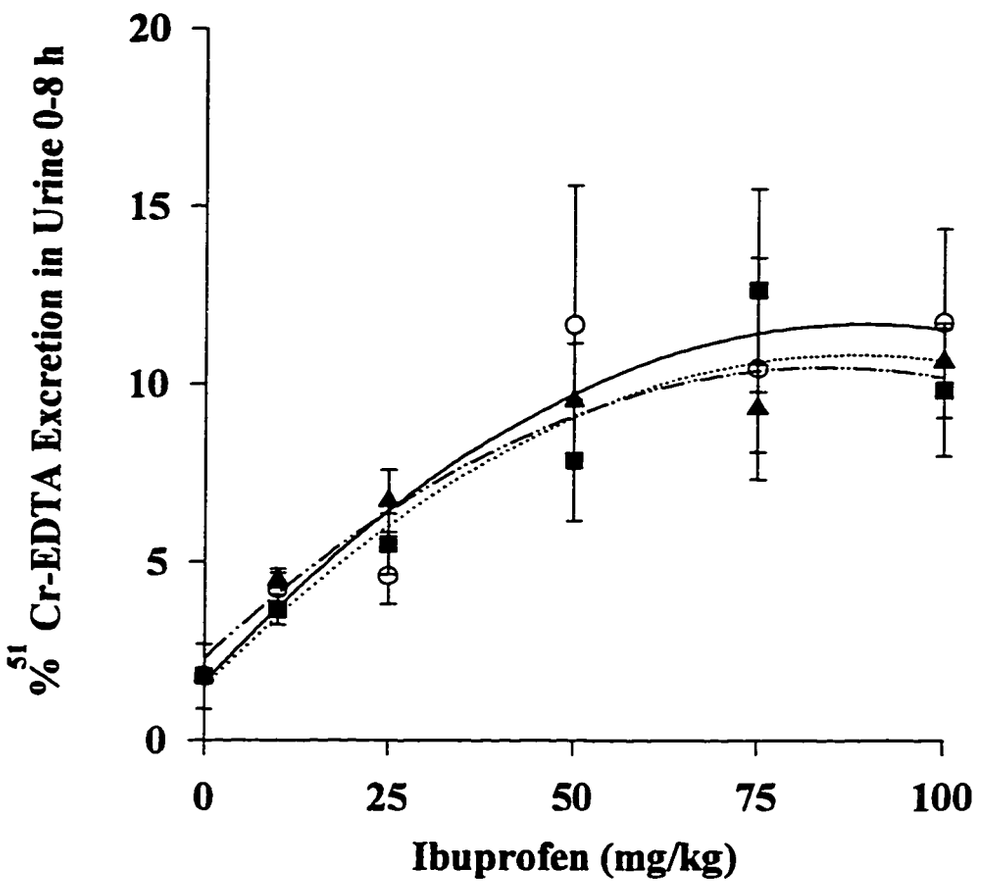


Figure 32. Effects of single racemic ibuprofen on ^{51}Cr-EDTA urinary excretion (n = 5 for each group except for controls n = 21; mean ± s.e.m.; s.c. ■, p.o. ▲, i.p. O). No significant differences between treatment groups (ANOVA, $p<0.05$). All treatments are significantly different from controls (ANOVA, $p<0.05$).

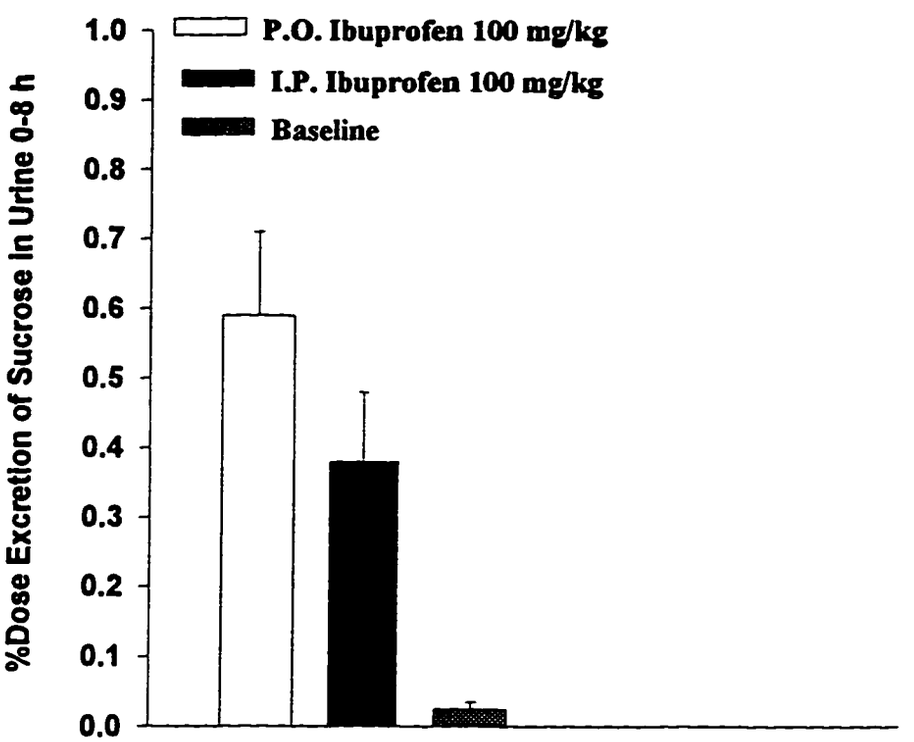


Figure 33. Effect of single doses of racemic ibuprofen on sucrose urinary excretion (n = 4 for each group; mean ± s.e.m.). No significant differences between treatments (ANOVA, $p<0.05$). Both treatments are significantly different from controls (ANOVA, $p<0.05$).

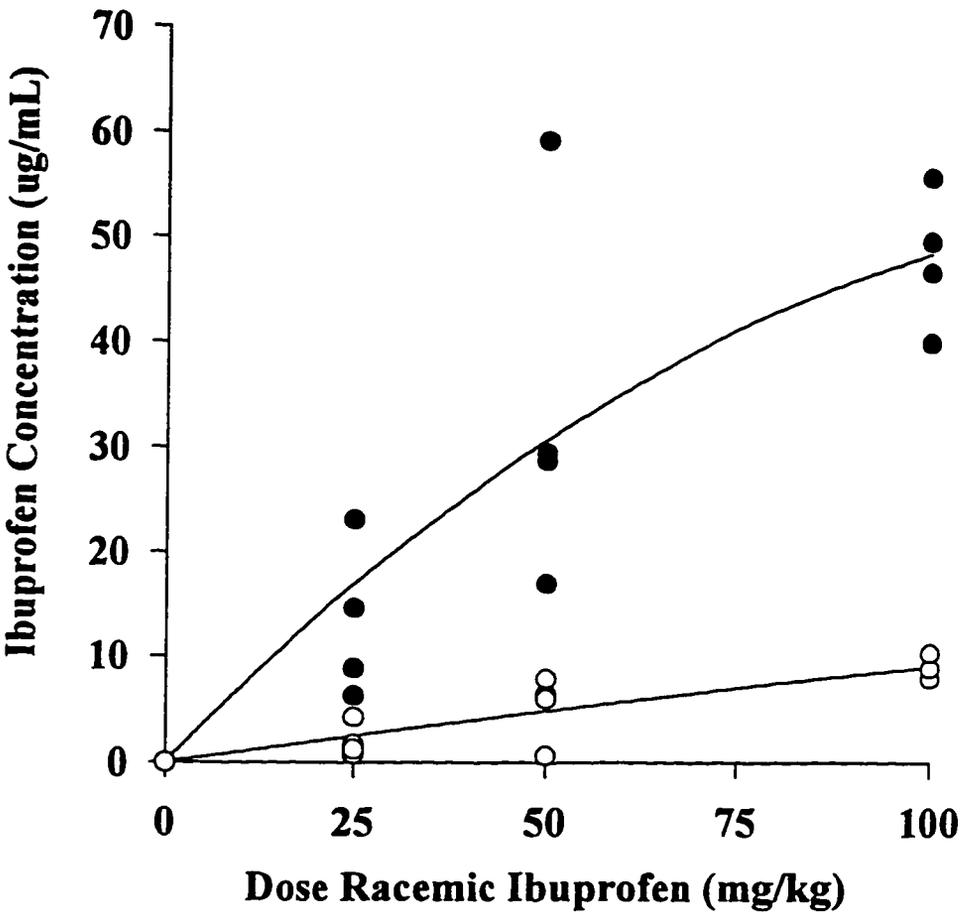


Figure 34. Intraperitoneal dose versus plasma ibuprofen concentration relationship for S- ibuprofen (closed circles) and R-ibuprofen (open circles) 3 h post-racemic ibuprofen dose (n = 4 for each dose).

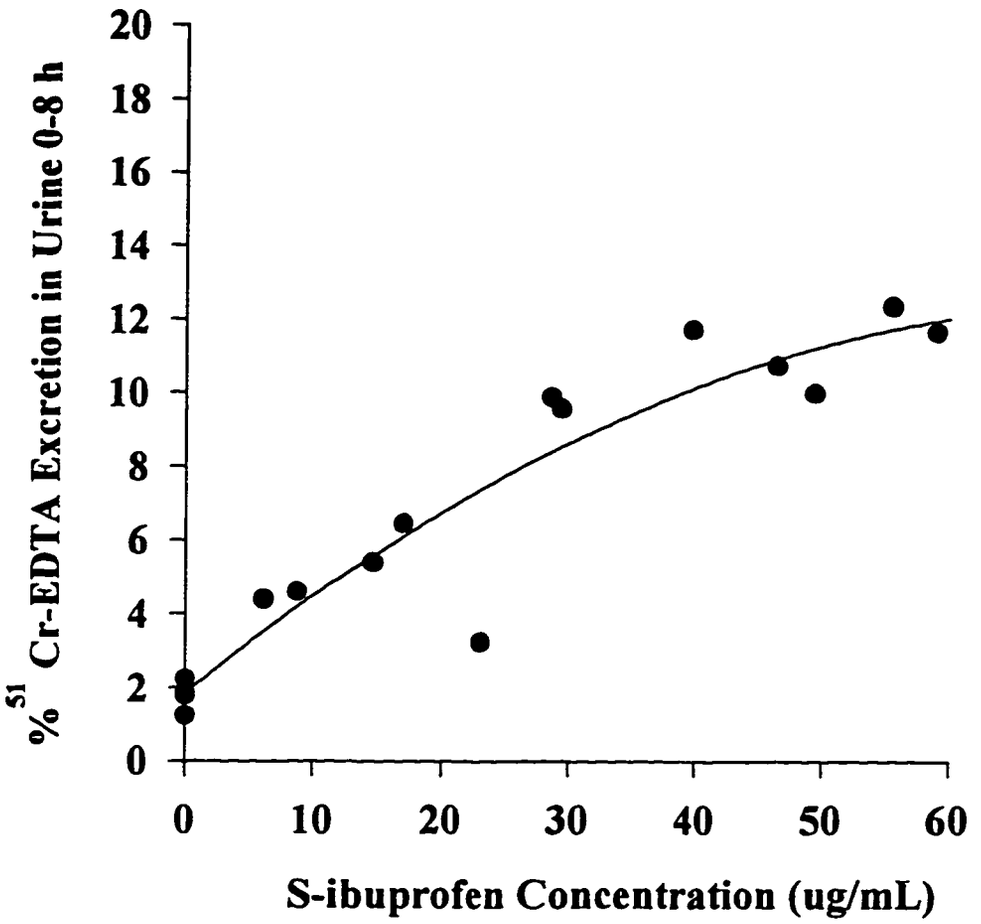


Figure 35. Concentration-effect relationship between S-ibuprofen at 3 h post i.p. dose and % ^{51}Cr-EDTA excretion in urine 0-8 h (n = 4; mean ± s.e.m.).

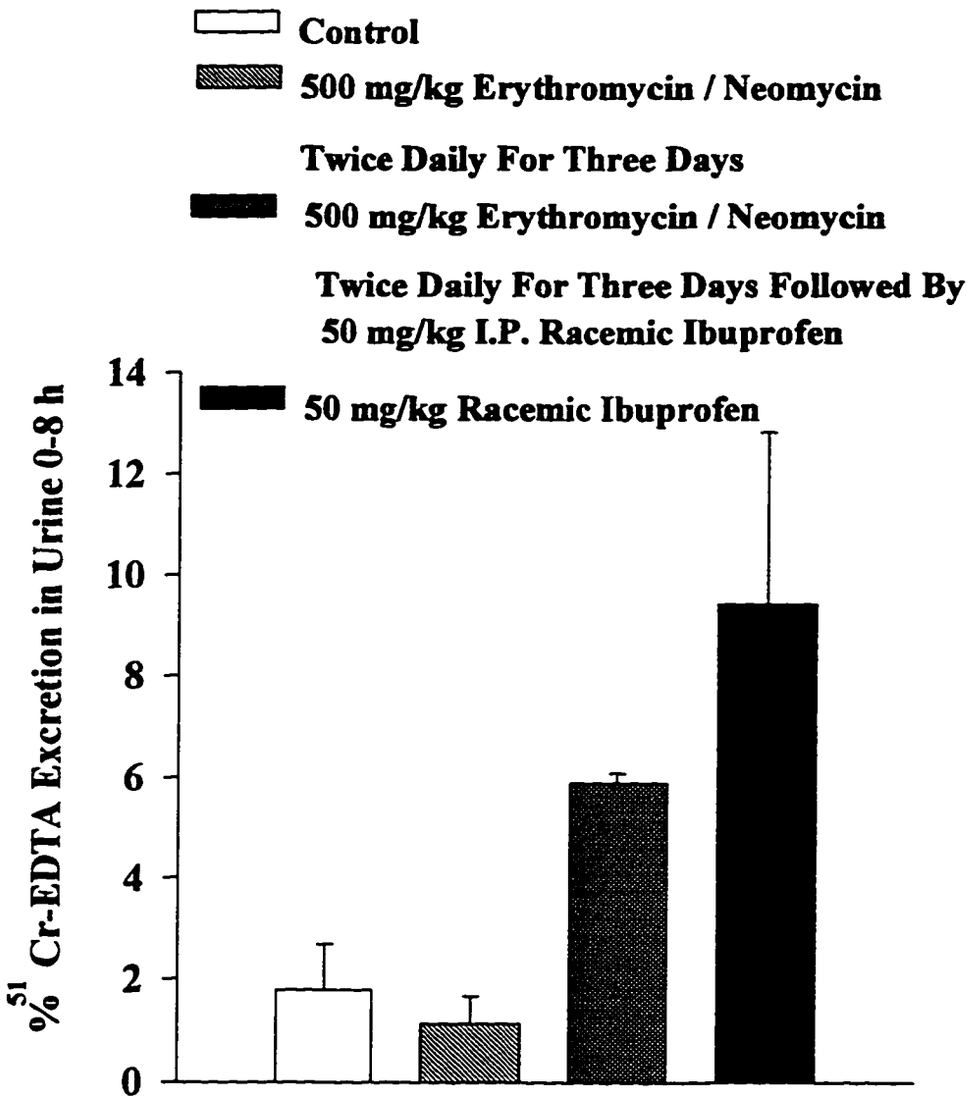


Figure 36. Effects of a single i.p. dose of racemic ibuprofen on ^{51}Cr-EDTA urinary excretion in antibiotic treated and control rats (n = 4 for each group; mean ± s.e.m.). No significant differences between untreated control and treated antibiotic control groups (ANOVA, $p<0.05$). Ibuprofen treatments are significantly different from control groups but not from each other (ANOVA, $p<0.05$).

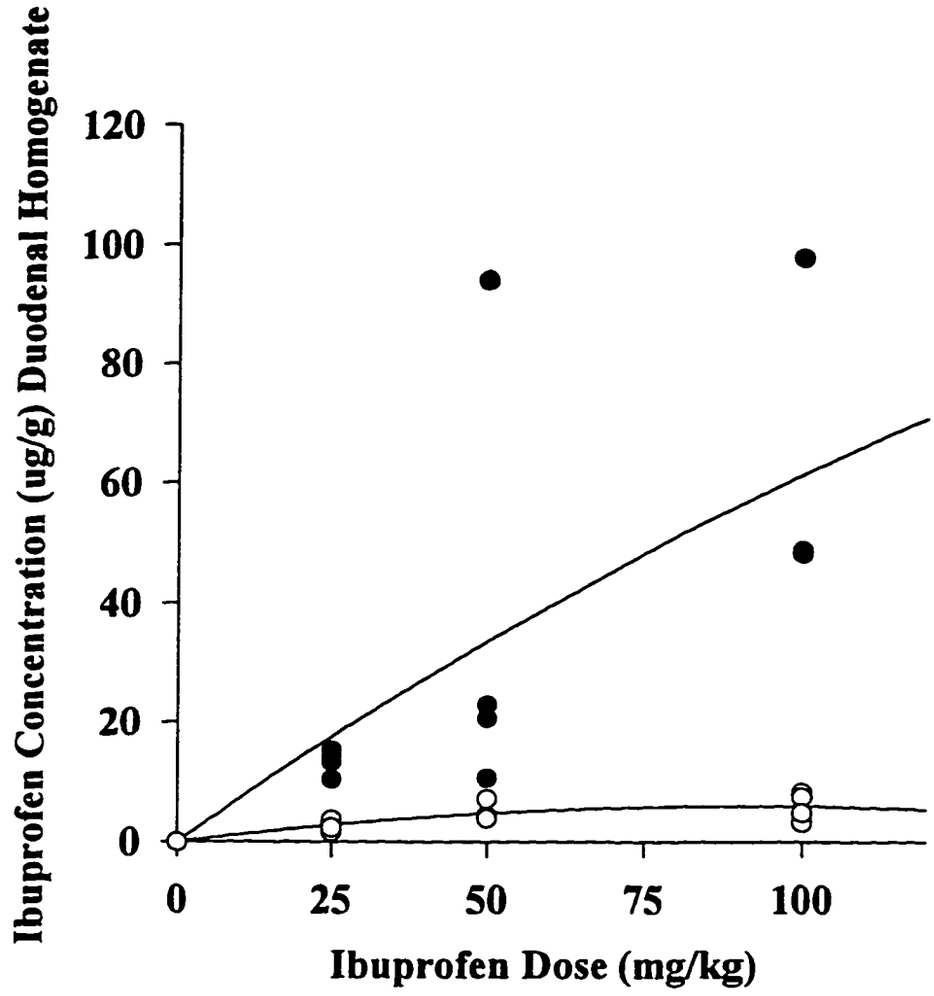


Figure 37. Effects of single i.p. doses of racemic ibuprofen 0-100 mg/kg on S-ibuprofen (closed circles) and R-ibuprofen (open circles) 3 h post-dose. Each point represents one rat duodenal homogenate.

