National Library of Canada
Acquisitions and Bibliographic Services Branch
395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada
Direction des acquisitions et des services bibliographiques
395, rue Wellington
Ottawa (Ontario)
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

UNIVERSITY OF ALBERTA

Influence of concentration time course on the gastrointestinal toxicity of selected non steroidal antiinflammatory drugs

By

Corinne CAMPANELLA 

A thesis submitted to the **Faculty of Graduate Studies and Research** in partial fulfillment of the degree of **Master of Science**

IN

PHARMACEUTICAL SCIENCES
(PHARMACOKINETICS)

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

EDMONTON, ALBERTA

FALL 1994

National Library of Canada
Acquisitions and Bibliographic Services Branch
395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada
Direction des acquisitions et des services bibliographiques
395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-95015-3

Canada

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: **Corinne CAMPANELLA**

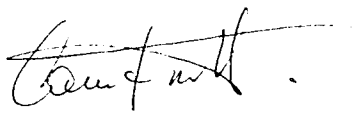
TITLE OF THE THESIS: **Influence of concentration time Course on the Gastrointestinal Toxicity of selected non steroidal antiinflammatory drugs.**

DEGREE: **Master of Science**

YEAR THIS DEGREE GRANTED: **1994**

Permission is hereby granted to the **University of Alberta Library** to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.



Permanent Address:
17 rue Albert Salmon
77000 Melun, FRANCE

Date: August 31st 1994

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **"Influence of concentration time course on the gastrointestinal toxicity of selected non steroidal antiinflammatory drugs"** submitted by **Corinne CAMPANELLA** in partial fulfillment of the requirements for the degree of **Master of Science in Pharmaceutical Sciences (Pharmacokinetics).**

Dr. F. JAMALI (Supervisor)

Dr. F.M. PASUTTO (Professor)

Dr. A.S. RUSSELL (Professor)

Date: August 31st 1994

To Thierry

To my parents

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. SUMMARY

In this work, the relationship of lower intestinal toxicity of NSAIDs with prolonged residence time was studied. Urinary excretion of ^{51}Cr-EDTA was used to measure intestinal permeability. ^{51}Cr-EDTA test has been successfully used to measure NSAID-induced intestinal permeability in humans. Recently rats have been described as a suitable model to study lower intestinal toxicity of NSAIDs.

The sex difference in the disposition of tenoxicam (TX) in the rat provided a model to compare long and short half-life. This model avoid the problem of intrinsic chemical differences between products. In females, who show lower clearance than males, toxicity was higher in terms of magnitude rather than in term of duration of the effect, but only at high doses. This may suggest minor prolongation of the effect when the residence time of the drug is prolonged. Due to the low toxicity of TX, this sex-related model could not lead to a clear interpretation of the effect of half-life. The selection of a more toxic drug, flurbiprofen, and the comparison of different routes and dosing frequencies was thus undertaken. Flurbiprofen has a short half-life in the rat, therefore implantation of osmotic pump was used to simulate a longer half-life. In this case, the infusion of the drug was found to cause lower toxicity than the corresponding multiple dose. This suggest that increasing the residence time of a drug does not necessarily increase its toxicity. More importantly, it seems that fluctuations in plasma concentrations (C_{max}) are a more prominent factor in determining toxicity.

Observation of adaptation during multiple dosing treatments with flurbiprofen was an interesting finding. While this has been shown with aspirin and upper GI tract side effects, it is the first observation of this phenomenon at the lower intestinal level. This adaptation has been observed with PO and IP treatments, suggesting involvement of a systemic process. These results are in contradiction with the higher incidence of GI side effects noticed in patients on long term NSAID therapy. However, based on our results, it seems that repeated exposure rather than accumulation of the drug is responsible for more toxi vents.

2. RATIONALE

The prevalence of rheumatic diseases is relatively high: approximately 8% of the population will experience rheumatic symptom in their life time (Brooks & Day 1991). The non steroidal antiinflammatory drugs (NSAIDs) are extensively used either as simple analgesics or as antiinflammatory agents in rheumatic diseases.

The principal mechanism of action of NSAIDs is cyclooxygenase inhibition and subsequent interference with prostaglandin synthesis (Abramson & Weissmann 1989). In addition, NSAIDs have pharmacological effects not dependent on prostaglandin metabolism such as inhibition of phospholipase C in mononuclear cells, or inhibition of neutrophil aggregation.

NSAID use is associated with high incidence of upper gastrointestinal (GI) toxicity (Hochberg 1992). Minor effects include gastric pain, heartburn, nausea, vomiting and diarrhea (Brogden 1986). In addition, more serious complications such as ulcerations, perforations, bleeding and even death have been reported after treatment with NSAIDs (Hayllar *et al.* 1992, Aabaken 1992). Although upper GI toxicity has been extensively studied, the effects of NSAIDs on the more distal part of the small intestine have only been recently examined, and it has been suggested that their frequency might be underestimated (Rampton 1987). Animal studies are lacking, and few human observations of lower intestinal lesions have been made as compared to upper GI injury (Bjarnason *et al.* 1986, Holt *et al.* 1993). This is in part due to less accessibility to the lower part of the GI tract by endoscopy. It has been suggested that distal damage could be of more serious consequences than previously thought (Rampton 1987). Disruption of the intestinal epithelium integrity with increased intestinal permeability augments the intestinal uptake of exogenous molecules, luminal antigens, toxins and bacteria and can lead to serious consequences (Jenkins *et al.* 1987).

Numerous studies report NSAID induced GI side effects. Nevertheless, there is still insufficient data to rank the different NSAIDs in terms of severity of GI toxicity. In addition, few of these studies have considered the pharmacokinetics of these agents. The time course of permeability change (as a measure of small intestinal toxicity of NSAIDs) and their relationship with drug concentration have only recently been examined. In the last 10 years, some speculations have been made regarding the relationship between longer elimination half lives ($t_{1/2}$) and higher toxicity (Adams 1988, 1992, Collier *et al.* 1985). However, relating toxicity to $t_{1/2}$ is an over

simplification of a rather complex situation: $t_{1/2}$ is a hybrid pharmacokinetic parameter and is dependent on the volume of distribution (V_d) and the total body clearance (Cl_{TB}). The extent of protein binding as well as mechanisms by which the drug is cleared should also be considered.

In the first part of the study, we investigated the impact of a long half-life NSAID on GI toxicity, using the rat as a model (Davies *et al.* 1994). TX belongs to the oxicam class of NSAIDs, with a long half-life in humans (70 h). Different studies have suggested that TX is better tolerated than other NSAIDs in rats (Al-Gahndi *et al.* 1991) and in humans (Bird *et al.* 1982) but its toxicity on the lower intestine has never been examined.