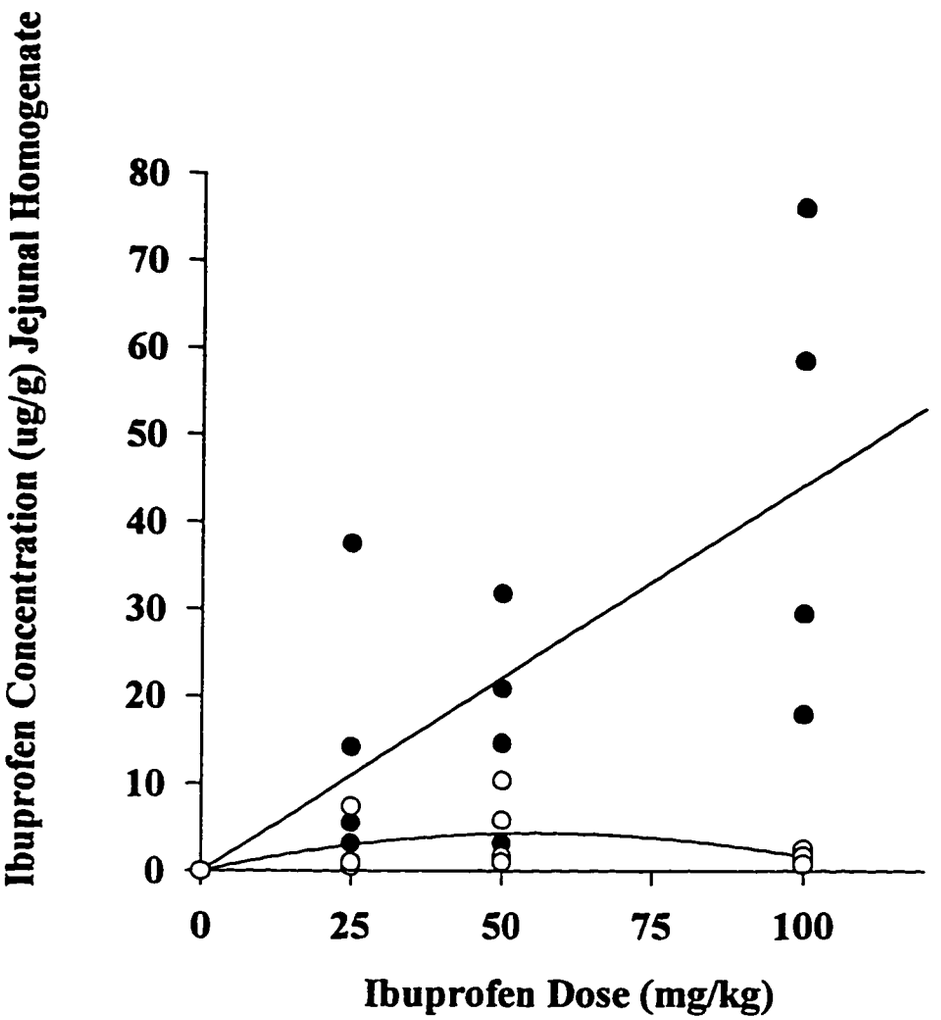


Figure 38. Effects of single i.p. doses of racemic ibuprofen 0-100 mg/kg on S-ibuprofen (closed circles) and R-ibuprofen (open circles) 3 h post-dose. Each point represents one rat jejunal homogenate.

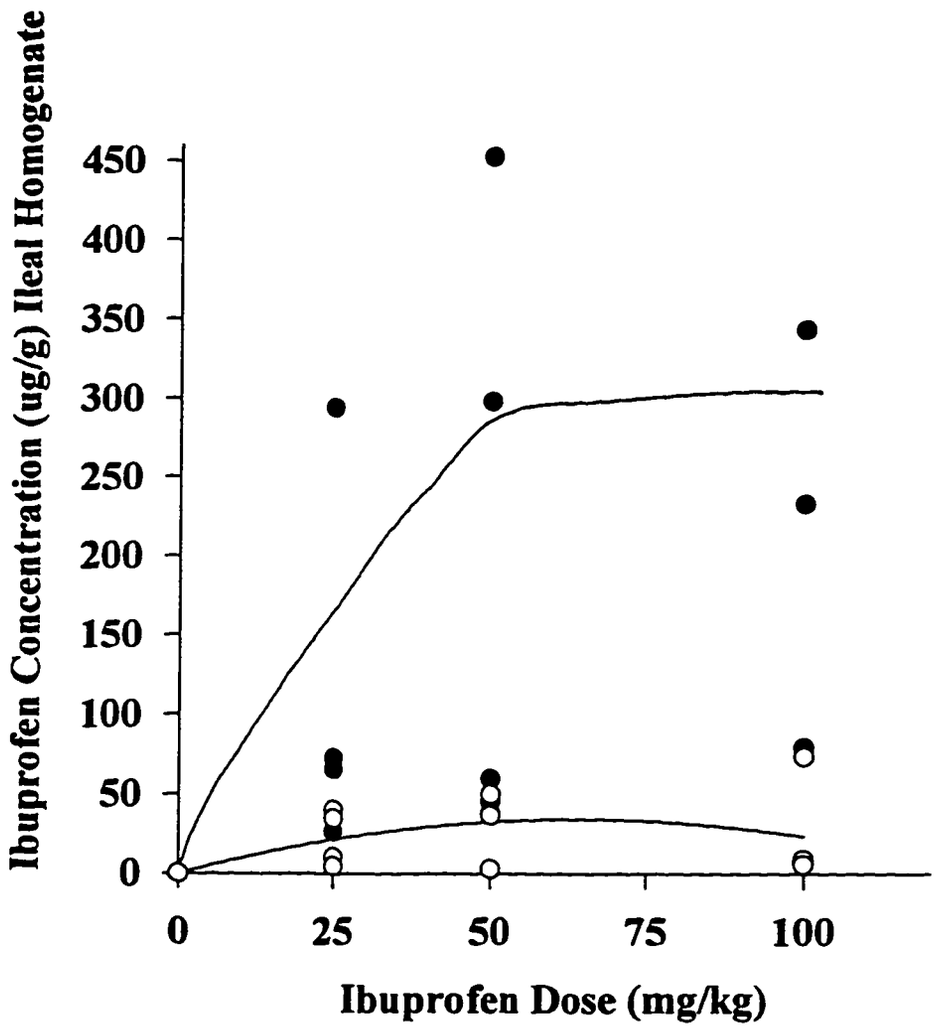


Figure 39. Effects of single i.p. doses of racemic ibuprofen 0-100 mg/kg on S-ibuprofen (closed circles) and R-ibuprofen (open circles) 3 h post-dose. Each point represents one rat ileal homogenate.

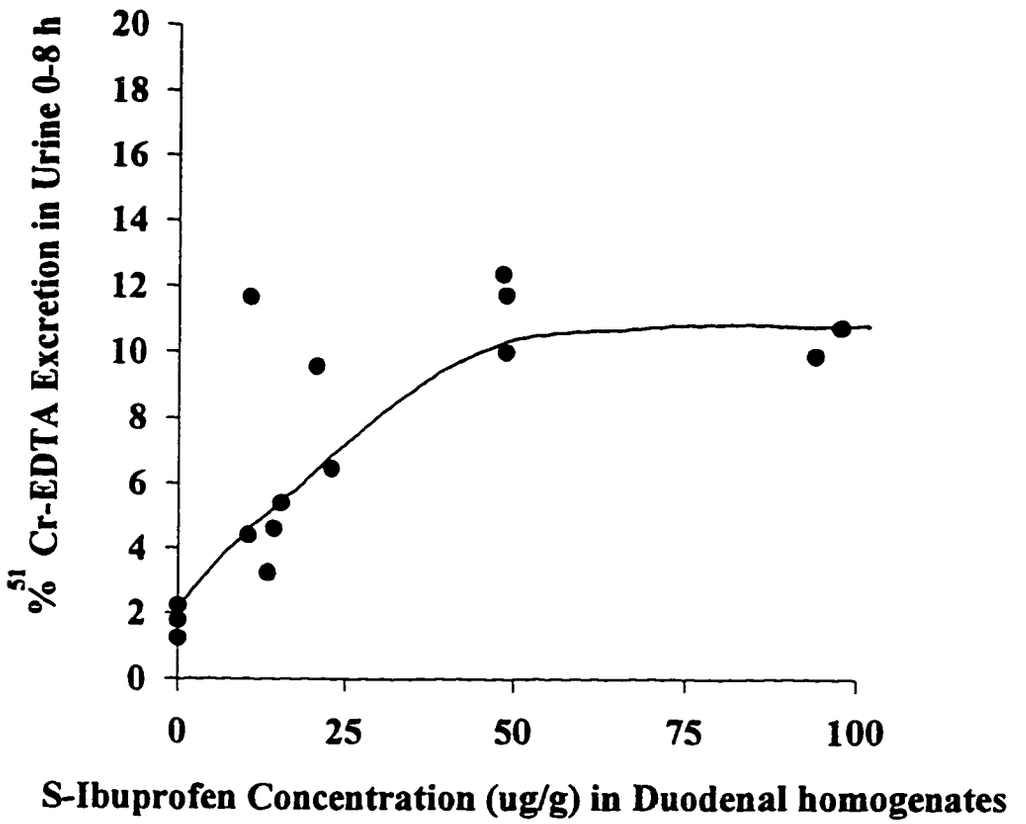


Figure 40. Concentration-effect relationship between S-ibuprofen (closed circles) at 3 h post i.p. dose in duodenal homogenates and % ^{51}Cr-EDTA excretion in urine 0-8 h (each point represents 1 rat).

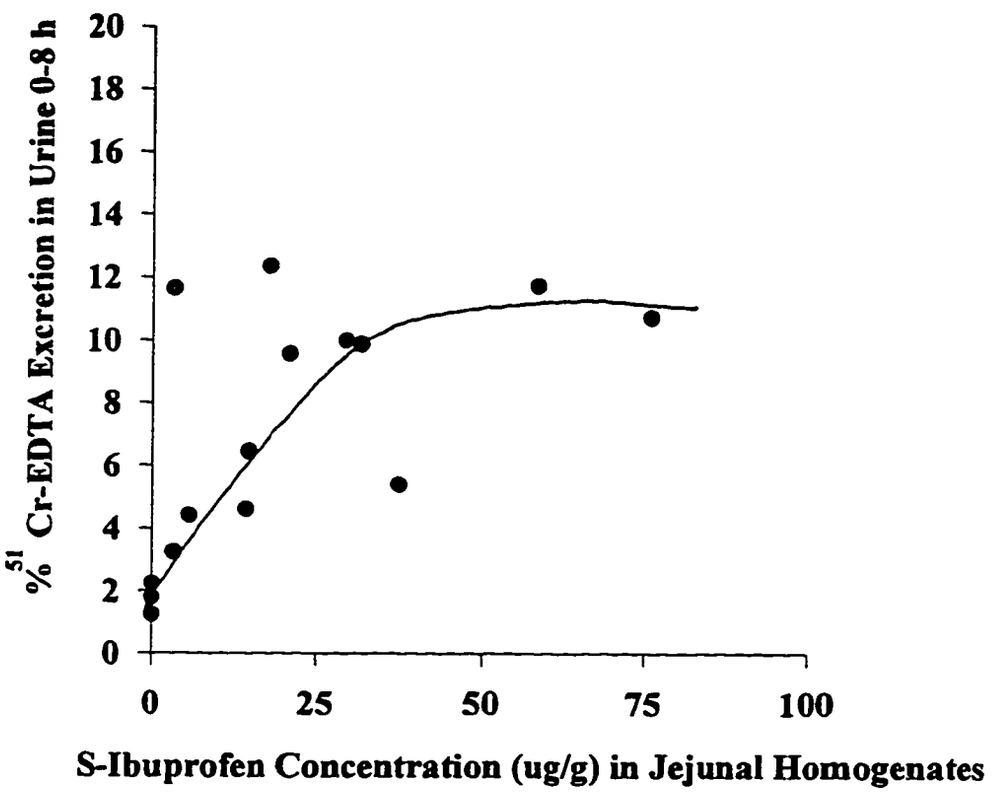


Figure 41. Concentration-effect relationshipbetween S-ibuprofen (closed circles) at 3 h post i.p. dose in jejunal homogenates and % ^{51}Cr-EDTA excretion in urine 0-8 h (each point represents 1 rat).

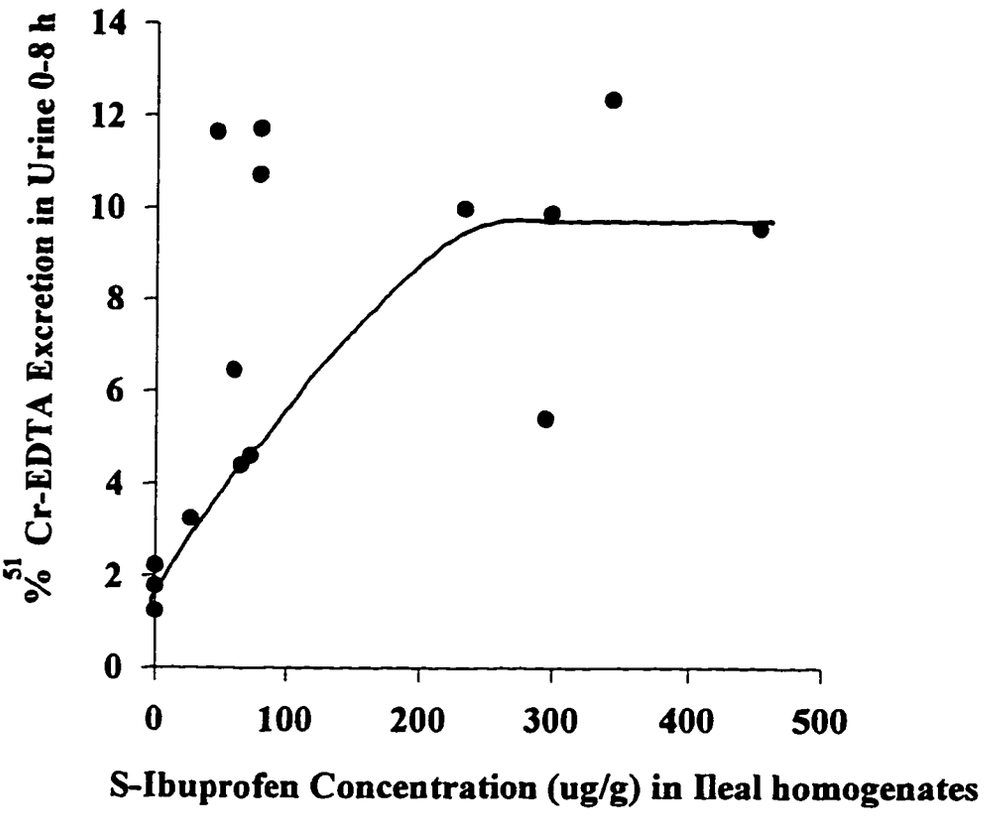


Figure 42. Concentration-effect relationship between S-ibuprofen (closed circles) at 3 h post i.p. dose in ileal homogenates and $\%^{51}$Cr-EDTA excretion in urine 0-8 h (each point represents 1 rat).

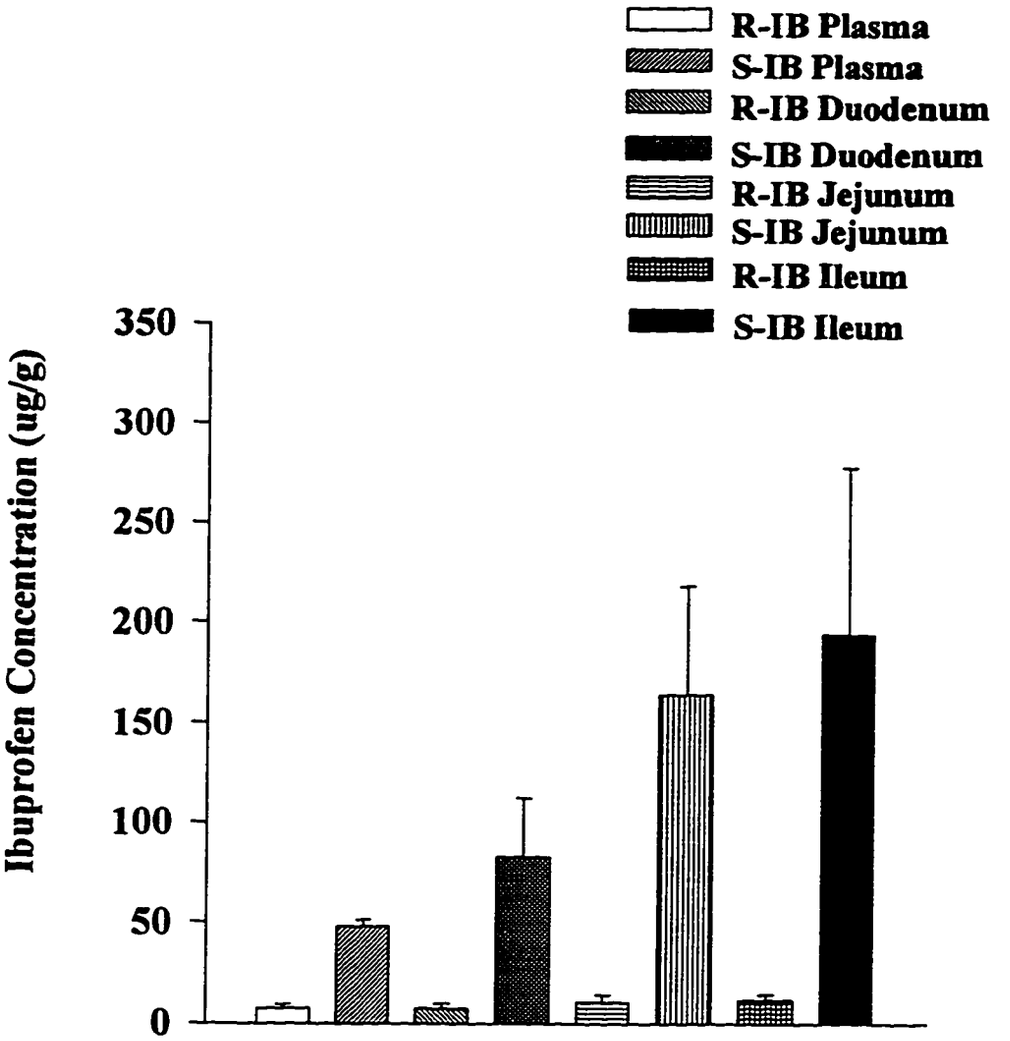


Figure 43. Concentration of R and S-ibuprofen at 3 h post 100 mg/kg i.p. dose in intestinal homogenates and plasma (n = 3 for each group; mean ± s.e.m.).

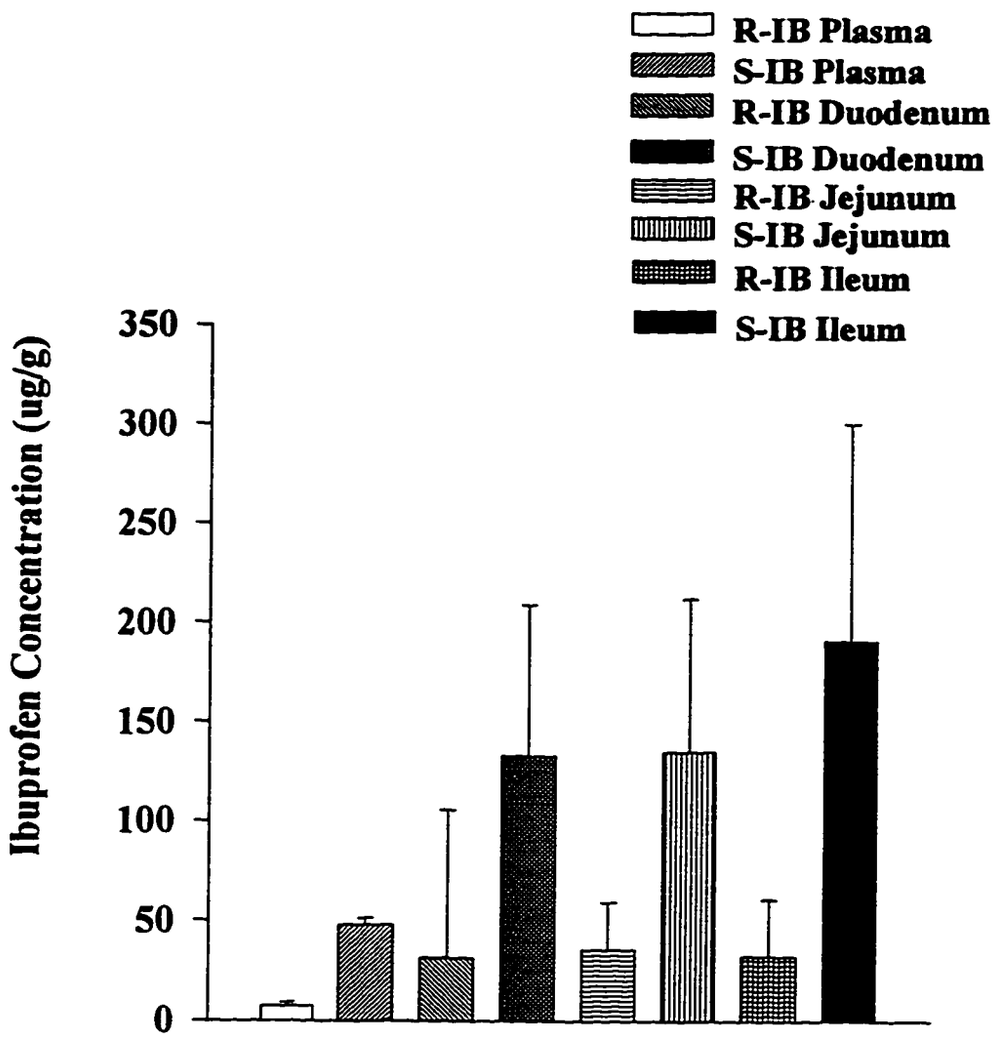


Figure 44. Concentration of R and S-ibuprofen at 3 h post 100 mg/kg p.o. dose in intestinal homogenates and plasma (n = 3 for each group; mean ± s.e.m.).