In the second part of the study, the sex difference in the disposition of TX was examined. Sex differences in metabolism, elimination, protein binding and disposition of numerous xenobiotics have been established (Bonate 1991, Wilson 1984). This may lead to sex-related differences in the pharmacological response as well as in the toxicity. Other oxicams, for instance sudoxicam and piroxicam, have shown longer elimination half-life in female rats (Woolf & Radulovic 1989). Our preliminary studies indicated that TX exhibits a longer half-lives in female as compared to male rats. Therefore, female rats provided a model for long half-life NSAIDs.

A comparison of various NSAIDs with different $t_{1/2}$ ignores the possibility of differences that are attributable to other properties of drugs such as intrinsic molecular toxicity. We, therefore, simulated a situation where the same drug was presented to the body with different residence times. This, as the third objective of the work, was achieved by comparing the toxicity after multiple intraperitoneal (IP) and oral (PO) dosing with that observed after infusion using osmotic pump implants. Due to the low toxicity profile of TX, flurbiprofen (FL) was chosen as the model drug.

3. INTRODUCTION

3.1. NON STEROIDAL ANTIINFLAMMATORY DRUGS (NSAIDs)

NSAIDs are effective in management of pain and inflammation, and are widely used in the treatment of rheumatoid diseases. They come from a variety of chemical classes (Table I). They exhibit different chemical structures, diverse mechanisms of action and physicochemical properties. Nevertheless, the vast majority of NSAIDs are weak acids with pKas ranging from 3 to 5. This property is of major importance regarding the distribution of NSAIDs into the synovial fluid of inflamed joints (Brooks & Day 1991).

Table I: Chemical classification of NSAIDs

Carboxylic acids	Acetic acids	Pyrano carboxylic acids	Propionic acids	Fenamic acids	Enolic acids	Oxicams	Non acidic compounds
Acetylated	Diclofenac	Etodolac	Ibuprofen	Flufenamic acid	Oxyphenbutazone	Piroxicam	Nabumetone
Aspirin	Indomethacin		Naproxen	Mefenamic acid	Phenylbutazone	Tenoxicam	Proquazone
Nonacetylated	Tolmetin		Fenoprofen	Meclofenamic acid	Trimethazone	Isoxicam*	Bufexamac
Sodium salicylate	Sulindac		Pirprofen	Niflumic acid		Lornoxicam	
Diflunisal			Indoprofen			Cinnoxicam	
Salicylamide			Ketoprofen			Sudoxicam	
			Flurbiprofen				
			Carprofen				
			Suprofen				
			Tiaprofenic acid				

*: withdrawn from the market.

3.1.1. MECHANISMS OF ACTION

The main mechanism by which NSAIDs exert their pharmacological activities is the reduction of prostaglandin (PG) synthesis via inhibition of cyclooxygenase activity. In addition, some of the 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 & Botting 1990).

This plethora of potential mechanisms may, in part, be due to the different methodologies used to explore NSAID activities (cell-free systems, animal models, etc.) and to the physicochemical properties of various NSAIDs (pKa, lipophilicity, protein binding, etc.).

3.1.1.1. Inhibition of cyclooxygenase activity

NSAIDs inhibit the transformation of arachidonic acid to stable PGs such as PGE_2 and prostacyclin (PGI_2) that elicit many signs of inflammation. Cyclooxygenase (COX) inhibition, through interaction with other mediators, may have secondary effects including inhibition of neutrophil activation, reduction of leukotriene production as well as diminution of T and B cell proliferation (Furst 1994). The PG role in inflammation is complicated by the fact that PGE_2 and PGI_2 possess both proinflammatory and antiinflammatory properties.

Recently, COX has been shown to exist as two iso-enzymes, COX1 and COX2 (Merlie *et al.* 1988). COX1 is constitutive, present in cells under physiological conditions and is thought to mediate the production of cytoprotective PGs. On the other hand, the biosynthesis of COX2 is stimulated in cells exposed to proinflammatory agents such as cytokines, mitogenes and endotoxines (Vane *et al.* 1994).

It is suggested that the antiinflammatory actions of NSAIDs are due to inhibition of COX2 whereas the unwanted side effects are due to COX1 inhibition (Appelton *et al.* 1994). NSAIDs have shown differential inhibitory effect *in vitro* on COX1 and COX2 (Meade *et al.* 1993, Mitchell *et al.* 1993). Indomethacin, sulindac and piroxicam preferentially decrease COX1 activity, whereas ibuprofen and

flurbiprofen inhibit both, and nabumetone preferentially diminished COX2. These results might explain, at least in part, the disparity in NSAID GI toxicity. However this selective effect on arachidonic acid metabolism should be interpreted cautiously due to the lack of *in vivo* data.

3.1.1.2. Prostaglandin independent processes

NSAIDs have been found to inhibit a variety of membrane associated processes such as superoxide formation by a cell-free NADPH oxidase in neutrophils and macrophage phospholipase C activity (Abramson & Weismann 1989). These effects may result from uncoupling protein-protein interactions within the lipid bilayer of the plasma membrane. A number of NSAIDs including indomethacin, ibuprofen, piroxicam and salicylates inhibit neutrophil function. Neutrophil aggregation is affected by all NSAIDs with the exception of piroxicam which only appears to reduce hydrogen peroxide formation from neutrophils (Brooks & Day 1991).

In addition, higher (antiinflammatory) doses of NSAIDs interfere with the synthesis of proteoglycan by chondrocytes, transmembrane ion fluxes and cell-cell binding. For example, piroxicam inhibits proteoglycanase production, while TX inhibits both proteoglycanase and collagenase production (Fries *et al.* 1991).

3.1.2. ADVERSE REACTIONS

The side effects of NSAIDs are well known and frequent. Nearly 25% of drug-related side effects are caused by NSAID treatment (Committee on Safety of Medicine 1986). The most frequent side effects associated with NSAIDs' treatment are gastrointestinal, dyspepsia being the most common one. Renal side effects are next in frequency, followed by skin reactions and central nervous system reactions.

3.1.2.1. Gastrointestinal side-effects

Since PGs have a major role in the maintenance of normal gastrointestinal function, it is not surprising that drugs inhibiting PGs synthesis will interfere with GI physiology. GI damages result from multiple and interrelated processes. In addition, NSAIDs vary in ulcerogenic activity within different regions of the GI tract.
These differences are due to different kinetics of absorption, intracellular distribution within mucosal cells, systemic distribution, and to different potency in COX inhibition (Fenner 1992).

Gastrointestinal side effects can be divided into two categories: upper GI toxicity and lower GI toxicity (more distal part of the intestine). Upper GI toxicity is relatively well documented, lower has only been recently studied.

3.1.2.1.1. Upper gastrointestinal side effects

A dual mechanism has been proposed for upper GI damage caused by NSAIDs: local effect and systemic interaction with PGs synthesis. Almost instantaneous mucosal damage after oral administration of aspirin illustrate the topical toxicity of this drug. Moreover, intravenous and rectal administrations induce lesions as well, suggesting involvement of a systemic component (Rowe 1987, Halter 1988, Ligumsky 1990,).