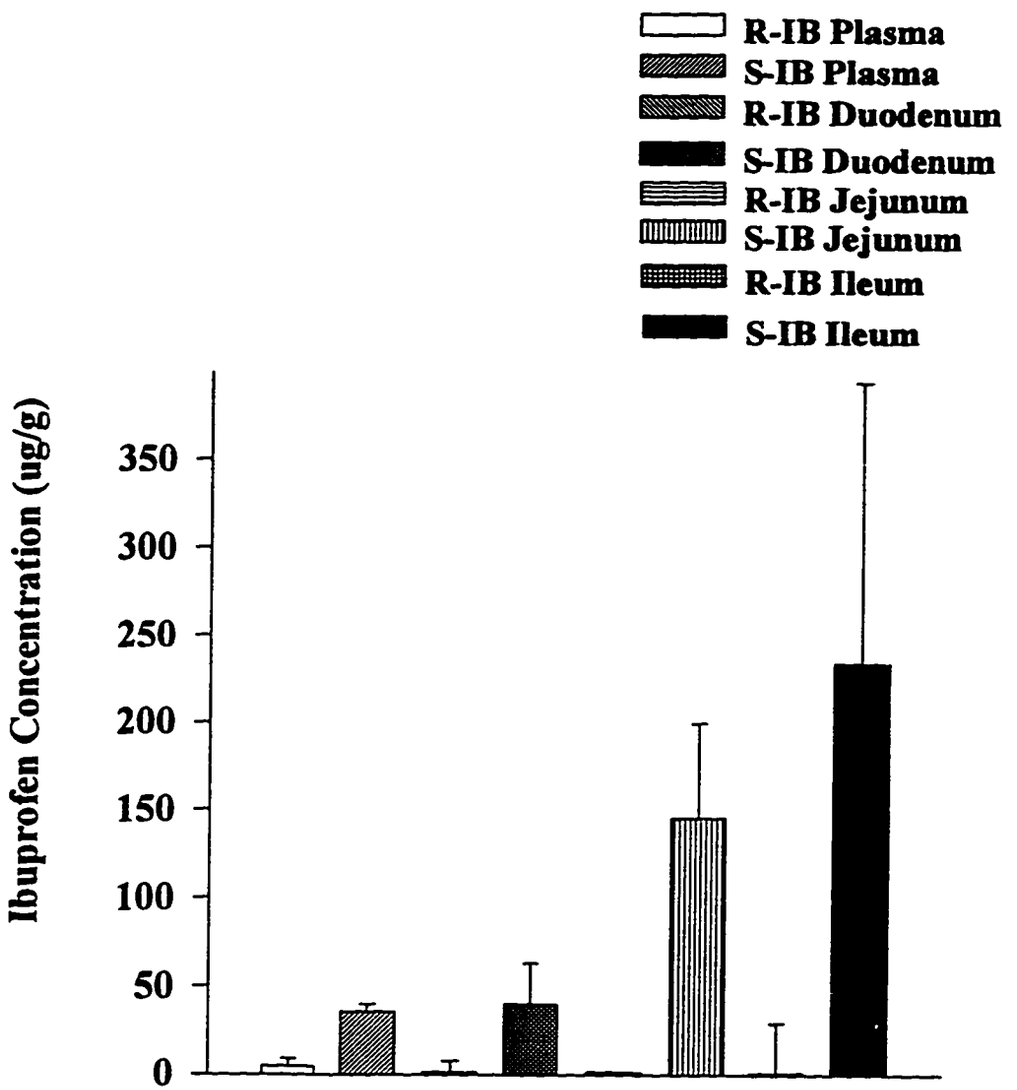


Figure 45. Concentration of R and S-ibuprofen at 3 h post 100 mg/kg s.c. dose in intestinal homogenates and plasma (n = 3 for each group; mean ± s.e.m.).

4.8 Toxicokinetics of Regular and Sustained Release Flurbiprofen Formulations

4.8.1 Results

Following administration of the regular release suspension, the plasma concentration of both enantiomers of flurbiprofen peaked rapidly at approximately 0.5 h (Figure 46, Table 12). Following administration of the sustained release formulation, on the other hand, both enantiomers of flurbiprofen exhibited slow absorption rates with a t_{max} between 2.25-3.5 h and a lower C_{max} (Figure 47, Table 12). There were no other formulation-dependent significant differences with respect to the kinetic parameters except for Vd/F for R-flurbiprofen which was significantly different ($p > 0.05$, unpaired t-tests). After the sustained release formulation, the plasma concentration profiles of the two enantiomers were non-superimposable (Figs. 46-47). similar to the regular release formulation.

Both the regular release and sustained release flurbiprofen treatment increased gastroduodenal permeability significantly above baseline values 0.09 ± 0.05% (Fig. 48). The sucrose excretion in urine was greater for regular release than sustained release ($p < 0.05$, unpaired t-tests).

The intestinal permeability of untreated animals was 1.8 ± 0.3 % (n = 21). For all treatment groups there was a rapid and significant increase in intestinal permeability beginning 1 h post-dose and reaching maximal values 1 h and 5 h post-dose for the regular and sustained release formulation, respectively (Fig. 49). Intestinal permeability did not return to control values for at least 24 h following the dose administration in all groups. The time course of permeability in the regular release

and sustained release treated groups did not parallel one another and the magnitude and duration of the changes in permeability were different. The intestinal permeability changes in the sustained release group appears to have a sustained level of permeability from 12 to 24 hours followed by a slow return to baseline values (Fig. 49).

When the mean time course of the change in intestinal permeability (Fig. 49) for the regular release formulation was plotted against the mean S-flurbiprofen plasma concentration (Fig. 46) a linear relationship r^2=0.95 was found (Fig. 50). However, when the mean time course of the change in intestinal permeability (Fig. 49) for the sustained release formulation was plotted against corresponding mean S-flurbiprofen plasma concentration (Fig. 46) a counterclockwise hysteresis was evident (Fig. 51).

4.8.2 Discussion

Following administration of a single oral dose of 10 mg/kg of flurbiprofen as both a regular release and a sustained release formulation, stereoselective concentration versus time profiles were observed in agreement with previous reports (Jamali *et al.*, 1988; Knihiniicki *et al.*, 1990) (Figs. 46-47). The concentrations of S-flurbiprofen were significantly higher than those observed for R-flurbiprofen. Accordingly, the oral clearance and Vd/F values of the S-enantiomer was significantly different from those values observed for the R-enantiomer (Table 12).

Flurbiprofen-induced intestinal permeability was previously demonstrated to follow an Emax model (Fig. 10). The doses used in this study were chosen as they had previously been shown to induce significant and measurable intestinal permeability, and

were also equivalent to those previously used by Jamali *et al.* (1988) for stereoselective pharmacokinetic studies in the rat.

The gastroduodenal permeability of sucrose after regular release flurbiprofen was significantly and substantially higher than after the sustained release formulation as depicted in Fig. 48. This indicates a substantial effect upon the upper GI tract after the regular release formulation as the upper GI tract is exposed to a very high concentrations of the NSAID due to immediate release of the active moiety. Regular release flurbiprofen is rapidly absorbed so that the only flurbiprofen that reaches the small intestine is mainly through biliary excretion and the systemic circulation. After administration of the sustained release formulation the permeability of the distal intestine was significantly higher than from the regular release formulation (Fig. 49) while a limited effect is seen on the upper GI tract (Fig. 48). There was limited release from the sustained release product in the gastroduodenum but gradual release in the intestine which coupled with the systemic effect resulted in a substantial increase in distal intestinal permeability. These results indicate that the GI damage of flurbiprofen is, indeed, formulation-dependent.

The mean pharmacokinetic parameters were used to relate the mean plasma concentrations of S-flurbiprofen to the time course of drug in the plasma. Simple examination of the time course of drug in the plasma and the time course of the changes in intestinal permeability shows that there appears to be a direct relationship between the peak plasma concentration and the occurrence of the peak effect. (Fig. 50). Further, when the intestinal permeability is plotted against S-flurbiprofen plasma

concentrations and the points connected in temporal order there is a evidence of a linear relationship for the regular release and an anticlockwise hysteresis for the sustained release formulation (Figs. 50-51). This may suggest that the intestinal permeability changes induced by flurbiprofen are augmented by the sustained release and increase intestinal presence and contact time of flurbiprofen with the intestinal epithelium.

A higher intestinal permeability persisted much longer than the presence of the drug in the body of the rat. This may be due to an apparently slow rate of healing or the persistence of drug in an effect compartment outside the detectable levels in plasma. The damage induced by NSAIDs may also induce a number of stochastic physiological and pharmacological processes and responses that may also modulate regulation of intestinal permeability changes (Milks *et al.*, 1986; Nash *et al.*, 1987; Winter *et al.*, 1991; Kubes *et al.*, 1992).

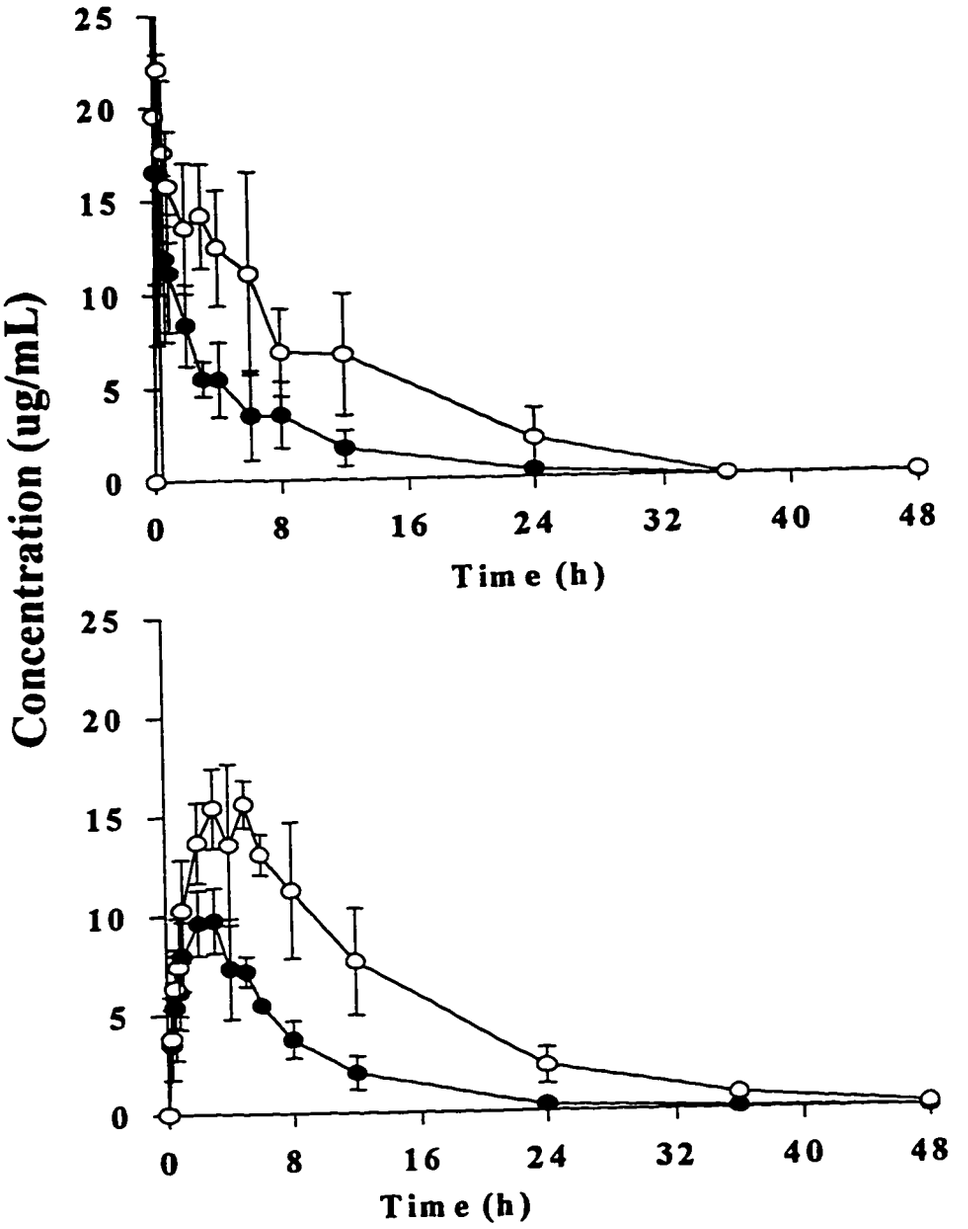
The results of intestinal permeability of the sustained release product appear to be more variable than the regular release product (Fig. 49) which is postulated to be due to the sustained release of drug and its presence within the intestinal tract. This may induce more local distal intestinal damage in addition to the systemic intestinal effects. These data are consistent with those reported by Choi *et al.* (1995) who found increased intestinal permeability with a sustained release formulation of diclofenac but did not see a statistically significant increase in intestinal permeability with regular release diclofenac. These observations in the rat also appear to agree with clinical observations of distal intestinal damage induced by SR and enteric-coated NSAIDs

(Table 7). Evidence for a presystemic distal intestinal damage has come from an osmotically activated constant-release formulation of indomethacin (Osmosin® Merck-Sharpe and Dohme, U.K.) which is no longer commercially available. Osmosin® tablets were located at the site of perforating colonic and ileal ulcers and were floating free in the peritoneal cavity (Day, 1983). However, a more recent report has also suggested the location of possible diclofenac pill fragments at the site of ulceration and strictures (Whitcomb *et al.*, 1992). This may lead to high local concentrations in the ileum and colon leading to lower GI damage induced by NSAIDs.

Although, the more distal intestinal toxicological manifestations of sustained release NSAID formulations have been largely ignored; the likelihood of its increased occurrence with more frequent use of NSAID medication has been previously predicted (Anonymous, 1989). Cost containment of pharmaceuticals is of current interest to health-care systems and the therapeutic rationale behind enteric-coated and sustained release formulations in terms of GI side-effects is not straight forward. These data suggest that sustained release NSAIDs do not solve the problem of NSAID-induced GI toxicity but merely shift the problem to a more distal site within the GI tract. The developed animal model also appears to be suitable for prediction of clinical events throughout the GI tract.

In summary, this study demonstrates the input rate and time-dependencies of flurbiprofen induced changes in GI permeability as estimated by urinary excretion of

^{51}Cr-EDTA and sucrose. In addition, the pharmacokinetic / pharmacodynamic relationship between the increase in intestinal permeability and plasma S-flurbiprofen concentrations have been shown. The present data suggest that the changes in GI permeability are dependent upon rate of input and that both presystemic and systemic components are involved in intestinal permeability changes. Gastroduodenal damage induced by NSAIDs is a clinically significant adverse drug reaction and should be evaluated parallel to concerns of damage in the more distal intestine in order to properly evaluate the contribution of the formulation on the overall toxicity in the entire GI tract. Therefore, this non-invasive model of assessing gastroduodenal and distal intestinal permeability using sucrose and ^{51}Cr-EDTA as markers, respectively, was an effective model to study the actions of regular release and sustained release formulations of flurbiprofen.



Figures 46-47. Mean plasma concentration (n=4 ± S.D.) versus time profiles for racemic flurbiprofen in rats dosed with 10 mg/kg flurbiprofen regular release suspension (upper panel) and sustained release granules (lower panel). S-flurbiprofen (open circles), R-flurbiprofen (closed circles).

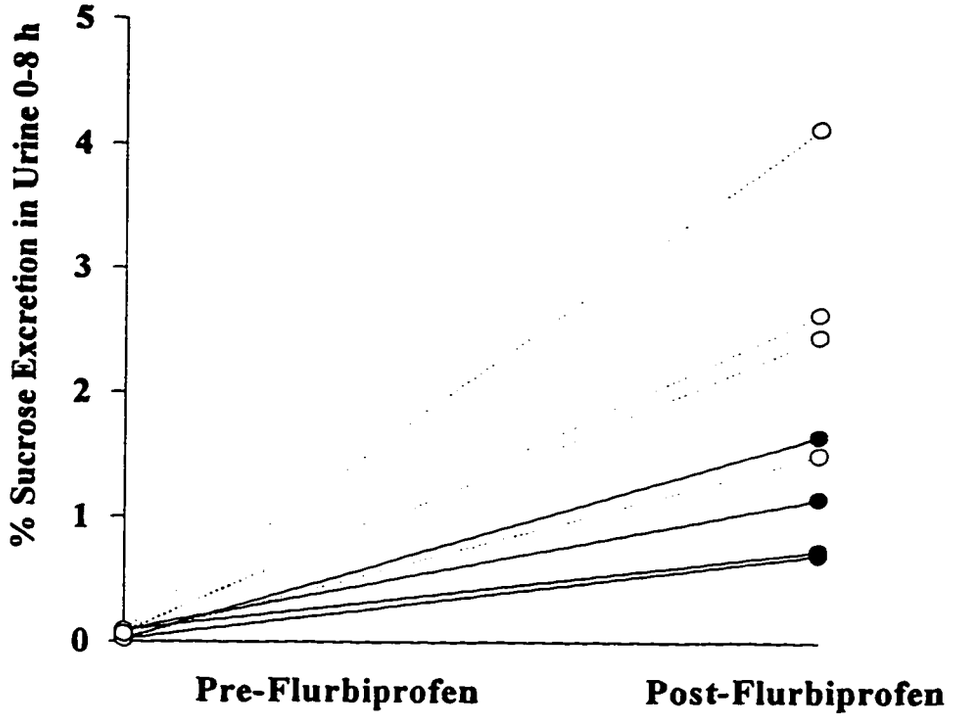


Figure 48. % Sucrose permeability after oral flurbiprofen 10 mg/kg. Each point represents one rat. Regular release suspension (open circles) and sustained release flurbiprofen (closed circles). Regular release is significantly different from sustained release.