For aspirin, the local damage has been attributed to the non-ionic diffusion at acidic pH (Davenport 1964). This pH dependent process leads to disruption of the gastric barrier and to back-diffusion of hydrogen ions into the mucosa. Consequently this phenomenon is responsible for local hemorrhage and cell necrosis via decrease in mitochondrial phosphorylation, and subsequent depletion in ATP.

In the stomach, PG-dependent and PG-independent factors influence the ulcerogenicity of NSAIDs (Rainsford 1989). The PG-dependent factors involve the role of PGs on mucus and bicarbonate secretion, regulation of acid secretion and blood flow. Consequently, their depletion will weaken the mucosal defense, leaving the mucosa more vulnerable to luminal aggressors. These changes may be of particular importance in acute damage, whereas impairment in cell growth may be critical for the development of chronic lesions (Rask-Masden 1987).

Inhibition of endogenous PG synthesis does not completely suppress the ability of the gastric mucosa blood vessel, to dilate when stimulated, thus PGs are not the sole mediator participating in regulation of blood flow (Halter 1988). Arachidonic acid metabolism involves two major pathways: the cyclo-oxygenase and the lipoxygenase. An inhibition of the first one would increase the involvement of the second one. It has been suggested that an increase in lipoxygenase pathway activity may produce more vasoconstrictor leukotrienes (like LTC_4, LTD_4), and this may, in turn, contribute to the vascular and mucosal changes induced by NSAIDs (Figure 3.1-1). Indeed it has been noted that LTC_4 exerts a profound vasoconstrictive action after local application to the gastric submucosal arteries and veinules in the rat (Wittle 1985). GI lesions induced by indomethacin in pigs and rats have been prevented by a prior injection of MK-886, a 5-lipoxygenase inhibitor (Rainsford 1993).

It seems likely that other factors, such as oxygen free radicals, could be involved in the pathogenesis of NSAID induced GI damage. Some compounds that can capture free radicals (aminopyrine, thiourea and its derivative, MK447) or that inhibit the lipoxygenase pathway (MK447) are able to ameliorate indomethacin induced injury (Del Soldato 1985). Similarly, gastric erosion is significantly decreased by treatment with superoxide dismutase or catalase. Pretreatment with allopurinol, a competitive inhibitor of xanthine oxidase considerably prevents gastric injury in various models (Yoshikawa 1990). These observations suggest that oxygen free radical and lipid peroxidation contribute to the formation of gastric mucosal lesions.

3.1.2.1.2. Lower gastrointestinal side effects

An important and recent consideration is the effect of NSAIDs on the lower GI tract. Approximately 47% of patients with occult GI bleeding while on regular NSAIDs were found to have jejunal or ileal ulceration (Morris 1991). Recent studies suggest that 60-70% of patients on long-term NSAID therapy may have an asymptomatic enteropathy (Bjarnason *et al.* 1986). NSAID-induced enteropathy appears to be associated with low-grade blood loss and protein loss, which may be utilized as an early diagnostic technique in these patients (Bjarnason *et al.* 1993).

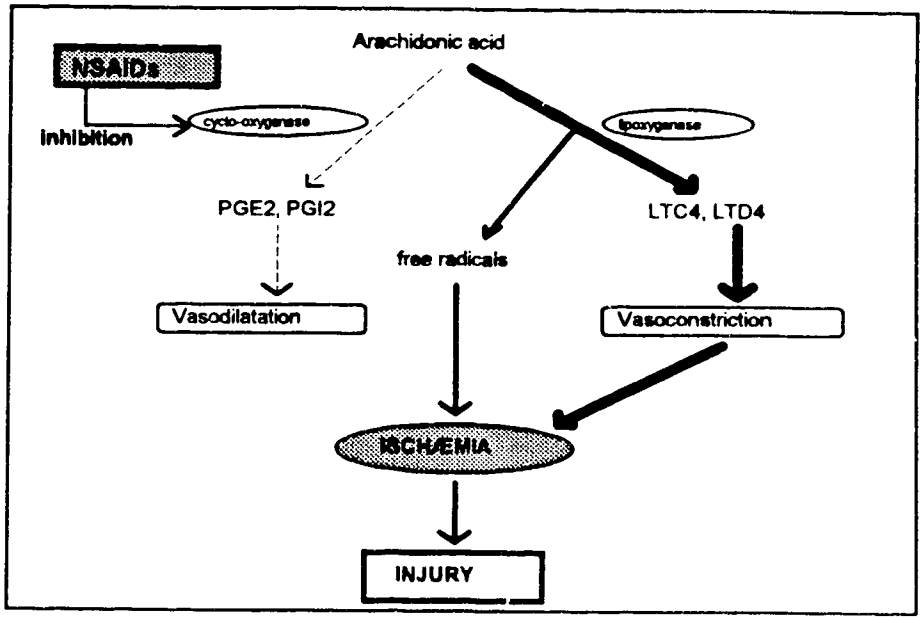


Figure 3.1-1: Postulated consequences of cyclo-oxygenase inhibition by NSAIDs (adapted from Rainsford 1989).

3.1.2.1.2.1. Small intestine permeability

The mucosa of the intestinal tract can be considered a protective boundary between the external and internal milieu. Nevertheless, this barrier is not complete. Water, electrolytes, and nutrients pass from the lumen of the gut into the portal circulation, and small amounts of macromolecules are known to cross this barrier in either direction by both active and passive mechanisms (Walker & Isselbacher 1974). The suggestion that the intestinal mucosa may be abnormally permeable, and that the absorption of antigens might be implicated in the pathogenesis of rheumatoid arthritis (RA) was first formulated by Bjarnason and colleagues (1984a). Their study showed that intestinal permeability was normal in untreated patients but almost invariably abnormal in patients treated with NSAIDs. The mechanism

responsible for increased intestinal permeability during NSAID therapy or RA remains unexplained. However, loss of mucosal integrity may be due to both functional or morphological alterations (Jenkins *et al.* 1987). The intercellular junctions appear to be particularly susceptible to a variety of noxious agents and consequently they may be the first organelle to suffer when the energy production of the enterocyte is compromised (Bjarnason & Peters 1989). This would result in disruption of intercellular integrity allowing permeation of macromolecules into mucosa. The increase in intestinal permeability is not restricted to the small intestine, thus, it has been recently demonstrated an increase in colonic permeability after NSAID use (Jenkins *et al.* 1991).

Permeability changes with NSAIDs are quantitatively similar to those found in inflammatory bowel diseases; evidence of small intestine inflammation has been observed in 45-70% of patients on long term NSAID therapy (Bjarnason & Peters 1989). The initial and immediate effect of NSAIDs to increase small intestine permeability is a prerequisite for the subsequent development of small intestine inflammation. This small intestine inflammation caused 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.* 1987). The blood loss is similar in magnitude to that observed in patients with intestinal malignancies, and may contribute to the iron deficiency which is so common in rheumatic patients.

3.1.2.1.2.2. Pathogenesis of small intestinal damages

In laboratory animals, large single doses of indomethacin given intramuscularly or intragastrically, produce multiple ulcerations of the small bowel and lead to subsequent perforations (Kent *et al.* 1969). It seems likely that reduced mucosal prostaglandin synthesis in these animals results in loss of intestinal integrity, followed by bacterial invasion. Microorganisms' toxins may penetrate the mucosa, damage the cells and produce ulcers and perforations (Satoh *et al.* 1983). Thus, macroscopic lesions are not seen in germ-free animals or when indomethacin is given with various antibiotics such as neomycin and polymyxin B (Kent *et al.* 1969, Robert and Asano 1977). Similarly, the beneficial effect of metronidazole on NSAID-induced intestinal damage confirms the role of intestinal bacteria in the

pathogenesis of the initial permeability increase (Davies *et al.* 1993, Bjarnason *et al.* 1992).

Misoprostol, a PG analogue, when given before indomethacin, had a significant protective effect on the intestinal cell integrity (Bjarnason *et al.* 1989, Rainsford 1988). This indicates that PG synthesis inhibition is an important pathogenic factor. However, many other plausible mechanisms have been suggested, hence, it should not be assumed that all observed intestine damages are due to prostaglandin inhibition. Hence, the naproxen-induced increase in small intestinal permeability was not prevented by administration of misoprostol (Rooney *et al.* 1988). Furthermore, some NSAIDs such as sodium salicylate do not inhibit prostaglandin synthesis but still damage the gastrointestinal mucosa (Ligumsky *et al.* 1982).

It has been suggested that early events leading to the permeability changes may involve inhibition of glycolysis and the tricarboxylic acid (TCA) cycle, resulting in reduced ATP production and cell death. The administration of glucose and citrate, metabolic precursors for glycolysis and the TCA cycle respectively, protect against increases in intestinal permeability induced by indomethacin in the experimental animal (Rainsford & Withehouse 1980). These observations have been corroborated by human studies (Bjarnason *et al.* 1992).

The effect of most NSAIDs on the small intestine can be envisaged as summation of local effect during absorption and systemic effect after distribution (Bjarnason & Peters 1989). The lower intestine might be exposed to NSAIDs following administration of enteric coated or sustained release formulations, and also via enterohepatic recirculation, resulting in local toxicity due to direct exposure of the small bowel mucosa (Aabakken 1992). However, the relative importance of local toxicity in the lower intestine is unknown. In this respect, the effect of available pro-NSAIDs would be of particular interest in distinguishing between the importance of local versus systemic toxicity, since these specific drugs could be only activated in the liver after absorption. Nabumetone exhibits minimal PG inhibitory properties before biotransformation into its active metabolite, 6-methoxy-2-naphthylacetic acid, which is not secreted into bile. Therefore, any effect of nabumetone on small intestine permeability is most likely resulting from systemic exposure. Nabumetone did not affect small intestine permeability over a period of one week, suggesting a minimal systemic toxicity for this product (Bjarnason *et al.* 1991). Similarly, sulindac, an indomethacin-like indole inactive COX inhibitor until its post-absorption reduction

to its sulfide derivative, did not increase mucosal permeability (Davies & Rampton 1991). These results show the importance of high local concentrations of drug during initial absorption. Moreover, the differing pattern of biliary excretion of these products might influence their potential for causing intestinal mucosal injury. While sulindac and nabumetone do not undergo enterohepatic recirculation as active metabolites, indomethacin is extensively recycled both as parent compound and inactive conjugates (Schneider *et al.* 1990). Nevertheless, a post market survey of sulindac did show some GI toxicity, suggesting the importance of a systemic effect (Carson *et al.* 1987).

There are no immediately apparent clinical implications of increased intestinal permeability, but it may play an important role in the pathogenesis of NSAID-induced enteropathy. Although the permeability changes are seen within hours, the inflammation may take longer to develop. However, once established the inflammation may remain up to 16 months after discontinuation of NSAID therapy (Bjarnason *et al.* 1987). Therefore, as a prerequisite to intestinal inflammation and enteropathy, the measurement of intestinal permeability might be useful in the early investigation of NSAID intestinal side effects.

3.1.2.2. Renal adverse effects

The other major and potentially serious side effects of NSAIDs are those affecting the kidney (Clive & Stoff 1984). They can cause reversible impairment of glomerular filtration, acute renal failure, edema, interstitial nephritis, papillary necrosis, chronic renal failure and hyperkalemia.

Prostaglandins participate in the autoregulation of renal blood flow and glomerular filtration, and also influence the tubular transport of ions and water (Brooks & Day 1991). Although PG mediated effects of NSAIDs on kidney is a characteristic feature of this class of drugs, it may be clinically more significant in patients with compromised kidney function.

The renal safety of pro-NSAIDs such as sulindac and nabumetone is controversial. Because of their unique metabolic pathways that limit renal exposure, they have been thought to cause less alteration in renal function than other NSAIDs. However, renal adverse reactions have been reported with both compounds (Skeith *et al.* 1994).

3.1.2.3. Cutaneous reactions

Although most cutaneous reactions are mild, a serious adverse effect, erythema multiform, has been frequently described with the use of NSAIDs (O'Brien & Bagby 1985a). The arylpropionic acid class of NSAIDs has been implicated in photosensitivity reactions. These reactions generally occur on exposed skin areas of patients taking ketoprofen, naproxen, tiaprofenic acid and piroxicam (Diffey *et al.* 1983).

3.1.2.4. Central nervous system reactions

Many NSAIDs have been implicated in adverse effects in the central nervous system (CNS). These generally mild reactions, including headaches, drowsiness and dizziness, have been most commonly associated with indomethacin (O'Brien & Bagby 1985b).

3.1.2.5. Hepatic adverse effects

Serious liver reactions to NSAIDs are rare, and unlike gastrointestinal and renal effects, cannot be anticipated from known pharmacological actions of the drugs. The mechanism by which hepatic damage is caused is not known and the problem appears to be unpredictable. However, with the great increase in the use of NSAIDs the possibility of drug-induced hepatocellular damage should be considered (Henry 1988).

Salicylates have been associated with dose-dependent hepatotoxicity, as shown by a marked transaminemia, particularly in patients with juvenile rheumatoid arthritis (Lewis 1984). Adverse events affecting the liver have been reported with other NSAIDs. In particular, mild transaminemia is occasionally seen, although these events are rarely considered to be clinically significant.

3.1.2.6. Hematologic adverse effects

Two of the most serious hematologic adverse effects observed after NSAIDs use are agranulocytosis and aplastic anemia. While these are very rare complications with most NSAIDs, phenylbutazone and indomethacin may cause these potentially catastrophic events more frequently than others (Furst 1994).