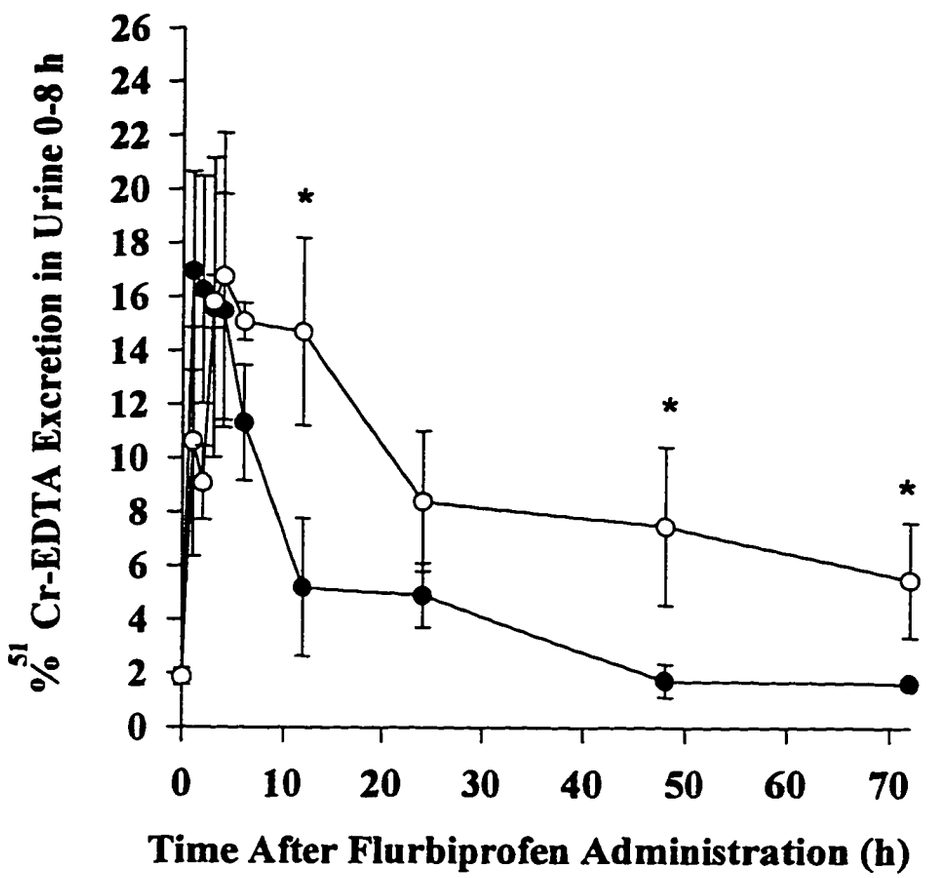
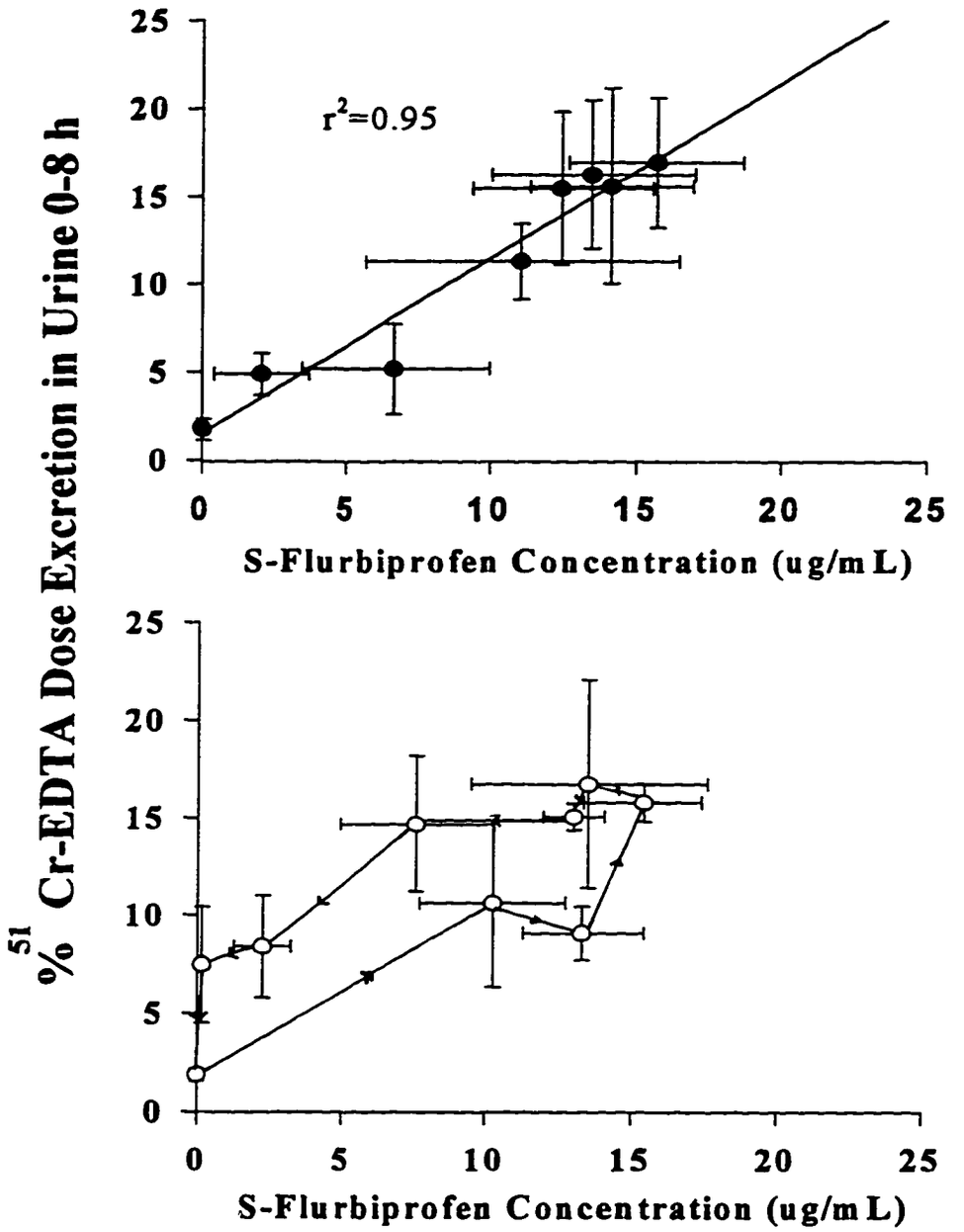


Figure 49. Time courses of intestinal permeability changes (mean ± s.e.m., n = 4 for each point) resulting from the oral administration of either 10 mg/kg regular release flurbiprofen (closed circles) or sustained release flurbiprofen (open circles) orally. * Denotes significantly different from regular release.



Figures 50-51. Change in intestinal permeability versus S-flurbiprofen plasma concentration for rats dosed with 10 mg/kg flurbiprofen regular release suspension (upper panel) or sustained release flurbiprofen (lower panel). The points are joined in temporal order (note counterclockwise hysteresis for sustained release).

Table 12. Pharmacokinetic parameters following oral doses of 10 mg/kg racemic flurbiprofen (mean ± S.D., n = 4 for each group).

Phamacokinetic Parameter	Regular Release	Sustained Release
AUC_{0-t} (μg h/mL) R	69.72±16.23	76.18±9.23
AUC_{0-t} (μg h/mL) S	197.35±45.82[a]	205.14±35.29[a]
$AUC_{0-\infty}$ (μg h/mL) R	75.77±19.88	83.56±12.104
$AUC_{0-\infty}$ (μg h/mL) S	214.86±54.85[a]	224.58±45.93[a]
$t_{1/2}$ (h) R	5.28±0.24	4.98±1.37 [a]
$t_{1/2}$ (h) S	8.02±0.84[a]	6.79±0.67[a]
Cl/F (mL/h) R	39.89±0.17	36.36±0.30
Cl/F (mL/h) S	13.27±0.13[a]	12.22±0.11[a]
Vd/F (mL) R	125.67±11.89	168.67±5.0
Vd/F (mL) S	63.88±5.5[a]	83.23±14a
Cmax (mg/L) R	17.36±4.89 [a]	10.90±1.014[b]
Cmax (mg/L) S	22.073±5.56	16.40±1.04
Tmax (h) R	0.44±0.11	2.25±0.83
Tmax (h) S	0.5±0	3.5±1.12
Mean Relative Bioavailability R		1.1
Mean Relative Bioavailability S		1.0

a, no signifcant stereoselectivity b, significant difference between formulations.

5. Conclusions

The NSAIDs as a therapeutic class exhibit dose-limiting side-effects in the GI tract. The purpose of this research was to investigate various toxicokinetic aspects of NSAID-induced GI damage in a suitable animal model.

Relatively little has been known about the more distal intestinal damage induced by NSAIDs. Perhaps the principal reason for this void has been the lack of suitable analytical methodology to accurately quantify this damage. The advent and clinical use of the permeability probes such as ^{51}Cr-EDTA and sucrose has become apparent over the last decade and allows the capability to carry out site-specific permeability studies. This test has led to an immense growth in gastrointestinal investigations both *in vivo* and *in vitro*. For the present studies, NSAIDs from various structural classes whose GI permeability had not been previously examined in the rat model were chosen. All drugs and antagonists tested in the rat model were initially chosen to parallel the studies already examined in human studies. The rat appears to be a suitable animal model for gastroduodenal and distal intestinal permeability studies as it responds in a similar fashion to those previously reported in humans.

The degree of gastric damage incorporated in the measurement of ^{51}Cr-EDTA appears to be negligible in these studies as a 42 mg/kg dose of aspirin did not cause measurable distal intestinal permeability changes, however, this dose was able to increase gastroduodenal permeability as measured by sucrose. These findings are consistent with results demonstrated in humans. Thus, aspirin, which is most

frequently implicated in causing gastric and duodenal mucosal damage and permeability (Meddings *et al.*, 1993; Rabassa *et al.*, 1994; Irvine *et al.*, 1995), only increased intestinal permeability slightly above baseline values (Bjarnason, 1986). In addition, the contact time and surface area of the gastroduodenum are considerably smaller than that of distal intestine which would also minimize any gastric permeability of ^{51}Cr-EDTA.

The permeation of ^{51}Cr-EDTA has been shown to be relatively specific to the distal intestine and a comparison of peroral and intraduodenal instillation showed no significant differences in the extent of urinary excretion (Simpson, 1985; Aabakken and Osnes, 1990). Intestinal permeability in the rat has also been shown to correlate with intestinal ulceration (Ford *et al.*, 1995). The use of excretory ratios of markers (i.e. lactulose / mannitol; ^{51}Cr-EDTA / mannitol; ^{51}Cr-EDTA / L-rhamnose; cellobiose/ L-rhamnose) to indicate intestinal permeability may have advantages over a single marker (Jenkins *et al.*, 1991; Bjarnason *et al.*, 1992a). Each probe acts as an internal standard for the other by minimizing some of the pre- and post-mucosal extramural factors which would affect the markers identically so their urinary excretion ratios are unchanged, decreasing the variability, and increasing the reliability of the intestinal permeability data. The contact time and surface area that ^{51}Cr-EDTA is exposed to in the GI tract influences the extent of intestinal permeability of the probe. In the present studies, several pharmacological approaches were employed which may have affected GI motility such as the administration of erythromycin or misoprostol. The urinary excretion ratios from 0 to 8 h and that of 0 to 24 h for control rats did not differ

significantly from those rats administered any of the pharmacological protective agents or NSAIDs suggesting that changes in pre-or post mucosal factors did not significantly affect intestinal permeability changes in these experiments. However, investigators should be inherently aware of these factors and future studies should incorporate another probe such as mannitol to act as an internal standard and to minimize any influence of these factors.

Recent studies have suggested that there may be some degree of colonic permeation of ^{51}Cr-EDTA (Elia *et al.*, 1987; Jenkins *et al.*, 1991). Bjarnason *et al.* (1984a) have suggested that this colonic permeation is small as ^{51}Cr-EDTA is incorporated into feces and, therefore, unavailable for colonic permeation. Histological evidence showed that NSAID damage was predominant in the jejunum and ileum and not the colon. It would appear that in these studies colonic permeation is minimal and would account for only a few percent of total gastrointestinal permeation. However, in the presence of significant colonic damage this contribution may become significant.

^{51}Cr-EDTA has been a useful tool as a intestinal permeability marker and has significantly contributed to important scientific understanding of the study of many GI diseases. However, ^{51}Cr-EDTA is not an ideal probe and other site-specific markers of the GI tract must continue to be developed and studied to overcome some of its apparent disadvantages.

A disparity between *in vitro* eudismic ratios of chiral NSAIDs was observed with *in vivo* intestinal permeability. The findings of etodolac and flurbiprofen contribute to the hypothesis that a stereochemically pure enantiomer may not be safer

on the GI tract than its corresponding racemate. In view of increasing evidence of other mediators and processes involved in intestinal permeability the effect of R enantiomers may suggest that intestinal permeability is not solely regulated by cyclooxygenase. Further detailed studies on the regulation of intestinal permeability as well as studies of COX_1/COX_2 ratios and inducible nitric oxide synthase and their possible relationship to GI permeability are clearly warranted.

The dose-response, time-course and pharmacokinetic / pharmacodynamic relationships of indomethacin were fully characterized. Indomethacin-induced intestinal permeability was both time and dose-dependent. There was a temporal lag between plasma concentrations and intestinal permeability, however, through the use of effect-compartment modeling and collapsing of the anti-clockwise hysteresis a linear concentration-effect relationship between indomethacin and intestinal permeability was apparent.

In the present work there are a number of observations of intestinal permeability changes induced in the rat though the administration of NSAIDs. Although it has been possible to measure both the effect and plasma NSAID concentration, attempting to establish a concentration-effect relationship was often difficult. Considerable efforts in the recent pharmacokinetic literature have been expended in an attempt to relate drug concentration and pharmacological effect(s), in particular when there is a temporal lag between the measured drug concentration and the observed pharmacological effect of the drug as has been demonstrated in the present work. Traditionally, the methods of analysis made several assumptions with

regard to both the pharmacodynamic response and the pharmacokinetics of the drug being measured. These methods require a single compartment model governed by first-order processes with pharmacological effect resulting solely from the drug (ie. no active metabolites) and a pharmacodynamic response that is linearly related to the logarithm of the amount of drug in the body. These assumptions may not always be upheld.

In attempts to overcome the aforementioned assumptions a mathematical approach to account for temporal lags between concentrations and observed effects has been utilized (Holford and Sheiner 1981). This method involves drug concentration estimation in a theoretical effect compartment using parameters obtained via pharmacokinetic modeling. The effect measurements that use a selected pharmacodynamic model (e.g. linear, E_{max}) and are related to the effect compartment concentrations via a parametric link. This effect compartment is of negligible volume and receives a negligible portion of the administered dose such that it is not apart of the pharmacokinetic model and the concentrations of drug within the effect compartment can never be measured. The estimates of the effect compartment drug concentrations are largely dependent on the adequacy of the pharmacokinetic model and on assumed estimated parametric constants and properties of the effect compartment (Holford and Sheiner, 1981). These methods of analysis also treat measured effect as a terminal event of a stochastic process of one or more steps. However, intestinal permeability may not be viewed as the terminal event of a stochastic process. Although the rise and subsequent fall of intestinal permeability

could be related to the plasma concentration of NSAID though the use of an effect compartment, such an analysis may also ignore concepts regarding control of the barrier function of the GI tract. The permeability of GI membranes may be regulated by many physiological factors and homeostatic mechanisms. If a response (e.g. increased intestinal permeability in the present study) is viewed as being influenced not only by the drug administered but also by homeostatic mechanisms, the observed response can become dependent on the physiological state of the model prior to drug administration. This speculation may be important in comparison of one or more studies or between different disease states or gender. The processes of homeostatic adaptations to physiological stresses are well documented and the assumption that the observed effect is solely the result of drug may ignore physiological reality. Other possible explanations for the lack of correlation between plasma drug concentration and effect (such as the production of an active metabolite, the production of an endogenous modulator, the modulation of effect by homeostatic mechanisms (ie. epithelial restitution) may be obscured. The use of pharmacokinetic / pharmacodynamic modeling may limit the interpretation of the effect of intestinal permeability solely to an immeasurable drug concentration and thereby exclude alternative interpretations.

An observation in the rat after administration of glucose / citrate with naproxen led to a further study to delineate the heterogeneity of the possible protective effect of the glucose / citrate formulation with several NSAIDs. It was found that glucose/citrate did not reduce indomethacin induced intestinal permeability when the

NSAID was administered subcutaneously. It was also found that the bioavailability of indomethacin was substantially reduced when this formulation was administered orally which emphasizes the need to consider both the route of administration as well as the pharmacokinetics of NSAID formulations in addition to the toxicological data when evaluating novel protective approaches to reduce NSAID-GI toxicity.

In recent years there has been intense investigations of the role of nitric oxide moieties and their protective properties in the GI tract. This work may further suggests that NO may be an important modulator of NSAID-induced GI permeability. Using the developed rat model the protective effect of metronidazole and tempo on NSAID-induced permeability was examined, and found to reduce both NSAID-induced intestinal permeability and mortality.

The developed rat model was also suitable to study the intestinal tissue concentrations of ibuprofen enantiomers and to characterize toxicokinetic relationships in the rat GI tract. There has been a general absence of a relationship between therapeutic response and plasma concentrations of the 2-APAs. These studies have demonstrated that a plasma and intestinal tissue concentration effect relationship may exist between the active enantiomer and a measure of GI toxicity.

Finally, the therapeutic value of sustained release formulations in terms of side-effects throughout the entire GI tract was addressed with a commercially available formulation of flurbiprofen. There is currently intense debate on the therapeutic rationale behind the marketing and administration of sustained release formulations of NSAIDs. These studies have demonstrated that a change in the

release pattern of NSAIDs may reduce the upper gastroduodenal damage but conversely may only shift this damage to a more distal site in the GI tract. This study questions the therapeutic rationale behind the modified release formulation of NSAIDs in terms of GI side-effects. These observations present several examples of the complex toxicokinetics of NSAID-induced gastrointestinal damage. Gastrointestinal permeability patterns of not even two of these chemically diverse groups are similar. It is evident that individual studies must be carried out with each member of this class in order to adequately ascertain their respective toxicokinetic relationships. The establishment of an animal model of NSAID-induced GI damage allows for a complete characterization of the pharmacokinetic / pharmacodynamic relationships of members of this therapeutic class. It is clear from these studies that it is not possible to make class-wide generalizations with respect to the magnitude of NSAID-induced GI permeability, extent of protection or site of GI damage.

6. References

Aabakken, L.: 1989. ^{51}Cr-Ethylenediaminetetraacetic acid absorption test: methodological aspects. Scand. J. Gastroenterol. 24: 351-358.

Aabakken, L., Larsen, S., Osnes, M.: 1989a. Sucralfate for prevention of naproxen-induced mucosal lesions in the proximal and distal gastrointestinal tract. Scand. J. Rheumatol. 18: 361-368.

Aabakken, L., Larsen, S., Osnes, M.: 1989b. Cimetidine tablets or suspension for the prevention of gastrointestinal mucosal lesions caused by non-steroidal anti-inflammatory drugs. Scand. J. Rheumatol. 18: 369-375.

Aabakken, L., Bjørnbeth, B.A., Hofstad, B., Olaussen, B., Larsen, S., Osnes, M.: 1989c. Comparison of the gastrointestinal side effects of naproxen formulated as plain tablets, enteric-coated tablets, or enteric-coated granules in capsules. Scand. J. Gastroenterol. 24: (suppl 163), 65-73.

Aabakken, L., Osnes, M.: 1989. Non-steroidal anti-inflammatory drug-induced disease in the distal ileum and large bowel. Scand. J. Gastroenterol. 24(suppl 163), 48-55.

Aabakken, L., Osnes, M.: 1990. ^{51}Cr-Ethylenediaminetetraacetic acid absorption test. Effects of naproxen, a non-steroidal, antiinflammatory drug. Scand J. Gastroenterol. 25: 917-924.

Aabakken, L., Bjørnbeth, B.A., Weberg, R., Vikmoen, L., Larsen, S., Osnes, M.: 1990. NSAID-associated gastroduodenal damage does famotidine protection extend into the mid and distal duodenum ? Aliment. Pharmacol. Therap. 4: 295-303.

Aabakken, L.: 1992. Non-steroidal anti-inflammatory drugs - the extending scope of gastrointestinal side effects. Aliment. Pharmacol. Ther. 6: 143-162.