The most predictable effect is NSAID-induced inhibition of platelet cycloxygenase resulting in reduced platelet adhesiveness and prolongation of bleeding time (Henry 1988). This effect is well known with aspirin and is exploited in prophylaxis of cardiovascular disease.

3.1.2.7. Hypertensive effects

Prostaglandins have a role in modulating vascular tone, and some diuretic agents and antihypertensive agents may stimulate the release of PGs. It is therefore not surprising that NSAIDs interfere with these agents. However, very few investigators have used rigorous experimental methodologies to properly assess the possibility of these drug-NSAID interactions (Radack & Deck 1987).

3.2. MEASUREMENT OF INTESTINAL PERMEABILITY

The degree of intestinal penetration by passively absorbed, water soluble, medium-sized molecules is called permeability (Csaky 1987). Tests of intestinal permeability were designed to assess the function of the intestine to act as a barrier as opposed to an absorptive organ. This is done noninvasively by measurement of urinary recovery of orally administered probes. A variety of probes are used to assess this permeability, and it has been suggested that their molecular weight is as important as their molecular geometry in terms of permeation (Hollander *et al.* 1988).

3.2.1. INTESTINAL PERMEABILITY

The small intestine is the principal site of absorption of most nutrients, and the large intestine is mainly involved in the absorption of water and electrolytes. Consequently, the small intestine is endowed with a more elaborate system which determines its permeability. The small intestine represents a large surface, estimated to be 200 m^2 in humans. Through this very large surface area, the body is exposed to the external environment.

Two routes of permeation exists across the intestinal barrier: a transcellular and a paracellular. The transcellular passage may proceed by simple diffusion, carrier mediation, or by pinocytosis. The paracellular passage is the most important for the macromolecular transfer. It has been shown that the morphological correlations of the damaging effect caused by a variety of chemical and physiological barrier breakers reside at the level of the intercellular junctions between enterocytes (Bjarnason *et al.* 1986).

3.2.2. INTESTINAL PERMEABILITY TESTS

The most commonly used probe and their respective permeabilities in normal human volunteers, are listed in Table II. The absorption from the gut and the excretion in the urine of these molecules (not metabolized in the body) are used to test small intestine permeability. Lactulose and the chromium-51 labeled ethylene diamine tetracetic acid (^{51}Cr-EDTA) specifically test the integrity of the intercellular junctions. Water soluble substances of a molecular weight less than 180 measure the permeability through aqueous pores, while polyethylene glycol (PEG 400) traverses the brush border via a lipid-soluble pathway.

The physical structure of an ideal probe for measuring intestinal integrity should be fully characterized. In addition, it should be biologically inert; that means it is neither metabolized in the body nor degraded prior to excretion, nor should it be recognized or influenced by the immune system. Ideally, it should be passively permeable, rapidly cleared, yet measurable with a rapid, accurate, precise and technically undemanding assay. As it could be anticipated, each of the probe molecules fits some, but not all of these criteria (Rooney *et al.* 1990).

Table II: Permeability of selected molecules in normal human volunteers (adapted from Hollander *et al.* 1988).

Probe	Molecular weight (Dalton)	Permeability (% of dose)
Rhamnose	164	9.5
Mannitol	182	17.6
Cellobiose	342	0.41
Lactulose	342	0.23
^{51}Cr-EDTA	359	1.3
PEG-400	242-594 (mean 400)	25.4

The inert sugars (lactulose, mannitol, cellobiose, L-rhamnose) have the advantage of being easily used in pairs as internal control. The combination of probes is utilized to reduce variability due to change in transit time. However, these sugars could be degraded by bacteria in the gut, and the assay is time consuming. Similarly, PEG is easy to administer, but also requires a technically demanding assay. The ^{51}Cr-EDTA seems to meet more criteria without too many disadvantages. The product is stable, it is easily quantified and the urine collection is made over 24 hours. One inconvenience could be its radioactivity.

3.2.3. ^{51}Cr-EDTA

^{51}Cr-EDTA was first introduced as a marker for evaluating gastrointestinal function in 1967 (Lökken & Sögnen 1967). It has the analytical advantage of the γ-ray emitting ^{51}Cr isotope in a water soluble, highly stable, chelated compound. In rats, absorption has not been seen from the stomach, and the proximal part of the small intestine seems to be the major site of absorption (Lökken & Sögnen 1967).

The limited passage of ^{51}Cr-EDTA through biologic membranes relates to its size and hydrophilicity and results in a very low permeability through intact intestinal

mucosa. However, there is a certain passive leakage, probably via intercellular junctions (Bjarnason *et al.* 1986). Therefore, any damage to these junctions could be associated with increased absorption of ^{51}Cr-EDTA.

The introduction of the ^{51}Cr-EDTA test of intestinal permeability in 1983 (Bjarnason *et al.* 1983a) and its successful use in the identification of patients with coeliac and Crohn's disease (Bjarnason *et al.* 1983b) made it a potential probe in the screening for small bowel disease. Later this test was found to be reliable to detect abnormalities due to cytotoxic chemotherapy (Selby *et al.* 1984), atopic eczema (Forget *et al.* 1985), dermatitis herpetiformis (Bjarnason *et al.* 1985), or cystic fibrosis (Leclercq-Foucart *et al.* 1986) in alcoholics (Bjarnason *et al.* 1984b) and in patients taking NSAIDs (Bjarnason *et al.* 1984a, Mielants *et al.* 1991).

The validity of the ^{51}Cr-EDTA permeability test has been questioned (O'Mahony *et al.* 1984, Peled *et al.* 1985, Elia *et al.* 1987). In particular the specificity of the absorption site, the dependence on renal function and the variation among healthy control subjects. However, the methodological aspects of the ^{51}Cr-EDTA permeability test have been studied, and the method was found to be suitable for the evaluation of NSAID-induced permeability changes in the gut (Aabakken 1989). Small bowel transit time and creatinine clearance seem unlikely as confounding factors in healthy subjects. The distal gut (at least in healthy controls) appears to be the main absorption area for ^{51}Cr-EDTA, since no difference was found between the absorption after intraduodenal instillation and after oral intake of a test dose. The urine sampling period is an important factor to consider, and a 24 h collection may be necessary to reduce the intra-individual variations.

All these observations in humans show the difficulty of standardization of the ^{51}Cr-EDTA test. Therefore, the development of a suitable animal model is desirable. The intestinal permeability of humans to sugars such as lactulose, rhamnose or mannitol, has been found to be considerably different from that of three common species of experimental animals (Delahunty & Hollander 1987). ^{51}Cr-EDTA has been shown to be stable in the alimentary tract and is a convenient marker for assessing intestinal permeability in rats with experimentally induced enteropathy (Bjarnason *et al.* 1985). Furthermore, rats have been found to be a suitable model to study NSAID-induced intestinal permeability (Davies *et al.* 1994). Thus ^{51}Cr-EDTA permeability tests might be used in the measurement of NSAID-induced intestinal permeability.

3.3. OVERVIEW OF TENOXICAM PROPERTIES

3.3.1. OXICAMS

Oxicam non steroidal anti-inflammatory drugs are a group of structurally related substances (figure 2) with antiinflammatory, analgesic and antipyretic activities. They have a weakly acidic character and are extensively bound to plasma albumin. Due to their extensive binding to plasma proteins their apparent volumes of distribution are small. They are well absorbed both orally and rectally.

Most of the NSAIDs introduced in the 1970s were relatively short acting and needed to be administered 2 or 3 times a day. Longer acting drugs were developed for symptomatic relief in patients with chronic pain. Excluding lornoxicam, oxicams have a long elimination half-life ($t_{½}$) which allows for once-daily administration. This has been the major advantage of oxicams in clinical practice.

Unlike other NSAIDs, which have a carboxylic acid moiety in their structure, the weakly acidic character of oxicams is conferred by a tautomeric enolic group. As weak acids, all oxicams are extensively bound to plasma protein (over 96%). Piroxicam and tenoxicam (TX) have the lowest unbound fractions with only 1% of the drug being free (Olkkola *et al.* 1994).

3.3.2. TENOXICAM

TX, 4 -hydroxy -2 -methyl -2H -thieno -[2,3-e] -1, 2 -thiazine -3 -carboxamide 1, 1 -dioxide, is a thienothiazine oxicam NSAID. The substitution of the benzothiazine ring by a thienothiazine ring gives TX a more hydrophilic character than other oxicams.

3.3.2.1. Physicochemical properties

TX is a weak acid with a molecular weight of 337.4 D and pKa values of 5.35 and 1.07. At physiological pH the drug is predominately ionized which limits its ability to distribute into tissues (Nilsen 1994).

The lipophilicity of TX is relatively low, with an octanol/buffer pH 7.4 partition coefficient of 0.3. In comparison piroxicam, flurbiprofen, indomethacin and diclofenac have partition coefficient of 1.9, 8.0, 9.1 and 15.6 respectively (Fenner 1989). This property may limit the tissue distribution of TX.

3.3.2.2. Pharmacology

In animal and *in vitro* models, TX and piroxicam (PX) have been demonstrated to be weaker prostaglandin inhibitors than indomethacin and diclofenac (Fenner 1992). The inhibitory activity reflects a 120 fold difference between indomethacin or diclofenac and PX, and a 260 fold difference with TX.

In addition, TX inhibits both proteoglycanase and collagenase production while PX inhibits only proteoglycanase production (Day *et al.* 1991).

3.3.2.3. Pharmacokinetics

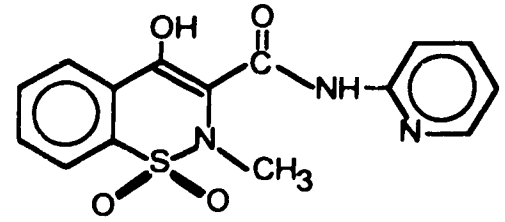
The pharmacokinetics of oxicam NSAIDs are unique because of their low lipophilicities, small V_ds, low hepatic extraction ratios and prolonged $t_{½}$ values.

3.3.2.3.1. Absorption and Distribution

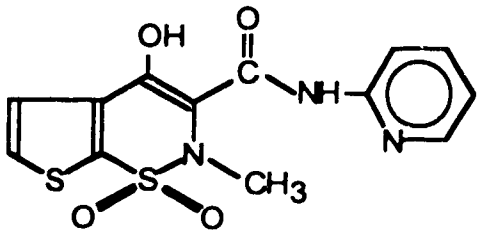
TX is rapidly and completely absorbed after oral administration (Guentert 1987). Intake of food delays absorption without altering the bioavailability. There is no evidence of enterohepatic recirculation of TX in humans.

More than 98.5% of the drug is bound to human plasma proteins, therefore the V_d after intravenous administration is only 0.15 l/kg (Olkkola *et al.* 1994).

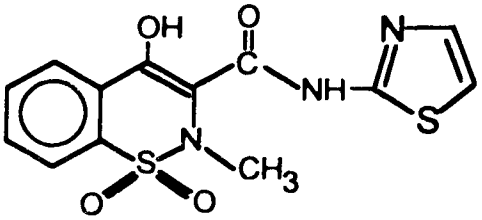
After oral administration of 40 mg of TX in patients with polyarthritis, the drug was found to enter the synovial fluid consistently. Synovial fluid concentrations were 50 to 60 % of plasma concentrations (Bird *et al.* 1985).



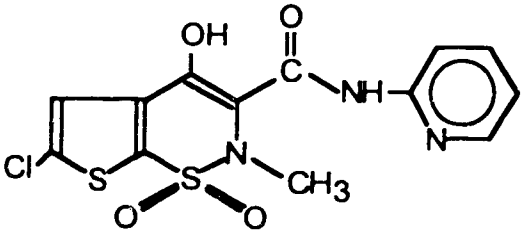
Piroxicam



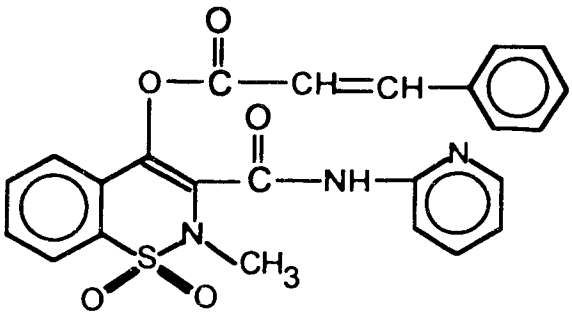
Tenoxicam



Sudoxicam



Lornoxicam



Cinnoxicam

Figure 3.3-1 : Chemical structures of oxicam non steroidal antiinflammatory drugs.

3.3.2.3.2. Excretion and Metabolism

Two main metabolites of TX have been identified in humans (figure 3.3-2). The 5'-hydroxy metabolite (5'-OHTX), representing 30-40 % of the dose, is excreted mainly in the urine. The 6-O-glucuronidated metabolite (G6-OTX), which may represent as much as 40-50 % of the dose, is mainly excreted in bile (Woolf & Radulovic 1989). These two metabolites do not have any pharmacological activity. Beside these two metabolites, six others have been identified in rats (Ichihara *et al.* 1984).

Only traces of drug are excreted unchanged in urine and unchanged TX has not been detected in bile. After IV administration, the mean total body clearance (Cl_{TB}) is 0.106 l/h. The ability of the liver to extract TX from blood is low, so the drug has a long $t_{½}$ with a mean value of about 70 hours in healthy volunteers, the longest of any of the oxicam NSAIDs (Nilsen 1994).