Abramson, S.B., Weissmann, G.: 1989. The mechanisms of action of nonsteroidal antiinflammatory drugs. Arth. Rheum. 32: 1-9.

Abramson, S.B., Leszczynska-Piziak, J., Haines, K., Reibman, J.: 1991. Non-steroidal anti-inflammatory drugs: effects on a GTP binding protein within the neutrophil plasma membrane. Biochem. Pharmacol. 41(11): 1567-1573.

Aeberhard, E.E., Henderson, S.A., Griscavage, J.M., Arabolos, N.S., Castro, F.E., Barrett, C.T., Ignarro, L.J.: 1995. Nonsteroidal antiinflammatory drugs inhibit expression of the nitric oxide synthase gene in rat alveolar macrophages. J. Invest. Med. 43(1) (Suppl 1) 133A.

Ahn, H.-Y., Jamali, F., Cox, S.R., Kittayanoud, D., Smith, D.E.: 1991. Stereospecific disposition of ibuprofen enantiomers in the isolated perfused rat kidney. Pharm. Res. 8: 1520-1524.

Akamatsu, H., Oguchi, M., Nishijima, S., Asada, Y., Takahashi, M., Ushijima, T., Niwa, Y.: 1991. The inhibition of free radical generation by human neutrophils through the synergistic effects of metronidazole with palmitoleic acid: A possible mechanism of action of metronidazole in rosacea and acne. Arch. Dermatol. Res. 282: 449-454.

Akarasereenont, P., Mitchell, J.A., Appleton, I., Thiermann, C., Vane, J.R.: 1994. Involvement of tyrosine kinase in the induction of cyclo-oxygenase and nitric oxide synthase by endotoxin in cultured cells. Br. J. Pharmacol. 113(4): 1522-1528.

Allison, M.C., Howatson, A.G., Torrance, C.J., Lee F.D., Russell, R.I.: 1992. Gastrointestinal damage associated with the use of nonsteroidal antiinflammatory drugs. N. Engl. J. Med. 327: (11) 751-756.

Alpan, G., Eyal, F., Vinograd, I., Udassin, R., Amir, G., Mogle, P., Glick, B.: 1985. Localized intestinal perforation after enteral administration of indomethacin in premature infants. J. Pediatr. 106: 277-281.

Anonymous.1989. NSAIDs and gut damage.[letter] Lancet 2: 600.

Anthony, A., Dhillon, A.P., Nygard, G., Hudson, M., Piasecki, C., Strong, P., Trevethick, M.A., Clayton, N.M., Jordan, C.C., Pounder, R.E., Wakefield, A.J.: 1993. Early histological features of small intestinal injury induced by indomethacin. Aliment. Pharmacol. Ther. 7: 29-40.

Ariens, E.J.: 1984. Stereochemistry, a basis for sophisticated nonsense in pharmacokinetics and clinical pharmacology. Eur. J. Clin. Pharmacol. 26: 663-669.

Aronson, A.L., Rogers, K.M.: 1972. Effects of calcium and chromium chelates of ethylenediaminetetraaccetate on intestinal permeability and collagen metabolism in the rat. Toxicol. Appl. Pharmacol. 21: 440-453.

Auer, I.O., Habscheid, W., Hiller, S., Gerhards, W., Eilles, C.: 1987. Nicht-steroidal antiphlogistika erhöhen die darmpermeabilität. Dtsch. Med. Wochenschr. 112: 1032-1037.

Averginos, A., Hutt, A.J.: 1990. Interindividual variability in the enantiomeric disposition of ibuprofen following the oral administration of the racemic drug to healthy volunteers. Chirality 2: 249-256.

Baert, F., Hart, J., Blackstone, M.O.: 1995. A case of diclofenac-induced colitis with focal granulomatous change. Am. J. Gastroenterol. 90(10): 1871-1873.

Balint, G.A.: 1991. Possibilities to reduce the unwanted gastric side-effects of orally administered indomethacin in the rat. Kitasato. Arch. Exp. Med., 73-75.

Banerjee, A.K., Sherwood, R., Rennie, J.A., Peters, T.J.: 1986. Sulphasalazine reduced indomethacin induced changes in small intestinal permeability in man. Brit. Soc. Gastro. A593.

Baum, C., Kennedy, D.L., Forbes, M.B.: 1985. Utilization of nonsteroidal antiinflammatory drugs. Arth. Rheum. 28: 686-692.

Beard, K., Walker, A.M., Perera, D.R., Jick, H.: 1987. Nonsteroidal anti-inflammatory drugs and hospitalization for gastroesophageal bleeding in the elderly. Arch. Int. Med. 147: 1621-1623.

Beck, W.S., Schneider, H.T., Dietzel, K., Nuemberg, B., Brune, K.: 1990. Gastrointestinal ulcerations induced by anti-inflammatory drugs in rats. Arch. Toxicol. 64: 210-217.

Behrens, R.H., Szaz, K.F., Northrop, C., Elia, M., Neale, G.: 1987. Radionuclide tests for assessment of intestinal permeability. Eur. J. Clin. Invest. 17, 100-105.

Bem, J.L., Mann, R.D., Coulson, R.: 1988. Fatal gastrointestinal damage associated with the use of osmotic mini pump indomethacin (Osmosin). Pharmaceut. Med. 3: 35-43.

Bergstrom, K., Havermark, G.: 1976. Enzymes in intestinal mucosa from patients with rheumatoid arthritis. Scand. J. Rheum. 5: 29-32.

Bernhard, G.C.: 1992. Worldwide safety experience with nambumetone. J. Rheumatol. (suppl 36): 19: 48-57.

Beró, T., Németh, A., Mózsik, T., Jávor, T.: 1984. The effects of gastric cytoprotective drugs (atropine, cimetidine, vitamin-A) on the indomrthacin induced intestinal ulcers in rats. Acta. Physiologica. Hungarica. 64: (3-4) 343-347.

Berry, B.W., Jamali, F.: 1988. Stereospecific high-performance liquid chromatographic (HPLC) assay of flurbiprofen in biological specimens. Pharm. Res. 5(2):123-125.

Berry, B.W., Jamali, F.: 1989. Enantiomeric interaction of flurbiprofen in the rat. J. Pharm. Sci. 78:(8), 632-634.

Bett, I.M.: 1966. Urinary tryptophan metabolites in rheumatoid arthritis and some other diseases. Ann. Rheum. Dis. 25: 556-562.

Bielecki, J.W., Gebbers, J.O., Filippini, L.: 1995. Segmentale dunndarmlasionen nach einnahme nicht-steroidaler antirheumatika. Dtsch. Med. Wschr. 120: 436-441.

Bjarnason, I., Peters, T.J., Veall, N.: 1983a. A persistent defect in intestinal permeability in coeliac disease demonstrated by a ^{51}Cr-labeled EDTA absorption test. Lancet 1: 323-325.

Bjarnason, I., O'Morain, C., Levi, A.J., Peters, T.J.:1983b. Absorption of ^{51}Chromium-labeled ethylenediaminetetraacetate in inflammatory bowel disease. Gastroenterology 85: 318-22.

Bjarnason, I., So, A., Levi, A.J., Peters, T.J., Williams, D., Zanelli, G.D., Gumpel, J.M., Ansell B.: 1984a. Intestinal permeability and inflammation in rheumatoid arthritis: Effects of non-steroidal anti-inflammatory drugs. Lancet 2: 1171-1173.

Bjarnason, I., Peters, T.J., Veall, N.: 1984b. ^{51}Cr-EDTA test for intestinal permeability. Lancet 2: 523.

Bjarnason, I., Ward, K., Peters, T.J.: 1984c. The leaky gut of alcoholism: possible route of entry for toxic compounds. Lancet 1: 179-182.

Bjarnason, I., Marsh, M.N., Price, A., Levi, A.J., Peters, T.J.: 1985a. Intestinal permeability in patients with coeliac disease and dermatitis herpetiformis. Gut 26: 1214-1219.

Bjarnason, I., Goolamali, S.K., Levi, A.J., Peters, T.J.:1985b. Intestinal permeability in patients with atopic eczema. Brit. J. Derm. 112: 291-297.

Bjarnason, I., Smethurst, P., Levi, A. J., Peters, T.J.: 1985c. Intestinal permeability to ^{51}Cr-EDTA in rats with experimentally induced enteropathy. Gut 26: 579-585.

Bjarnason, I., Williams, P., Smethurst, P., Peters, T.J., Levi, A.J. 1986a. Effect of non-steroidal anti-inflammatory drugs and prostaglandins on the permeability of the human small intestine. Gut 27: 1292-1297.

Bjarnason, I., Peters, T.J, Levi, A.J.:1986b. Intestinal permeability : Clinical correlates. Dig. Dis. 4: 83-92.

Bjarnason, I., Zanelli, G., Prouse, P., Williams, P., Gumpel, M.J., Levi, A.J.:1986c. Effect of non-steroidal Anti-inflammatory drugs on the human small intestine. Drugs 32 (Suppl): 35-41.

Bjarnason, I., Zanelli, G., Smith, T., Prouse, P., Williams, P., Smethurst, P., Delacey, G., Gumpel, M.J., Levi, A.J.: 1987a. Nonsteroidal antiinflammatory drug-induced intestinal inflammation in humans. Gastroenterology. 93: 480-489.

Bjarnason, I., Prouse, P., Smith, T., Gumpel, M.J., Zanelli, G., Smethurst, P., Levi, S., Levi, A.J.: 1987b. Blood and protein loss via small intestinal inflammation induced by non-steroidal anti-inflammatory drugs. Lancet 2: 711-714.

Bjarnason, I., Zanelli, G., Smith, T., Smethurst, P., Price, A.B., Gumpel, M.J., Levi, A.J.:1987c. The pathogenesis and consequence of non-steroidal anti-inflammatory drug induced small intestinal inflammation. Scand. J. Rheumatol. suppl 64: 55-62.

Bjarnason, I., Peters, T.J.: 1987d. Helping the mucosa make sense of macromolecules. Gut 28: 1057-1061.

Bjarnason, I., Price, A.B., Zanelli, G., Smethurst, P., Burke, M., Gumpel, M., Levi, A.J.: 1988a. Clinicopathological features of nonsteroidal antiinflammatory drug-induced small intestinal strictures. Gastroenterology 94: 1070-1074.

Bjarnason, I., Levi, S., Smethurst, P., Menzies, I.S., Levi, A.J.: 1988b. Vindaloo and you. Br. Med. J. 297: 1629-1631.

Bjarnason, I., Smethurst, P., Levi, A.J., Price, A.B.: 1988c. Indomethacin induced chronic small intestinal ulceration in the rat. Gastroenterology 94: A37.

Bjarnason, I., Peters, T.J.: 1989. Intestinal permeability, non-steroidal anti-inflammatory drug enteropathy and inflammatory bowel disease: an overview. Gut 30 Spec No: 22-28.

Bjarnason, I., Smethurst, P., Fenn, C.G., Lee, C.E., Menzies, I.S., Levi, J.: 1989. Misoprostol reduces indomethacin- induced changes in human small intestinal permeability. Dig. Dis. Sci. 34: 407-411.

Bjarnason, I.: 1990. Experimental evidence of the benefit of misoprostol beyond the stomach in humans. J. Rheumatol. (suppl 20) 17: 38-41.

Bjarnason, I., Hopkinsson, N., Zanelli, G., Prouse, P., Smethurst, P., Gumpel, J.M., Levi, A.J.: 1990a. Treatment of non-steroidal anti-inflammatory drug induced enteropathy. Gut 33: 777-780.

Bjarnason, I., Fehilly, B., Smethurst, P., Menzies, I.S., Levi, A.J.: 1991a. Importance of local versus systemic effects of non-steroidal anti-inflammatory drugs in increasing small intestinal permeability in man. Gut. 32: 275-277.

Bjarnason, I., Hayllar, J., Smethurst, P., Price, A., Gumpel, M., Levi, J.: 1991b. Studies on the pathogenesis of non-steroidal anti-inflammatory drug (NSAID) induced enteropathy in man. Gastroenterology 100: A198.

Bjarnason, I., Smethurst, P., Levi, A.J., Menzies, I.S., Peters, T.J.: 1991c. The effect of polyacrylic acid polymers on small-intestinal function and permeability changes caused by indomethacin. Scand. J. Gastroenterol. 26: 685-688.

Bjarnason, I., Smethurst, P., Macpherson, A., Walker, F., McElnay, J.C., Passmore, P., Menzies, I.S.: 1992a. Glucose and citrate reduce the permeability changes caused by indomethacin in humans. Gastroenterology 102:1546-1550.

Bjarnason, I., Hayllar, J., Smethurst, P., Price, A., Gumpel, M.J.: 1992b. Metronidazole reduces intestinal inflammation and blood loss in non-steroidal anti-inflammatory drug induced enteropathy. Gut 33: 1204-1208.

Bjarnason, I., Hayllar, J., Macpherson, A.J., Russell, A.S.: 1993a. Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine. Gastroenterology 104: 1832-1847.

Bjarnason, I., Macpherson, A., Somaslindaram, S., Teahon, K.: 1993b. Nonsteroidal anti-inflammatory drugs and inflammatory bowel disease. Can. J. Gastroenterol. 7: (2) 160-169.

Blower, P.R.: 1993. Nonsteroidal anti-inflammatory drugs. Brit. J. Rheumatol. 32(Suppl 4): 35-8.

Boyd, E.J.S., Wormsley, K.G.: Gastrointestinal side-effects of prostaglandins. In: Side effects of anti-inflammatory Drugs. eds. Rainsford, K.D. & Vels, G.P., M.T.P. Press, Lancaster, 1987, pp. 143-149.

Brett, M.A., Greb, W.H., Kurth, H.J., Rulander, G., Haddock, R.E., Thawley, A.R.: 1988. Fehlende enterohepatische zirculation des aktiven metaboliten von nambumetone. Fortschr. Med. 106: 736

Brodie, D.A., Cook, P.G., Bauer, B.J., Dagle, G.E.: 1970. Indomethacin-induced intestinal lesions in the rat. Toxicol. Appl. Pharmacol. 17: 615-624.

Brody, T.M.: 1956. The uncoupling of oxidative phosphorylation as a mechanism of drug action. Pharmacol. Rev. 7: 335-365.

Brooks, P.M., Day, R.O.: 1991. Nonsteroidal antiinflammatory drugs- differences and similarities. New Engl. J. Med. 324: (24), 1716-1724.

Brune, K., Beck, W.S., Geisslinger, G., Menzel-Soglowek, S., Peskar, B.M., Peskar, B.A.: 1991. Aspirin-like drugs may block pain independently of prostaglandin synthesis inhibition. Experientia 47: 257-261.

Brune, K., Geisslinger, G., Menzel-Sogolowek, S. 1992. Pure enantiomers of 2-arylpropionic acids: Tools in pain research and improved drugs in rheumatology. J. Clin. Pharmacol., 32, 944-952.

Caldwell, J.: 1986. Therapeutic approaches to and management of NSAID induced gastritis. Pract. Gastroenterol. 10(3): 64-86.

Caldwell, J., Hutt, A.J., Fournel-Gigleux, S.: 1988. The metabolic chiral inversion and disposition enantioselectivity of the 2-arylpropionic acids and their biological consequences. Biochem. Pharmacol., 37: 105-114.

Carson, J.L., Strom, B.L., Soper, K.A., West, S.L., Morse, L. 1987. The association of nonsteroidal antiinflammatory drugs with upper gastrointestinal tract bleeding. Arch. Int. Med. 114: 85-88.

Carson, J., Notis, W.M., Orris, E.S.: 1989. Colonic ulceration and bleeding during diclofenac therapy. N. Eng. J. Med. 323: 135.

Carratù, R., Parisi, P., Agozzino, A.: 1993. Segmental ischemic colitis associated with nonsteroidal antiinflammatory drugs. Gastroenterology 16(1): 31-34.

Ceuppens, J.L., Rodriguez, M.A., Goodwin, J.S.: 1982. Nonsteroidal antiinflammatory agents inhibit the synthesis of IgM rheumatoid facto *in vitro*. Lancet 1: 528-530.

Chadwick, R.G., Hossenbocus, A., Colin-Jones, D.G.: 1976. Steatorrhea complicating therapy with mefenamic acid. Br. Med. J. 397.

Chadwick, V.S., Phillips, S.F., Hoffman, A.F.: 1977. Measurements of intestinal permeability using low molecular weight polyethylene glycols (PEG 400) I. Chemical analysis and biological properties of PEG 400. Gastroenterology 73: 241-246.

Chan, C.C., Gordon, R., Brideau, C., Rodger, I.W., Li, C.S., Prasit, P., Tgari, P., Ethier, D., Vickers, P., Boyce, S., Rupniak, N., Webb, J., Hill, R., Ford-Hutchinson, A.W.: 1994 In Vivo pharmacology of L-745, 337: a novel non-steroidal anti-inflammatory agent (NSAID) with an ulcerogenic sparing effect in rat and monkey stomach. Can. J. Physiol. Pharmacol. 72(Suppl. 1): 266.

Chlud, K.: 1995. Evaluation of tolerance and efficacy of S(+)-ibuprofen (Seractil®) in daily practice: a post-marketing-surveillance study in 1400 patients. J. Clin. Pharmacol. 35: 921-940.

Choi, V.M.I., Coates, J.E., Chooi, J., Thomson, A.B.R., Russell, A.S.: 1995. Small bowel permeability - a variable effect of NSAIDs. Clin. Invest. Med. 18(5): 357-361.

Citi, S.: 1993. The molecular organization of tight junctions. J. Cell Biol. 121: 485-489.

Claude, P.: 1978. Morphological factors influencing transepithelial permeability: a model for the resistance of the zonula occludens. J. Membrane Biol. 39, 219-232.

Collins, A.J., Davies, J., Dixon, A.: 1988. A prospective endoscopic study of the effect of Orudis and Oruvail on the upper gastrointestinal tract in patients with osteoarthritis. Br. J. Rheumatol. 27: 106 -109.

Collins, A.J., Reid, J., Soper, C.J., Notarianni, L.J.: 1995. Characteristics of ulcers of the small bowel induced by non-steroidal anti-inflammatory drugs in the rat: Implications for clinical practice. Br. J. Rheumatol. 34: 727-731.

Committee on the Safety of Medicines.: 1986. Non-steroidal anti-inflammatory drugs and serious gastrointestinal adverse reactions-2. Br. Med. J. 292: 1190-1191.

Cox, P.G.F., Moons,W.M., Russel, F.G.M., van Ginneken, C.A.M.: 1991. Renal handling and effects of S(+)-ibuprofen and R(-)-ibuprofen in the rat isolated perfused kidney. Br. J. Pharmacol.,103, 1542-1546.

Coutrot, S., Roland, D., Barbier, J., van der Marq, P., Alcalay, M., Matchuansky, C.: 1978. Acute perforation of colonic diverticula associated with short-term indomethacin. Lancet 2: 1055-1056.

Cooper, B.T.: 1984. Small intestinal permeability in clinical practice. J. Clin. Gastroenterol. 6: 499-501.

Cree, I.A., Walker, M.A., Wright, M., Forrester, J.S.: 1985. Osmosin and ileal ulceration: a case report. Scot. Med. J. 30: 40-41.

Csaky, T.Z.: 1981. Factors involved in the integrated mechanism of intestinal absorption. In: Advances in physiological sciences. Proceedings of the 28th international congress of physiological sciences, Budapest, 1980, Pergamon, Oxford, vol 12.

Cutler, C., Rex, D.K., Cummings, O.W.: 1993. Per anus enteroscopic demonstration of a non-steroidal anti-inflammatory drug-induced ileal stricture. Gastroint. Endos. 39 (4): 601-603.