3.3.2.3.3. Effect of age and disease

In elderly, the mean $t_{½}$ was similar to that observed in healthy young volunteers (Shmitt & Guenter 1989). However, interindividual differences were larger in the elderly population than in younger volunteers.

Renal disease does not affect the pharmacokinetics of TX and haemodialysis does not increase its elimination (Al-Ghamdi *et al.* 1992). Interestingly, although TX is predominantly eliminated through metabolic processes, liver disease does not significantly affect the pharmacokinetic profile of TX (Crevoisier *et al.* 1989).

3.3.2.3.4. Efficacy and tolerability

The efficacy and tolerability of TX have been investigated following different dosage regimens (Visher 1987). Due to the long $t_{½}$ of TX, a daily dose of 20 mg has been defined as an optimal dosage regimen. In comparative studies with indomethacin (75 mg), naproxen (500 mg), ibuprofen (2.4 g) and diclofenac sodium (75 mg), a daily dose of TX (20 mg) proved to be as effective as these other NSAIDs.

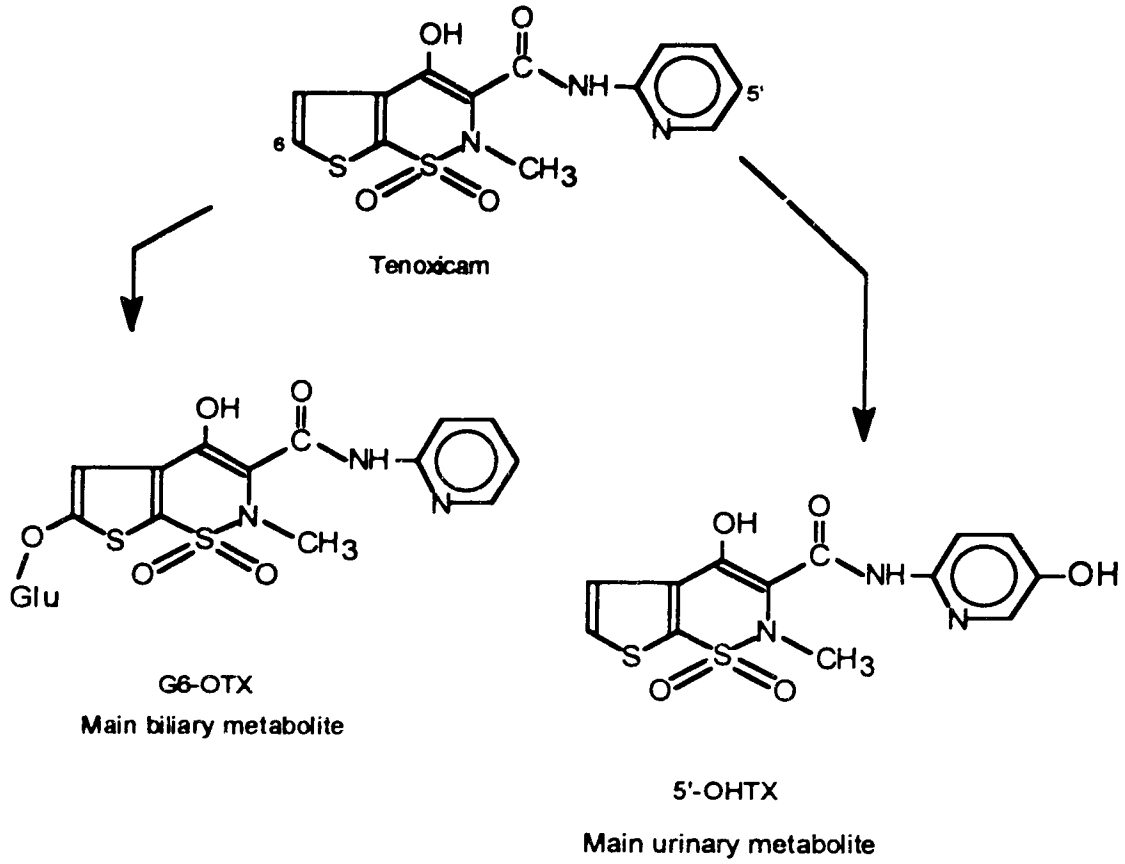


Figure 3.3-2: Main metabolites of tenoxicam.

In short-term and long-term comparative studies versus piroxicam, the types of side effects were very similar (gastrointestinal disturbances and skin reactions) and generally mild and reversible. However the incidence of adverse reactions was lower with TX (Table III).

TX appears to be better tolerated than other NSAIDs such as aspirin and ketoprofen (Bird *et al.* 1983, Ejstrup *et al.* 1989). Twenty-nine percent of the TX treated patients experienced at least one adverse reaction compared to 47.3 % of those treated with ketoprofen. Similar results have been shown in rats (Whitehouse 1986, Al-Ghamdi *et al.* 1991).

Table III: Incidence of side-effects in patients treated with 20 mg of TX or 20 mg of piroxicam (from Wisher 1987).

	Short-term studies (up to 6 weeks)		**Long-term studies (6-12 weeks)**	
	Tenoxicam	Piroxicam	Tenoxicam	Piroxicam
Number of patients	800	801	177	177
Incidence of side-effects (%)	10.75	16.85	14.7	24.3

3.4. OBJECTIVES OF THE STUDY

The objective of this work was to examine the influence of the residence time of the drug on intestinal toxicity of NSAIDs. In the first part, the lower GI toxicity of TX, a long-half life NSAID, was studied using the rat as a model. Preliminary studies showed that in male rats TX exhibits a short half-life. However, in female rats the elimination half-life of TX is much longer. Hence, we delineated the pharmacokinetics of TX in both sexes. Subsequent comparison of the TX-induced intestinal permeability in both sexes provided insight into the effect of residence time of TX on GI toxicity. We established, in both sexes, the time courses of the GI

effect as well as the dose effect relationship. In this model, however, any observed difference could be attributed to other sex-related differences.

The effect of alteration of the residence time of NSAIDs (long *vs* short $t_{1/4}$) has been an object of debate in the last several years (Adams 1987, Furst 1992). In previous studies the efficacy and toxicity of long $t_{1/4}$ NSAIDs have been compared with those of short lived drugs. This approach ignores intrinsic molecular differences between drugs. In male rats, we studied the effect of flurbiprofen on the intestinal permeability following multiple pulse IP and continuous IP infusion via osmotic pumps. The latter is a simulation of prolongation of the residence time without repeated GI exposure or fluctuation in the drug plasma concentration.

It has become apparent that acute gastric mucosal lesions caused by a single exposure of the gastric mucosa to NSAIDs or other irritants decrease with repeated insults indicating the ability of the mucosa to adapt to these damaging agents (Konturec & Konturek 1994). This concept of adaptation during continuous treatment has been most extensively studied for aspirin (St John *et al.* 1973, Graham *et al.* 1983). However, this concept has never been examined in the lower GI tract. We followed the changes in lower GI toxicity during multiple pulse treatments (IP and PO) and during continuous IP infusion.