Cuzzolin, L., Conforti, A., Adami, A., Lussignoli, S., Menestrina, F., Del Soldato, P., Benoni, G.: 1995. Anti-inflammatory potency and gastrointestinal toxcity of a new compound, nitronaproxen. Pharmacol. Res. 31(1): 61-65.

D'Alteroche, L., Boulier, P., Barre, I., Metman, E-H.: 1994. Les lesions coliques droites sont-elles specifiques des AINS a liberation prolongee? Gastroenterol. Clin. Biol. 18: 1042-1043.

D'Arienzo, M., Pennisi, M., Zanolo, G., Borsa, M.: 1984. Ketoprofen lysine: ketoprofen serum levels and analgesic activity. Drugs. Clin. Res. 12: 863-866.

Dahinden, C., Fehr, J.: 1980. Receptor directed inhibition of chemotactic factor induced neutropenic hyperactivity by pyrazolone derivatives. J. Clin. Invest. 66: 884-91.

Davies, D.R., Brightmore, T.: 1970. Idiopathic and drug-induced ulceration of the small intestine. Br. J. Surg. 57(2): 134-139.

Davies, G.R., Rampton, D.S.: 1991. The pro-drug sulindac may reduce the risk of intestinal damage associated with the use of conventional non-steroidal anti-inflammatory drugs. Aliment. Pharmacol. Therap. 5: 593-598.

Davies, G.R., Wilkie, M.E., Rampton, D.S.: 1993. Effects of metronidazole and misoprostol on indomethacin-induced changes in intestinal permeability. Dig. Dis. Sci. 38 (3): 417-425.

Davies, N.M., Jamali, F., Skeith, K.J.: 1996. Nonsteroidal antiinflammatory drug (NSAID)-Induced Enteropathy and Severe Chronic Anemia in a Patient with Rheumatoid Arthritis. Arth. Rheum. 39(2): 321-324.

Davies, NM., Anderson, K., Stevenson, B.R., Jamali, F.: 1995b. Non-Steroidal Antiinflammatory Drug (NSAID)-Induced Damage: *In Vitro* Cell Culture Models of Toxicity. Pharm. Res. 12(9): A8351 S-414.

Davis, S.S., Hardy, J.G., Fara, J.W.: 1986. Transit of pharmaceutical dosage forms through the small intestine. Gut 27: 886-892.

Day, R.O., Furst, D.E., Dromgoole, S.H.: 1982. Relationship of serum naproxen concentration to efficacy in rheumatoid arthritis. Clin. Pharmacol. Ther. 31: 733-740.

Day, T.K.: 1983. Intestinal perforation associated with osmotic slow release indomethacin capsules. Br. Med. J. 287: 1672-1672.

Day, R.O., Graham, G.G., Williams, K.M., Champion, G.D., De Jager, J.: 1987. Clinical pharmacology of Non-Steroidal Anti-inflammatory Drugs. Pharmacol. Ther., 33, 383-433.

Day, R.O., Graham, G.G., Williams, K.M., Brooks, P.M.: 1988. Variability in response to NSAIDs fact or fiction? Drugs 36: 643-651.

Day, R.O., Graham, G.G., Bieri, D.: 1989. Concentration-response relationships for salicylate-induced ototoxicity in normal volunteers. Br. J. Clin. Pharmacol. 28: 695-702.

Deakin, M.: 1988. Small bowel perforation associated with an excessive dose of slow release diclofenac sodium. Br. Med. J. 297: 488-489.

Del Soldato, P., Foschi, D., Benoni, G., Velo, G.P.: Early and late phases in the formation by anti-inflammatory drugs of intestinal lesions in rats. In: Rainsford KD, Velo GP, Eds. Side-effects of antiinflammatory drugs. Lancaster: MTP Press, 67-81.

Del Soldato, P., Foschi, D., Benoni, G., Scarpignato, C.: 1985. Oxygen free radicals interact with indomethacin to cause gastrointestinal injury. Agents and Actions, 17, 484-488.

Dietzel, K., Beck, W.S., Schneider, H-T., Geisslinger, G., Brune, K.: 1990. The biliary elimination and enterohepatic circulation of ibuprofen in rats. Pharm. Res. 7: 87-90.

Dobrinska, M.R., Furst, D.E., Spigel, T. Vincet, W.C., Tompkins, R., Duggan, D.E., Davies, R.C., Paulus, H.E.: 1983. Biliary secretion of sulindac and metabolites in man. Biopharm. Drug. Disp. 4: 347-358.

Duffy, M.E., Hamau, B., Ho, S., Bentzel, C.J.: 1981. Regulation of epithelial tight junction permeability by cyclic AMP. Nature 204: 451-453.

Duggam, D.E., Hooke, K.F., Noll, R.M., Kwan, K.C.: 1975. Enterohepatic circulation of indomethacin and its role in intestinal irritation. Biochem. Pharmacol. 25: 1749-1754.

Dujovone, C.A., Pitterman, A., Vincek, W.C., Drorinska, M.R., Davies, R.O., Duggan, D.E.: 1983. Enterohepatic circulation of sulindac and metabolites. Clin. Pharmacol. Therap. 33: 172-177.

Dunagan, F.M., McGill, P.E., Kelman, A.W., Whiting, B.: 1986. Quantitation of dose and concentration-effect relationships for fenclofenac in rheumatoid arthritis. Br. J. Clin. Pharmacol. 21: 409-416.

Dunagan, F.M., McGill, P.E., Kelman, A.W., Whiting, B.: 1988. Naproxen dose and concentration: response relationship in rheumatoid arthritis. Br. J. Rheum. 27: 48-53.

Dusci, L.J., Hackett, L.P.: 1979. Determination of some anti-inflammatory drugs in serum by HPLC. J. Chromatogr. 172: 516-520.

Dwivedi, S.K, Sattari, S., Jamali, F., Mitchell, A.G.: 1992. Ibuprofen racemate and enantiomers: phase diagram, solubility and thermodynamic studies. Int. J. Pharm. 87: 95-104.

Dyer, N.H., Kendall, M.J., Hawkins, C.F.: 1971. Malabsorption in rheumatoid arthritis. Ann. Rheum. Dis. 30: 626-630.

Ecknauer, R., Buck, B., Breitig, D.: 1983. An experimental model for measuring intestinal permeability. Digestion 26: 24-32.

Edwards, A.L., Heargerty, A.M., Bing, R.F.: 1983. Enteritis and colitis associated with mefenamic acid. Br. Med. J. 287: 1627.

Elia, M., Behrens, R., Northrop, C., Wraight, P., Neale, G.: 1987. Evaluation of mannitol, lactulose and ^{51}Cr-labeled ethylenediaminetetra-acetate as markers of intestinal permeability in man. Clin. Sci. 73: 197-204.

Epstein, A.M., Read, J.L., Winickoff, R.: 1984. Physician beliefs, attitudes, and prescribing behavior for anti-inflammatory drugs. Am. J. Med. 77: 313-318.

Elliot, G.A., Purmalis, A., Vander Meer, D.A., Denlinger, R.H.: 1988. The Propionic acids. Gastrointestinal toxicity in various species. Toxicol. Pathol., 16: 245-249.

Evans, A.M., Nation, R.L., Sansom, L.N., Bochner, F., Somogyi, A.A.: 1991. Effect of racemic ibuprofen dose on the magnitude and duration of platelet cyclo-oxygenase inhibition: relationship between inhibition of thromboxane synthesis and the plasma unbound concentration of S(+)-ibuprofen. Br. J. Clin. Pharmacol. 31: 131-138.

Evans, A.M.: 1992. Enantioselective pharmacodynamics and pharmacokinetics of chiral non-steroidal anti-inflammatory drugs. Clin. Pharmacol. 42: 237-256.

Fälth-Magnuson, K., Jansson, G., Stenhammar, L., Sundqvist, T., Magnusson, K.E.: 1989. Intestinal permeability assessed with different-sized polyethylene glycols in children undergoing small-intestinal biopsy for suspected celiac disease. Scand. J. Gastroenterol. 24: 40-46.

Fang, W-F., Broughton, A., Jacobson, E.D.: 1977. Indomethacin-induced intestinal inflammation. Dig. Dis. 22(9): 749-760.

Farquharson-Roberts, M.A., Giddings, A.E.B., Nunn, A.J.: 1975. Perforation of small bowel due to slow release potassium chloride (slow-K). Br. Med. J. 3: 206.

Fellows, I.W., Clarke, J.M.F., Roberts, P.F.: 1992. Non-steroidal anti-inflammatory drug-induced jejunal and colonic diaphragm disease: a report of two cases. Gut 33: 1424-1426.

Fink, M.P., Kaups, K.L., Wang, H., Rothschild, H.R.: 1992. Ibuprofen improves survival but does not ameliorate increased gut mucosal permeability in endotoxic pigs. Arch. Surg. 127: 49-54.

Fleischmann, R.M.: 1992. Clinical efficacy and safety of nambumetone in rheumatoid arthritis and osteoarthritis. J. Rheumatol. 19 (suppl 36) 32-40.

Ford, J., Martin, S.W., Houston, J.B.: 1995. Assessment of intestinal permeability changes induced by nonsteroidal anti-inflammatory drugs in the rat. J. Pharm.Toxicol. Meth. 34: 9-16.

Foster, R.T., Jamali, F.: 1988. Stereoselective pharmacokinetics of ketoprofen in the rat: Influence of route of administration. Drug. Metab. Dispos. 16(4): 623-626.

Foster, P.N., Axon, A.T.R., Packman,L., Harper, A.: 1989. Nonsteroidal anti-inflammatory drugs and the bowel. Lancet 2: 1048.

Freeman, H.J.: 1986. Sulindac-associated small bowel lesion. J. Clin. Gastroenterol. 8(5): 569-571.

Fries, J.F.: 1991. NSAID Gastropathy: Epidemiology. J. Musculoskeletal. Med. 8: (2), 21-28.

Argot, D., Chaussade, S., D'Alteroche, L., Desbazeille, F., Grandjouan, S., Louvel, A., Douun, J., Causse, J., Festin, D., Chapuis, Y., Legoux, J.L.: 1995. Nonsteroidal anti-inflammatory drug-induced colonic strictures: two cases and literature review. Am. J. Gastroenterol. 90(11) 2035-2038.

Geisslinger, G., Schuster, O., Stock, K.P., Loew, D., Bach, G.L., Brune, K.: 1990. Pharmacokinetics of S(+)-and R(-)-ibuprofen in volunteers and first clinical experience of S(+)-ibuprofen in rheumatoid arthritis. Eur. J. Clin. Pharmacol. 38: 493-497.

Geisslinger, G., Ferreira, S.H., Menzel, S., Schlott, D., Brune, K.: 1994. Antinociceptive actions of R(-)-Flurbiprofen-A non-cycloxygenase inhibiting 2-arylpropionic acid- in rats.
Life Sciences 54(10): 173-177.

Gibson, G.R., Whitacre, E.B., Ricotti, C.A.: 1992. Colitis induced by nonsteroidal antiinflammatory drugs. Arch. Int. Med. 152: 625-632.

Giercksky, K.-E., Huseby, G., Rugstad, H.-E.: 1989. Epidemiology of NSAID-related gastrointestinal side effects. Scand. J. Gastroenterol. 24: (Suppl. 163), 3-8.

Glaborg-Jorgensen, T., Weis-Fogh, U.S., Olsen, H.P.: 1976. Influence of acetylsalicylic acid (aspirin) on gastric mucosal content of energy-rich phophate bonds. Scand. J. Clin. Lab. Invest. 36: 771-777.

Glavin, G.B., Szabo, S.: 1992. Experimental gastric mucosal injury: laboratory models reveal mechanisms of pathogenesis and new therapeutic strategies. FASEB J. 6: 825-831.

Going, J., Canvin, J., Sturrock, R.: 1993. Possible precursor of diaphragm disease in the small intestine. Lancet 341: 638-639.

Gosh, P.: 1988. Anti-rheumatic drugs and cartilage. Baillieres Clin. Rheumatol. 2(2): 309-328.

Graham, D.Y., Smith, J.L., Holmes, G.I., Davies, R.O.: 1985. Nonsteroidal anti-inflammatory effect of sulindac sulfoxide and sulfide on gastric mucosa. Clin. Pharmacol. Ther. 38: 65-70.

Granerus, G., Aurell, M.: 1981. Reference values for ^{51}Cr-EDTA clearance as a measure of glomerular filtration rate. Scand. J. Clin. Invest. 41: 611-616.

Grennan, D.M., Aarons, L., Siddiqui, M., Richards, M., Thompson, R., Higham, C.: 1983. Dose-response study with ibuprofen in rheumatoid arthritis: clinical and pharmacokinetic findings. Br. J. Clin. Pharmacol. 15: 311-316.

Gumbiner, B.: 1987. Structure, biochemistry, and assembly of epithelial tight junctions. Am. J. Physiol. 253: C749-C758.

Hall, R.I., Pelty, A.H., Cobden, I., Lendrum, R.: 1983. Enteritis and colitis associated with mefenamic acid. Br. Med. J. 287: 1182.

Halter, F., Weber, B., Huber, T., Eigenmann, F., Frey, M.P., Ruchti, C.: 1993. Diaphragm disease of the ascending colon associated with sustained release diclofenac. J. Clin. Gastroenterol. 16: 74-80.

Hamilton, I., Cobden, I., Rothwell, J., Axon, A.T.R.: 1982. Intestinal permeability in coeliac disease. The response to gluten withdrawal and single dose gluten challenge. Gut 23: 202-210.

Haque, S., Haswell, J.E., Dreznick, J.T., West, A.B.: 1992. A cecal diaphragm associated with the use of nonsteroidal anti-inflammatory drugs. J. Clin. Gastroenterol. 15(4): 332-335.

Haslock, I.: 1990. Prevalence of NSAID-induced gastrointestinal morbidity and mortality. J. Rheumatol. (suppl. 20) 17: 2-6.

Hayllar, J., Macpherson, A., Bjarnason, I.: 1992. Gastroprotection and nonsteroidal anti-inflammatory drugs (NSAIDs). Drug Safety 7: 86-105.

Hayllar, J., Smith, T., Macpherson, A., Price, A.B., Gumpel, M., Bjarnason, I.: 1994. Nonsteroidal anti-inflammatory drug-induced small intestinal inflammation and blood loss. Arth. Rheum. 37(8): 1146-1150.

Helleberg, L.: 1981. Clinical pharmacokinetics of indomethacin. Clin. Pharmacokin. 6: 245-258.

Hershfield, N.B.: 1992. Endoscopic demonstration of non-steroidal anti-inflammatory drug-induced small intestinal strictures. Gastroint. Endosc. 38(3): 388-390.

Hervas, J.A., Masip, M.C., Alomar, A., Bergante, J.I.: 1986. Localized intestinal perforation after intravenous indomethacin in a premature infant. Helv. Paediat. Acta. 41: 437-440.

Holford, N.H.G., Sheiner, L.B.: 1981. Understanding the dose-effect relationship: Clinical application of pharmacokinetic-pharmacodynamic models. Clin. Pharmacokin. 6: 429-453.

Holford, N.H.G., Sheiner, .L.B.A. Mathematical Modeling Tool for the PROPHET System. In PROPHET Public Procedures Notebook. Perry, H. Med. Bolt, Bernak and Newman Inc, Cambridge, MA, 1982.

Hollander, D., Vadheim, C.M., Bretholz, E., Peterson, M., Delahunty, T., Rotter, J.I.: 1986. Increased intestinal permeability in patients with Crohn's disease and their relatives. Ann. Intern. Med. 105: 883-885.

Hollingsworth, J., Alexander-Williams, J.: 1991. Nonsteroidal anti-inflammatory drugs and stercoral perforation of the colon. Ann. R. Col. Surg. Eng. 73: 337-340.

Holt, S., Rigoglioso, V., Sidhu, M., Irshad, M., Howden, C.W., Mainero, M.: 1993. Nonsteroidal antiinflammatory drugs and lower gastrointestinal bleeding. Dig. Dis. Sci. 38: 1619-1623.

Huber, T., Ruchti, C., Halter, F.: 1991. Nonsteroidal antiinflammatory drug-induced colonic strictures: a case report. Gastroenterology 100: 1119-1122.

Hucker, H.B., Hoffman, E.A.: 1971. Physiologic disposition and metabolic fate of indomethacin analogs. I. J. Pharm. Sci. 60(7): 1049-1052.

Hudson, N., Wilkinson, M.J., Swannell, A.J., Steele, R.J., Hawkey, C.J.: 1993. Ileo-cecal ulceration associated with the use of diclofenac slow release. Aliment. Pharmacol. Ther. 7: 197-200.

Hunt, O.F., Giordani, A.B., Rhodes, G., Herold, D.A.: 1982. Mixture analysis by triple quadruple mass spectrometry. Clin. Chem. 28: 2387-2392.

Hutt, A.J., Caldwell, J.: 1983. The metabolic chiral inversion of 2-arylpropionic acids-a novel route with pharmacological consequences. J. Pharm. Pharmacol. 35:693-704.

Hutt, A.J., Caldwell, J.: 1984. The importance of stereochemistry in the clinical pharmacokinetics of the 2-arylpropionic acid non-steroidal anti-inflammatory drugs. Clin. Pharmacokin. 9(4): 371-373.

Irvine, E.J., Goodacre, R.L., McCullough, H.N., Thoma, E.M.: 1995. Damaged surface area of gastric mucosa correlates with urinary sucrose excretion. Arth. Rheum. 38(9): A57 S162.

Isaacs, P.E.T., Sladen, G.E., Filipe, I.: 1987. Mefenamic acid enteropathy. J. Clin. Pathol. 40: 1221-1227.

Iwakawa, S., He, X.W., Hashimoto, S., Volland, C., Benet, L.Z., Lin, E.T.: 1991. Stereoselective disposition of ketoprofen in rats. Drug. Metab. Disp. 19(3): 717-718.

Jamali, F., Singh, N.N., Pasutto, F.M., Coutts, R.T., Russell, A.S.: 1988. Pharmacokinetics of ibuprofen enantiomers in man following oral administration of tablets with different absorption rates. Pharm. Res. 5: 40-43.

Jamali, F.: 1988. Pharmacokinetics of Enantiomers of Chiral Non-Steroidal Anti-inflammatory Drugs. Eur. J. Drug Metab. Pharmacokin. 13, 1-9.

Jamali, F., Berry, B.W., Tehrani, M.R., Russell, A.S.: 1988. Stereoselective pharmacokinetics of flurbiprofen in humans and rats. J. Pharm. Sci. 77(8): 666-669.

Jamali, F., Berry, B.W.: 1990. Pharmacokinetics of ibuprofen enantiomers in the rat: effect of route of administration. Eur. J. Drug. Metab. Pharmacokin. 15(2):A402, 2.

Jamali, F., Mehvar, R., Russell, A.S., Sattari, S., Yakimets, W.W., Koo, J.: 1991a. Human pharmacokinetics of ibuprofen enantiomers following different doses and formulations: Intestinal chiral inversion. J. Pharm. Sci. 81: 221-225.

Jamali, F., Collins, D., Berry, B., Molder, S., Cheung, R., McColl, K., Cheung, H.: 1991b. Comparative bioavailability of two flurbiprofen products: stereospecific versus conventional approach. Biopharm. Drug. Disp. 12: 435-445.

Jenkins, R.T., Rooney, R.T., Jones, D.R., Bienenstock, J., Goodacre, R.L.: 1987a. Increased intestinal permeability in patients with rheumatoid arthritis: a side-effect of oral non-steroidal antiinflammatory drug therapy ? Br. J. Rheumatol. 26: 103-107.

Jenkins, R.T., Jones, D.B., Goodacre, R.L., Collins, S.M., Coates, G., Hunt, R.H., Bienenstock, J.: 1987b. Reversibility of increased intestinal permeability to ^{51}Cr-EDTA in patients with gastrointestinal inflammatory diseases. Am. J. Gastroenterol. 82, 11, 1159-1164.

Jenkins, R.T., Rooney, P.J., Hunt, R.H.: 1988. Increased bowel permeability to [^{51}Cr]EDTA in controls caused by Naproxen is not prevented by cytoprotection. Arth. Rheum., 31 (Suppl 1): R11.

Jenkins, A.P., Trew, D.R., Crump, B.J., Nukajam, W.S., Foley, J.A., Menzies, I.S., Creamer B.: 1991. Do non-steroidal anti-inflammatory drugs increase colonic permeability? Gut 32: 66-69.

Johnston, F.: 1987. Recurrent small bowel obstruction associated with piroxicam. Br. J. Surg. 74:654.

Juby, L.D., Rithwell, J., Axon, A.T.R.: 1989. Lactulose / mannitol test: an ideal screen for celiac disease. Gastroenterology 96: 79-85.

Kantor, H.S., Hampton, M.: 1978. Indomethacin in submicromolar concentrations inhibits cyclic AMP-dependent protein kinase. Nature 276: 841-842.

Kauffman, G.L., Foley, K.T., Grossman, M.I.: 1979. Cimetidine does not inhibit Indomethacin-induced small bowel ulceration in the rat. Proc. Soc. Exp. Biol. Med. 161: 512-514.

Keating, J.P., Rees, M.: 1992. Diaphragm disease: a rare cause of small-bowel obstruction. Hospital Update 1: 48-49.

Keating, J.P., McIlwaine, J.: 1993. Simultaneous small and large bowel ulceration associated with short term NSAID use. N.Z. Med. J. 106 (965): 438.

Kendall, M.J., Hawkins, C.F.: 1971. Xylose test; effect of aspirin and indomethacin. Br. Med. J. I: 535-553.

Kent, T.H., Cardelli, R.M., Stamler, F.W.:1969. Small intestinal ulcers and intestinal flora in rats given indomethacin. Am. J. Pathol. 54: 237-245.

Kim, C.D., Hong, K.W.:1995. Preventative effect of rebamipide on gastric lesions induced by ischemic-reperfusion in the rat. J. Pharm. Exp. Ther. 275: 340-344.

Knihiniicki, R.D., Day, R.O., Graham, G.G., Williams, K.M.: 1990. Stereoselective disposition of ibuprofen and flurbiprofen in rats. Chirality. 2: 134-140.

Knoben, J.E., Anderson, P.O. Handbook of Clinical Drug Data, 6th ed.; Drug Intelligence Publications, Inc., Hamilton, 1988.

Köhler, G., Primbs, P., Morand, J., Rubelt, C.H.: 1985. Correlation between ketoprofen plasma levels and analgesic effect in acute lumbar pain and radicular pain. Clin. Rheumatol. 4: 399-404.

Krupińska, J., Sobański, H., Piotrowicz, J., Cebo, B., Mazur, J.: 1980. Antioxidants as agents reducing the toxicity of indomethacin. Acta. Biol. Med. Germ. 39: 717-721.

Kubes, P.: 1992. Nitric oxide modulates epithelial permeability in the feline small intestine. Am. J. Physiol. 262: G1138-G1142

Kubes, P. Szuki, M, Granger, D.N.: 1991. Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc. Natl. Acad. Sci. USA 88: 4651.

Kühl, G., Willie, L., Bolkenius, M., Seyburth, H.W.: 1985. Intestinal perforation associated with indomethacin treatment in premature infants. Eur. J. Pediatr. 143: 213-216.

Kwo, P.Y., Tremaine, W.J.: 1995. Nonsteroidal anti-inflammatory drug-induced enteropathy: case discussion and review of the literature. Mayo Clin. Proc. 70: 55-61.

Laidler, P., Maslin, S.C., Gilhome, R.W.: 1985. What's new in Osmosin and intestinal perforation? Pathol. Res. Pract. 180: 74-76.

Lang, J., Price, A.B., Levi, A. J., Burk, M., Gumpel, M.J., Bjarnason, I.: 1988. Diaphragm disease: the pathology of non-steroidal anti-inflammatory drug induced small intestinal strictures. J. Clin. Pathol. 41: 516-526.

Langenbach, R., Morham, S.G., Tiano, H.F., Loftin, C.D., Ghanayem, B.I., Chluda, P.C., Mahler, J.F., Lee, C.A., Goulding, E.H., Kluckman, K.D., Kim, H.S., Smithies, O.: 1995. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. Cell 83: 483-492.

Langman, M.J.S., Morgan, L., Worall, A.: 1985. Use of inflammatory drugs by patients admitted with small or large bowel perforations and haemorrhage. Br. Med. J. 290: 347-349.

Lanza, F.L., Royer, G.L., Nelson, R.S.: 1980. Endoscopic evaluation of the effects of aspirin, buffered aspirin, and enteric-coated aspirin on gastric and duodenal mucosa. New Engl. J. Med. 303(3): 136-138.

Laska, E.M., Sunshine, A., Marrero, I.: 1986. The correlation between blood levels of ibuprofen and clinical analgesic response. Clin. Pharmacol. Ther. 40(1): 1-7.

Leclerq-Foucart, J., Forget, P., Sadoyez-Goffavy, F., Zappitelli, A.: 1986. Intestinal permeability to [^{51}Cr] EDTA in children with cystic fibrosis. J. Ped. Gastro. Nutr. 5:384-387.

Lee, E.J.D., Williams, K., Day, R., Graham, G., Champion, D.: 1985. Stereoselective disposition of ibuprofen enantiomers in man. Br. J. Clin. Pharmacol. 19: 669-674.

Lennert, V.K.A., Kootz, F.: 1967. Nil nocre! Arzneimittelbedingte dünndardmulzera. Münch. Med. Wochenschr. 40: 2058-2062.

Levi, S., de-Lacey, G., Price, A.B., Gumpel, M.J., Levi, A.J., Bjarnason, I.: 1990. 'Diaphragm-like' strictures of the small bowel in patients treated with non-steroidal anti-inflammatory drugs. Br. J. Radiol. 63: 186-189.

Levi, S., Shaw-Smith, C.: 1994. Nonsteroidal anti-inflammatory drugs: how do they damage the gut? Brit. J. Rheumatol. 33(7): 605-612.

Lichtenberger, L.M., Wang, Z.M., Romero, J.J., Ullo, C., Perez, J.C., Giraud, M.N., Barreto, J.C.: 1995. Non-steroidal anti-inflammatory drugs (NSAIDs) associate with zwitterionic phospholipids: insight into the mechanism and reversal of NSAID-induced gastrointestinal injury. Nature Medicine 1(2): 154-158.

Ligumsky, M., Golanska, E.M., Hansen, D.G., Kauffman, G.L.: 1984. Aspirin can inhibit gastric mucosal cycloxygenase without causing lesions in the rat. Gastroenterology 84: 756-761.

Ligumsky, M., Sestieri, M., Karmeli, F., Zimmerman, J., Okon, E., Rachmilewitz, D.: 1990. Rectal administration of non-steroidal anti-inflammatory drugs. Effect on rat gastric ulcerogenicity and prostaglandin E_2 synthesis. Gastroenterology 98: 1245-1249.

Lim, S.G., Menzies, I.S., Lee, C.A., Johnson, M.A., Pounder, R.E.: 1993. Intestinal permeability and function in patients infected with human immunodeficiency virus. Scand. J. Gastroenterol. 28: 573-580.

Lindsey, J.R.: 1979. Historical Foundations, In, The Laboratory Rat. Vol. I . Baker, H.J., Lindsey, J.R., Weisbroth, S.H.(eds.). PP.1-36, Academic press, Toronto.

Lökken, P., Sögnen, E.: 1967. ^{51}Cr-EDTA as a reference substance in research on gastrointestinal functions. Acta. Pharmacol. Toxicol. 25(4): 39.

Løkken, P.: 1970. Studies on ^{51}Cr-EDTA and its evaluation as a reference substance in gastro-intestinal research. Universitetsforlaget, Oslo.

Lopez-Belmonte, J., Whittle, B.J.R., Moncada, S.: 1993. The actions of nitric oxide donors in the prevention or induction of injury to rat gastric mucosa. Br. J. Pharmacol. 108: 73-79 (1993).

Madara, J.L., Trier, J.S.:1980. Structural abnormalities of jejunal epithelial membranes in coeliac sprue. Lab Invest. 43: 254-261.

Madara, J.L., Moore, R., Carlson, S.: 1987. Alteration of intestinal tight junction structure and permeability by cytoskeletal contraction. Am. J. Physiol. 253: G854:G861.

Madara, J.L., Stafford, J.: 1989. Interferon-γ directly affects barrier function of cultured intestinal epithelial monolayers. J. Clin. Invest. 83: 724-727.

Madok, R., Mackenzie, J.A., Lee, F.D., Bruckner, F.E., Terry, T.R. Sturrock, R.D.: 1986. Small bowel ulceration in patients receiving NSAIDs for rheumatoid arthritis. Quart. J. Med. 58: 53-58.

MacKenzie, J.A., Lee, F.D., Bruckner, F.E., Terry, T.R., Sturrock, R.D.: 1986. Small bowel ulceration in patients receiving non-steroidal anti-inflammatory drugs for rheumatoid arthritis. Quart. J. Med. 58(225): 53-58.

Magnusson, K.-E., Sundquist, T., Sjödahl, R., Tagesson, C.: 1983. Altered intestinal permeability to low-weight polyethylene glycols (PEG 400) in patients with Crohn's disease. Acta. Chir. Scand. 149: 323-327.

Mahmud, T., Wrigglesworth, J.M., Rafi, S., Scott, D.L., Bjarnason, I.: 1995. Uncoupling of oxidative phophorylation by non-steroidal anti-inflammatory drugs (NSAIDs) is related to ionizable moiety and can be altered by modification of carboxylic group. Arth. Rheum. 38(9): A326 S207

Malandrino, S., Bestetti, A., Fumagalli, G., Borsa, M.: 1987. Role of endogenous prostaglandins in protection of rat gastric mucosa by tripotassium dicitrate bismuthate. Scand. J. Gastroenterol. 22: 943-948.

Malmberg, A.B., Yaksh, T.L.: 1992. Hyperalgesia mediated by spinal glutamate or substance P receptor blocked by spinal cyclooxygenase inhibition. Science 257:1276-1278.

Malmberg, A.B., Yaksh, T.L.: 1994. Antinociception produced by spinal delivery of the S and R enantiomers of flurbiprofen in the formalin test. Eur. J. Pharmacol. 256: 205-209.

Markey, C.M., Alward, A., Weller, P.E., Marnett, L.J.: 1987. Quantitative studies of hydroperoxide reduction by prostaglandin H synthase. Reducing substrate specificity and the relationship of peroxidase to cyclooxygenase activities. J. Biol. Chem. 262(13): 6266-6279.

Marlow, N., Chiswick, M.L.: 1982. Neonatal bartter's syndrome, indomethacin and necrotising enterocolitis. Acta. Paediatr. Scand. 71: 1031-1032.

Masferrer, J.L., Zweifel, B.S., Manning, P.T., Hauser, S.D., Leahy, K.M., Smith, W.G., Isakson, P.C., Seibert, K.: 1994. Selective inhibition of inducible cyclooxygenase in vivo is antiinflammatory and nonulcerogenic. Proc. Natl. Acad. Sci. U.S.A. 91: 3228-3232.

Marshall, T.A.: 1985. Intestinal perforation following enteral administration of indomethacin. J. Pediatr. 107: 484-485.

Matsuhashi, N., Yamada, A., Hiraishi, M., Konishi, T., Minota, S., Saito, T., Sugano, K., Yazaki, Y., Mori, M., Shiga, J.: 1993. Multiple Strictures of the small intestine after long-term nonsteroidal anti-inflammatory drug therapy. Am. J. Gastroenterol. 87:(9) 1183-1186.

Maxton, D.G., Bjarnason, I., Reynolds, A.P., Catt, S.D., Peters, T.J., Menzies, I.S.: 1986. Lactulose ^{51}Cr-labelled ethylenediaminetetra-acetate, L-rhamnose and polyethylene glycol 500 as probe markers for assessment in vivo of human intestinal permeability. Clin. Sci. 71: 71-80.

May, G.R., Crook, P., Moore, P.K., Page, C.P.: 1991. The role of nitric oxide as an endogenous regulator of platelet and neutrophil activation within the pulmonary circulation of the rabbit. Br. J. Pharmacol. 102: 759-766.

McCarthy, D.M.: 1989. Nonsteroidal antiinflammatory drug-induced ulcers: management by traditional therapies. Gastroenterology 96: 647-655.

McCune, K.H., Allen, D., Cranley, B.: 1992. Small bowel diaphragm disease - strictures asscoiated with non-steroidal anti-inflammatory drugs. Ulster Med. J. 61(2): 182-184.

Meade, E.A., Smith, W.L., De Witt, D.L.: 1991. Differential inhibition of prostaglandin endoperoxide synthetase (cylooxygenase) isoenzymes by aspirin and other Non steroidal antiinflammatory drugs. J. Biol. Chem. 268: 6610-6614.

Meddings, J.B., Sutherland, L.R., Byles, N.I., Wallace, J.L.: 1993. Sucrose: A novel permeability marker for gastroduodenal disease. Gastroenterology 104: 1619-1626.

Meddings, J.B., Kirk, D., Olson, M.E.: 1995. Non-invasive detection of nonsteroidal anti-inflammatory drug-induced gastropathy in dogs. Am. J. Vet. Res. 56(8): 977-981.

Melarange, R., Moore, G., Blower, P.R., Coates, M.E., Ward, F.W., Ronaasen, V.:1992. A comparison of indomethacin with ibuprofen on gastrointestinal mucosal integrity in conventional and germ-free rats. Aliment. Pharmacol. Ther. 6: 67-77.

Menzies, I.S. In: Intestinal absorption and secretion, Skadhauge, H.; MTP Press: Lancaster, pp. 527-543, 1984.

Menzel-Soglowek, S., Geisslinger, G., Beck, W.S., Brune. K.: 1993. Variability of inversion of (R)-flurbiprofen in different species. J. Pharm. Sci. 81(9): 888-891.

Metzler, C.M., Weiner, D.L.: 1992. PCNONLIN-Version 4.1. Statistical Consultants, Inc. Lexington, K.Y.

Mielants, H., Goemaere, S., De Vos, M., Schelstraete, K., Goethal, K., Maertens, M., Ackerman, C., Veys, E.M.: 1991a. Intestinal mucosal permeability in inflammatory rheumatic diseases. I. Role of antiinflammatory drugs. J. Rheumatol. 18: 389-393.

Mielants, H., De Vos, M., Goemaere, S., Schelstraete, K., Cuvelier, C., Goethals, K., Maertens, M., Ackerman, C., Veys, E.M.: 1991b. Intestinal mucosal permeability in inflammatory rheumatic diseases. II. Role of Disease. J. Rheumatol. 18: 394-400.

Mielants, H., Vey, E.M.: 1985. NSAID and the leaky gut. Lancet i: 218.

Milks, L.C., Conyers, G.P., Cramer, E.B.: 1986. The effect of neutrophil migration on epithelial permeability. J. Cell Biol. 103: 2729-2738.

Mitchell, J.B., Samuni, A., Krishna, M.C., Glass, J., Wilson, L., Samuni, U., Russo, A.: 1990. Biologically active metal-independent superoxide dismutase mimics. Biochemistry 29: 2802-2807.

Mitchell, J.A., Akarasereenont, P., Thiemermann, C., Flower, R.J. and Vane, J.R.: 1994. Selectivity of non-steroidal antiinflammatory drugs as inhibitors of constitutive an inducible cylooxygenase. Proc. Natl. Acad. Sci. 90: 11693-11697.

Miyachi, Y., Yoshioka, A., Imamura, S., Niwa, Y.: 1987. Effect of sulphasalazine and its metabolites on the generation of reactive oxygen species. Gut 28: 190-195.

Miyahara, J.T., Karler, R.: 1965. Effect of salicylate on oxidative phophorylation and respiration of mitochondrial fragments. Biochem. J. 97: 194-198.

Moncada, S., Palmer, R.M.J., Higgs, E.A.: 1991. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol. Rev 43: 109-141.

Morris, A.J., Madhok, R., Sturrock, R.D., Capell, H.A., MacKenzie, J.F.: 1991. Enteroscopic diagnosis of small bowel ulceration in patients receiving non-steroidal anti-inflammatory drugs. Lancet 337: 520.

Morris, A.J., Wasson, L.A., Mackenzie, J.F.: 1992. Small bowel enteroscopy in undiagnosed gastrointestinal blood loss. Gut 33: 887-889.

Morris, A.J., Murray, L., Sturrock, R.D., Madhok, R. Capell, H.A., Mackenzie, J.F.: 1994. Short report: the effect of misoprostol on the anemia of NSAID enteropathy. Aliment. Pharmacol. Ther. 8: 343-346.

Müller, M.: 1986. Reductive activation of nitroimidazoles in anaerobic microorganisms. Biochem. Pharmacol. 35(1): 37-41.

Myers, A.B.R.: 1876. Salicin in acute rheumatism. Lancet 2: 676-677.

Nagaraj, H.S., Sandhu, A.S., Cook, L.N., Buchino, J.T., Groff, D.: 1981. Gastrointestinal perforation following indomethacin therapy in low birth weight infants. J. Pediatr. Surgery. 166: 1003-1007.

Nash, S., Stafford, J., Madara, J.L.: 1987. Effects of polymorphonuclear leukocyte transmigration on the barrier function of cultured intestinal epithelial monolayers. J. Clin. Invest. 123-134.

Neoptolemos, J.P., Locke, T.J.: 1983. Recurrent small bowel obstruction associated with phenylbutazone. Br. J. Surg. 70: 244-245.

Nilsson, U.S., Olsson, L.I., Carlin, G., Bylund, F.A.: 1989. Inhibition of lipid peroxidation by spin labels. Relationships between structure and function. J. Biol. Chem. 264: 11131-11135.

Nowak, K.J., Wennmalm, A.: 1978. Influence of indomethacin and of prostaglandin E1 on total and regional blood flow in man. Acta. Physiol. Scand. 102: 484-491.

O'Mahoney, S., Ferguson, A.: 1991. Small intestinal mucosal protection mechanisms and their importance in rheumatology. Ann. Rheum. Dis. 50: 331-336.

Palylyk, E.L., Jamali, F.: 1991. Simultaneous determination of ketoprofen enantiomers and probenecid in plasma and urine by high-performance liquid chromatography. J. Chromatogr. 568: 187-196.

Parke, A.L., Fagan, E.A., Chadwick, V.S., Hughes, G.R.V.: 1983. Gut permeability in Sjögren's syndrome. RA, and coeliac disease. Ann. Rheum. Dis. 42: 216.

Pearson, A.D.J., Craft, A.W., Pledger, J.V.: 1985. Small bowel function in acutely lymphoblastic leukemia. Aron. Dis. Chilh. 59: 460-465.

Peled, Y., Watz, C., Gilat, T.: 1985. Measurement of intestinal permeability using ^{51}Cr-EDTA. Am. J. Gastroenterol. 80(10): 770-773.

Perreard, M., Monges, D., Iconomidis, N.: 1989. Colite ulcereuse secondaire a la prise de diclofenac (Voltarene) per os. Gastroenterol. Clin. Biol. 13: 314-315.

Peskar, B.M., Kleien, A., Pyras, F., Muller, M.K.: 1986. Gastrointestinal toxicity. Role of prostaglandins and leukotrienes. Med. Toxicol. 1(suppl 1): 39-43.

Peters, T.J., Bjarnason, I.: 1988. Uses and abuses of intestinal permeability measurements. Can. J. Gastroenterol. 2(3):127-132.

Powell, D. In: Mechanisms of Mucosal Protection in the Upper Gastrointestinal Tract; Allen, A., Ed.; Raven Press: New York, pp 1-5., 1984.

Powell, D.W.: 1981. Barrier function of epithelia. Am. J. Physiol. 241: 275-288.

Pucius, R.J., Charles, A.K., Adair, H.M., Rowe, R.C.G., Hacking, J.C.: 1993. Diaphragm-like strictures of the colon induced by nonsteroidal anti-inflammatory drugs. Brit. J. Surg. 80:395-396.

Rabassa, A.A., Goddame, R., Sutton, F.M., Ou, C.N., Rognerud, C., Graham, D.Y.: 1994. Effect of H. Pylori infection on gastroduodenal mucosal permeability. Am. J. Gastroenterol. 89(8): A181 1330.

Rachmilewitz, D., Karmeli, F., Okon, E., Samuni, A.: 1994. A novel antiulcerogenic stable radical prevents gastric mucosal lesions in rats. Gut 35: 1181-1188.

Rafi, S., Somansandarum, S., Mahmud, T., Scott, D., Wrigglesworth, J.H., Bjarnason, I.: 1995. The importance of uncoupling of mitochondrial oxidative phophorylation in NSAID-induced gastrointestinal damage. Arth. Rheum. 38(9): A325 S207.

Rainsford, K.D., Whitehouse, M.W.: 1980. Biochemical gastroprotection from acute ulceration induced by aspirin and related drugs. Biochem. Pharmacol. 29: 1281-1289.

Rainsford, K.D.: 1987. Prevention of indomethacin-induced gastro-intestinal ulceration in rats by glucose-citrate formulations: Role of ATP in mucosal defenses. Br. J. Rheumatol. 26: (suppl 2): 81.

Rainsford, K.D., Whitehouse, M.W.: 1987. A pharmaceutical formulation containing indomethacin. Eur. Pat. App. EP 239332.

Rainsford, K.D.: 1987a. The effects of 5-lipoxygenase inhibitors and leucotriene antagonists on the development of gastric lesions induced by non-steroidal anti-inflammatory drugs in mice. Agents Actions 21: 316-319.

Rainsford, K.D. 1987b. Introduction and historical aspects of the side-effects of anti-inflammatory analgesic drugs. In Rainsford KD & Velo JP (eds) Side-effects of Anti-inflammatory Drugs. Part 1: Clinical and Epidemiological Aspects, pp 3-26. Lancaster: MTP Press.

Rainsford, K.D. In Handbook of Animal Models for Rheumatic Diseases; RA Greenwald, R.A.; Diamond, H.S., Eds.; CRC Press: Boca Raton, pp. 181-206., 1988.

Rainsford, K.D.: 1989. Mechanisms of gastrointestinal toxicity of non-steroidal anti-inflammatory drugs. Scand. J. Gastroentrol. 24: 9-16.

Rainsford, K.D.: 1990. NSAID Gastropathy : Novel physiochemical approaches for reducing gastric mucosal injury by drug complexation with cyclodextrins. Drug Invest. 2 (Suppl. 4): 3-10.

Rainsford, K.D., Dieppe, P.A., Pritchard, M.H., Rhodes, J., Leach, H., Russell, R.I. Walker, F.S. Upadhyay, R., Hort, J.F.: 1991. Protection from gastrointestinal side-effects of azapropazone by its incorporation into a glucose sodium acid citrate formulation. Aliment. Pharmacol. Ther. 5(4): 419-33.

Ramage, J.K., Jenkins, R.T., Hunt, R.H., Perdue, M.H.: 1988. Permeability of inflamed jejunum to ^{51}Cr-EDTA in the *in vivo* rat. Gastroenterology 94: A1261.

Rampton, D.S.: 1987. Non-steroidal anti-inflammatory drugs and the lower gastrointestinal tract. Scand. J. Gastoenterol. 22: 1-4.

Redfern, J.S., Lee, E., Feldman, M.: 1987. Effect of indomethacin on gastric mucosal prostaglandins in humans: correlation with mucosal damage. Gastroenterol. 1987, 92, 969-977.

Reuter, B.K., Cirino, G., Wallace, J.L.: 1994. Markedly reduced intestinal toxicity of a diclofenac derivative. Life Sciences 55(1): 1-8.

Riddell, R.H., Tanaka, M., Mazzoleni, G.: 1992. Nonsteroidal anti-inflammatory drugs as possible cause of collagenous colitis: a case control study. Gut 23: 683-686.

Ritschard, T., Fillippini, L.: 1986. Nebenwirkungen nicht-steroidaer antirheumatika auf den unteren intestinaltrakt. Dtsch. Med. Woch. 111: 1561-1564.

Robert, A., Asano, T.: 1977. Resistance of germfree rats to indomethacin-induced intestinal lesions. Prostaglandins 14(2): 333-341.

Rogers, C., Pihan, G., Szabo, S.: 1986. Role of leucotrienes in the pathogenesis of haemorrhagic gastric mucosal lesions induced by ethanol or HCl in the rat. Gastroenterology 90: 1797.

Rooney, P.J., Jenkins, R.T., Buchanan, W.W.: 1990. A short review of the relationship between intestinal permeability and inflammatory joint disease. Clin. Exp. Rheum. 8: 75-83.

Rooney, P.J., Jenkins, R.T., Smith, K.M., Coates, G.: 1986. ^{111}Indium-labelled polymorphonuclear leucocytes scans in rheumatoid arthritis-an important clinical cause of false positive results. Br. J. Rheumatol. 25: 167-170.

Rowland, M., Riegelman, S.: 1968. Pharmacokinetics of acetylsalicylic acid and salicylic acid after iv administration in man. J. Pharm. Sci. 2: 1313-1319.

Rubanyi, G.M., Ho, E.H., Cantor, E.H., Lumma, W.C.: 1991. Cytoprotective function of nitric oxide: inactivation of superoxide radicals produced by human leukocytes. Biochem. Biophys. Res. Commun. 181(3): 1392-1397.

Salvemini, D., Misko, T.P., Mansferrer, J.L., Seibert, K., Currie, M.G., Needleman, P.: 1993. Nitric oxide activates cyclooxygenase enzymes. Proc. Natl. Acad. Sci. USA. 90: 7240-7244.

Samuni, A., Godinger, D., Aronovitch, J., Russo, A Mitchell, J.B.: 1991. Nitroxides block DNA scission and protect cells from oxidative damage. Biochemistry 30: 555-561

Satterwhite, J.H., Boudinot, D.: 1990. Effects of age and dose on the pharmacokinetics of
ibuprofen in the rat. Drug Metab. Disp. 19: 61-66.

Satoh, H., Guth. P.H., Grossman, M.I.: 1983. Role of bacteria in gastric ulceration produced by indomethacin in the rat: cytoprotective action of antibiotics. Gastroenterology 84(3): 483-489.

Saverymuttu, S.H., Thomas, A., Grundy, A., Maxwell, J.D.: 1986. Ileal stricturing after long-term indomethacin treatment. Postgrad. Med. J. 62: 967-968.

Saw, K.C., Quick, C.R.G., Higgins, A.F.: 1990. Ileocaecal perforation and bleeding - are non-steroidal anti-inflammatory drugs (NSAIDs) responsible? J. Royal Soc. Med. 83: 114-115.

Schneeberger, E.E., Lynch, R.D.: 1992. Structure, function, and regulation of cellular tight junctions. Am. J. Physiol. 262: L647-L661.

Selby, P., McElwain, T.J., Crofts, M., Lopes, N., Mundy, J.: 1984. ^{51}Cr-EDTA test for intestinal permeability. Lancet 38-39.

Shah, A., Jung, D.: 1987. Dose-dependent pharmacokinetics of ibuprofen in the rat. Drug Metab. Disp. 15(2): 151-154.

Shack, M.E.: 1966. Drug induced ulceration and perforation of small intestine. Ariz. Med. 517-523.

Shea-Donohue, T., Steel, L., Montreal, M.E., Dubois, A.: 1986. Gastric protection by sucralfate. Role of mucous and prostaglandins. Gastroenterology 91: 660-666.

Sheers, R., Williams, W.R.: 1989. NSAIDs and gut damage. Lancet 2: 1154.

Shriver, D.A., White, C.B., Sandor, A., Rosenthale, M.E.: 1975. A profile of the rat gastrointestinal toxicity of drugs used to treat inflammatory diseases. Toxicol. Appl. Pharmacol. 32: 73-83.

Silvoso, G.,Ivey, K.J., Butt, J.: 1979. Incidence of gastric lesions in patients with rheumatic disease on chronic aspirin therapy. Ann. Intern. Med. 91: 517-520.

Simpson, L.O.: 1985. NSAID and the leaky gut. Lancet i: 218-219.

Skoldstam, L., Larsson, L., Lindstrom, F.D.: 1979. Effects of fasting and lactovegetarian diet on rheumatoid arthritis. Scand. J. Rheumatol. 8: 249-255.

Smith, M.D., Brooks, P.M.: 1984. Increased bowel permeability in ankylosing spondylitis and active RA. Aust. N.Z. J. Med. 14 (suppl 1): 345-46.

Somasundaram, S., Macpherson, A.J., Hayllar, J., Saratchandra, P., Bjarnason, I.: 1992. Enterocyte mitochondrial damage due to NSAID in the rat. Gut 33: S5 W18.

Speed, C.A., Bramble, M.G., Corbett, W.A., Haslock, I.: 1994. Nonsteroidal anti-inflammatory induced diaphragm disease of the small intestine: complexities of diagnosis and management. Br. J. Rheum. 33: 778-780.

Stamm, C.P., Pearce, W.A., Larsen, B.A., Willis, S.M., Kirkendall, J.W., Moses, F.M., Rosen, H.M., Wong, R.K.H.: 1991. Colonic ulcerations associated with non-steroidal anti-inflammatory ingestion. Gastrointest. Endosc. 37: 260.

Steinhart, A.H., Detsky, A.S., Bombardier, C.: 1996. Economic analysis of the sucrose test: a non-invasive test of gastroduodenal mucosal damage. Can. J. Gastroenterol. 10(Suppl A): S100, 45A.

Stevenson, B.R., Anderson, M.A., Bullivant, S.: 1988. The epithelial tight junction: Structure, function and preliminary biochemical characterization. Mol. Cell. Biochem. 83, 129-145.

Stewart. J.T., Pennington, C.R., Pringle, R.: 1985. Anti-inflammatory drugs and bowel perforations and haemorrhage. BMJ 290: 787-788.

Stock, K.P., Geisslinger, G., Lowe, D., Beck, W.S., Bach, G.L., Brune, K.: 1991. S-ibuprofen versus ibuprofen-racemate. A randomized double-blind study in patients with rheumatoid arthritis. Rheumatoid. Int. 11: 199-202.

Struthers, G.R., Andrews, D.J., Gilson, R.J.R., Reynolds, G.A., Low-Beer, T.: 1985. Intestinal Permeability. Lancet 2: 587-588.

Sturges, H.F., Krone, C.L.: 1973. Ulceration and stricture of the jejunum in a patient on long-term indomethacin therapy. Am. J. Gastroenterol. 59: 162-169.

Sukumar, L.: 1987. Recurrent small bowel obstruction associated with piroxicam. Br. J. Surg. 74: 186.

Sunshine, A., Zighelboim, I., Olson, N., Laka, E.: 1987. Flurbiprofen, flurbiprofen dextrorotatory component (BTS 24332) and placebo in postepisiostomy pain. J. Clin. Pharmacol. 41: 162

Sundquist, T., Magnusson, K.E., Sjödahl, R., Stjerndtröm, I., Tagesson, C.: 1980. Passage of molecules through the wall of the gastrointestinal tract. II. Application of low-molecular weight polyethylene glycol and a deterministic mathematical model for determining intestinal permeability in man. Gut 21: 208-214.

Sutherland, L.R.,Verhoef, M., Wallace, J.L., Van Rosendaal, R., Crutcher, R., Meddings, J.B.: 1994. A Simple, Non-invasive Marker of Gastric Damage: Sucrose Permeability. Lancet 343: 998-1000.

Tagesson, C., Sjödahl, R.: 1984. Passage of molecules through the wall of the gastrointestinal tract. Urinary recovery of different-sized polyethylene glycols after intravenous and intestinal deposition. Scand. J. Gastroenterol. 19: 315-320.

Teahon, K., Smethurst. P., Levi, A.J., Bjarnason, I.: 1992. Intestinal permeability in patients with Crohn's disease and their first degree relatives. Gut 33: 320-323.

Tibdall, C.S.: 1964. Magnesium and calcium as regulators of intestinal permeability. Am. J. Physiol. 206: 243-246.

Trevethick, M.A., Clayton, N., Strong, P., Harman, I.: 1991. Indomethacin induced small intestinal damage in rats resembles NSAID induced enteropathy of the human small intestine. Gut 32: A1224.

Trondstad, R.I., Aadland, E., Holler, T., Olaussen, B.: 1987. Gastroscopic findings after treatment with enteric-coated and plain naproxen tablets in healthy subjects. Scand. J. Gastroenterol. 20: 239-242.

Turk, D., Ythier, H., Maquet, E., Devaux, M., Marchandise, X., Farriaux, J.P., Fontaine, G.: 1987. Intestinal permeability to [^{51}Cr] EDTA in children with Crohn's disease and celiac disease. J. Ped. Gastroent. Nutrit. 6: 535-537.

Ukabam, S.O., Homeida, M.A.: 1983. Small intestinal permeability in Sudanese with and without parasitic infections of the gut. Clin. Sci. 65:10.

Ukabam, S.O., Cooper, B.T.: 1984. Small intestinal permeability to mannitol, lactulose and polyethylene glycol 400 in coeliac disease. Digest. Dis. Sci. 29: 809-816.

Vakily, M., Corrigan, B.W., Jamali, F.: 1995. The problem of racemization in the stereospecific assay and pharmacokinetic evaluation of ketorolac in humans and rats. Pharm. Res. 12: 1652-1657.

Vane, J.R.: 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biol. 231: 232-235.

Vane, J.R., Mitchell, J.A., Appleton, I., Tomlinson, A., Bishop-Bailey, Croxtall, J., Willoughby, D.A.: 1994. Inducible isoforms of cyclooxygenase and nitric-oxide synthatase in inflammation. Proc. Natl. Acad. Sci. 91: 2046-2050.

Vaughan, W.G., Horton, J.W., Walker, P.B.: 1992. Allopurinol prevents intestinal permeability changes after ischemia-repurfusion injury. J. Pediat. Surg. 27(8): 968-973.

Villaneuva, M; Heckenberger, R.; Strobach, H.; Palmer, M.; Schrör, K.: 1993. Equipotent inhibition by R(-)-ibuprofen, S(+)-ibuprofen and racemic ibuprofen of human polymorphonuclear cell function in vitro. Br. J. Clin. Pharmac. 35: 235-242.

Walker, W.A., Isselbacher, K.J.: 1974. Uptake and transport of macromolecules by the intestine. Possible role in clinical disorders. Gastroenterology 67: 531-550.

Walker, F.S., Pritchard, M.H., Jones, J.M., Owen, G.M., Rainsford, K.D. 1987. Inhibition of indomethacin-induced gastrointestinal bleeding, both immediate and persistent, in man by citrate-glucose formulations. Br. J. Rheumatol. 26 (suppl 2): 12 A 20.

Wallace, J.L., Keenan, C.M., Cucala, M., Mugridge, K.G., Parente, L.: 1992. Mechanisms underlying the protective effects of interleukin 1 in experimental non-steroidal anti-inflammatory drug gastropathy. Gastroenterology 102: 1176-1185.

Wallace, J.L.: 1993. Gastric ulceration: critical events at the neutrophil-endothelium interface. Can. J. Physiol. Pharmacol. 71: 98-99.

Wallace, J.L., Reuter, B., Cicala, C., Mcknight, W., Grisham, M., Cirino, G.: 1994a. A diclofenac derivative without ulcerogenic properties. Eur. J. Pharm. 257: 249-255.

Wallace, J.L., Reuter, B., Cicala, C., McKnight, W., Grisham, M., Cirino, G.: 1994b. Novel nonsteroidal anti-inflammatory drug derivatives with markedly reduced ulcerogenic properties in the rat. Gastroenterology 107: 173-179.

Wallace, J.L., Reuter, B., Cirino, G.: 1994c. Nitric oxide-releasing nonsteroidal antiinflammatory drugs: a novel approach for reducing gastrointestinal toxcity. J. Gastroenterol. Hepatol. 9. S40-S44.

Wallace, J.L.: 1994. Mechanisms of nonsteroidal anti-inflammatory drug (NSAID) induced gastrointestinal damage - potential for development of gastrointestinal tract safe NSAIDs. Can. J. Physiol. Pharmacol. 72: 1493-1498.

Wallace, J.L., Cirino, G.: 1994. The development of gastrointestinal-sparing nonsteroidal anti-inflammatory drugs. TIPS 15: 405-406.

Wallace, J.L., Tigley, A.W.: 1995. New insights into prostaglandins and mucosal defence. Aliment. Pharmacol. Ther. 9: 227-235.

Warner, P.J., West, G.B.: 1978. Inhibition of leucocyte migration by salicylates and indomethacin. J. Pharm. Pharmacol. 30: 783-785.

Wax, J., Clinger, A., Varner, P., Bass, P., Winder, C.V.: 1970. Relationship of the enterohepatic cycle to ulcerogenesis in the rat small bowel with flufenamic acid. Gastroenterology 58(6): 772-779.

Wechter, W.J., Loughead, D.G., Reischer, R.J., Van Giessen, G.J., Kaiser, D.G.: 1974. Enzymatic

inversion of R(-) p-iso-butyl hydratopic acid. Biochem. Biophys. Res. Comm. 61(3): 833-837.

Whitcomb, D.C.: 1993. Pathophysiology of nonsteroidal antiinflammatory drug-induced intestinal strictures. Gastroenterology 105(5): 1590.

Whitham, R.: 1991. Voltaren (diclofenac sodium)-induced ileocolitis. Am. J. Gastroenterol. 86: 246-247.

Whittle, B.J.R.: 1981. Temporal relationship between cycloxygenase inhibition, as measured by prostacyclin biosynthesis, and the gastrointestinal damage induced by indomethacin in the rat. Gastroenterology 80: 94-98.

Whittle, B.J.R., Vane J.R.: 1984. A biochemical basis for the gastrointestinal toxicity of non-steroid anti-rheumatoid drugs. Arch. Toxicol. (suppl) 7: 315-322.

Wilkens, R.F.: 1992. The selection of a nonsteroidal antiinflammatory drug. Is there a difference? J. Rheumatol. (suppl 36) 19: 9-12.

Williams, K.M., Day, R.O.: 1988. The contribution to variability in response to anti-inflammatory drugs. Agents Actions 24: 76-84.

Williams, K.M., Browne, G., Nelson, C.J., Nguyen, T., Day, R.O.: 1995. Effects of 2-arylpropionic acids on the β-oxidation of fatty acids. J. Clin. Pharmacol. 35: 921-940.

Winter, M.C., Peterson, M.W., Shasby, D.M.: 1991. Synergistic effects of a calcium ionophore and activators of protein kinase C on epithelial paracellular permeability. Am. J. Respir. Cell Mol. Biol. 4: 470-477.

Wright, M.R., Sattari, S., Brocks, D.R., Jamali, F.: 1992. Improved high-performance liquid chromatographic assay method for the enantiomers of ibuprofen. J. Chromatogr. 583: 259-265.

Wright, M.R., Jamali, F.: 1993. Limited extent of stereochemical conversion of chiral NSAIDs induced by derivitization methods employing ethylchloroformate. J. Chromatogr.
616: 59-65.

Zaphiropoulous, G.C.: 1986. Rheumatoid arthritis and the gut. Br. J. Rheumatol. 5: 138-140.

Zhao, B., Geisslinger, G., Hall, I., Day, R.O., Williams, K.M.: 1992. The effect of the enantiomers of ibuprofen and flurbiprofen on the beta-oxidation of palmitate in the rat. Chirality 4: 137-141.