4. MATERIALS

4.1. CHEMICALS

TX was supplied by Hoffmann-La Roche LTD. (Basle, Switzerland), piroxicam, flurbiprofen and ketoprofen were purchased from Sigma Chemical Company (St. Louis, MO, USA).

Potassium hydrogen phosphate, acetonitrile and triethylamine were obtained from BDH Chemicals Canada LTD (Edmonton, Canada), L-leucinamide was purchased from Sigma Chemical Company (St. Louis, MO, USA). Solvents were analytical grade.

Methyl cellulose was purchased from BDH Chemicals Canada LTD. (Edmonton, Canada). ^{51}Cr-EDTA (specific activity 570 MCi/mg) was purchased from DUPONT (Wilmington DE, USA).

4.2. ANIMALS

Mature male (300-350 g) and female (250-300 g) Sprague-Dawley rats were housed at ambient temperature and humidity, with a 12 hours light-dark cycle in individual metabolic cages (Fisher Scientific, Edmonton, AB, Canada). Rats were fed *ad libitum* with standard chow (Purina rat chow, Ralston Purina, St. Louis, MO) and allowed free access to water.

5. METHODS

5.1. TENOXICAM PHARMACOKINETIC STUDIES

Right jugular vein was canulated with silastic tubing (0.58 mm i.d. x 0.965 mm o.d., Clay Adams, Parsippany, NJ) under methoxyflurane anesthesia (Janssen, Mississauga, Ontario, Canada) on male and female rats (n=3 in each group). Surgery was performed the day before the pharmacokinetic experiment. Animals were fasted overnight with free access to water. Food was allowed 3 hours after drug administration.

On the morning of the study, rats were dosed orally with 5 or 20 mg/kg of TX suspended in 2% methyl cellulose solution. Whole blood samples (200 µl) were collected at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48 and 72 hours after TX administration and immediately centrifuged. Plasma was separated and transferred to a 1 ml polypropylene microcentrifuge vial (Fisher Scientific, Edmonton, Canada) and frozen at -18 °C until analysis.

5.2. TENOXICAM PROTEIN BINDING STUDY

One ml aliquots of male or female rat plasma were spiked with TX in order to obtain concentrations of 10, 50, 100 and 150 µg/ml. Spiked plasma were incubated for 1 hour at 37°C in a shaking water bath. Separation of free from protein-bound drug was accomplished by ultrafiltration of free microsolute through an anisotropic, hydrophilic YMT ultrafiltration membrane (Amicon, Beverly, MA, USA). The driving

force for filtration is provided by centrifugation at 3000 rpm (1800 g) on a Clay-Adams centrifuge, for 15 min. Macromolecular components larger than the membrane pores are retained above the membrane, while ultrafiltrate containing free drug is collected in the filtrate cup. Collected samples were transferred into a 1 ml polypropylene vials and frozen at - 18 °C until analysis.

TX was shown to have no affinity to the ultrafiltration membrane by measuring the concentration on each side of the membrane after centrifugation.

5.3. TENOXICAM ^{51}Cr-EDTA PERMEABILITY STUDIES

5.3.1. INTESTINAL PERMEABILITY ASSAY

On the morning of the study each rat received a single oral dose of TX suspended in 0.5 ml of 2% solution of methyl cellulose. To test intestinal permeability, 0.5 ml of an aqueous solution containing 10 μCi/ml of ^{51}Cr-EDTA was administered orally after the dose of TX.

Urine was collected at 0-4, 4-8 and 8-24 hours following the administration of ^{51}Cr-EDTA solution in cups containing 1 ml of H_2SO_4 (1M) to prevent microbial growth. Before each collection, metabolic cages were rinsed with 10 ml of tap water and added to urine cups. Contents of urine cups were then transferred to scintillation vials and counted directly.

5.3.1.1. Baseline determination

For each experiment the ^{51}Cr-EDTA solution was administered to untreated animals (males and females) in order to determine the baseline value of relative permeability. Results were pooled and their mean was used as control.

Neither the suspending agent, nor the animal handling had any effect on the intestinal permeability.

5.3.1.2. Time course of permeability changes

TX, suspended in 2 % methyl cellulose solution, was orally administered to male and female rats at a dose of 5 or 20 mg/kg. TX administrations were followed by single doses of ^{51}Cr-EDTA solution at 1, 2, 3, 6, 12, 24, 36, 48, 72 and 96 hours (n=5 for each time point).

5.3.2. DOSE EFFECT STUDY

Each group of male and female rats (n=3) received increasing oral doses of TX (suspended in 2 % methyl cellulose): 0.5, 1, 2, 5, 10, 15, 20, 30 or 40 mg/kg followed by a single dose of ^{51}Cr-EDTA, 3 hours after drug administration for the males and 6 hours after for the females. The 3 and 6 hour time were selected as the maxima of the time course of toxicity in males and females respectively.

5.3.3. MULTIPLE DOSE STUDY

Male rats were randomized to receive TX at the dose of 0.5 mg/kg (n=8) or 1 mg/kg (n=8). Rats were then distributed into two treatment groups: single dose (n=4) at 9 AM, or multiple dose (n=4) every 12 hours (at 9 AM and 9 PM) for 3 days.

Intestinal permeability was assessed at both 3 and 48 h after the last TX administration, for each treatment group.

5.4. FLURBIPROFEN STUDIES

5.4.1. MULTIPLE DOSE / CONTINUOUS INFUSION STUDY

Male rats were randomized into two flurbiprofen (FL) treatments: multiple dose of 2.5 mg/kg each 12 hours via intraperitoneal injection (n=8), and continuous intraperitoneal infusion of 5 mg/kg/day (n=8). In each group, FL was dissolved in PEG 400. The total duration of study for both treatments was 13 days.

In the continuous infusion group, osmotic pumps (Alzet pump model 2002, ALZA Scientific Products, Palo Alto, USA) were implanted under methoxyflurane anesthesia, into the rat peritoneal cavity. Alzet pumps were filled with 200 µl of a solution of FL in PEG (135 mg/ml); the stability of the solution was tested after incubation in a 37°C water bath for 14 days. The delivering rate was 0.5 µl/h. These pumps start infusing two days after implantation. Therefore, the first day of treatment was considered to be the third day after implantation.

Urine samples (24 hours) were collected on day 6, 10, 12, to verify the achievement of steady state drug levels in animals. Lower intestinal permeability tests were performed at different times after the beginning of treatments (Figure 5.4-1). At the time of the sacrifice, by CO_2 asphyxiation, 1ml whole blood samples were withdrawn from each animal. Samples were immediately centrifuged, the plasma was separated, transferred to a 1 ml polypropylene vial and frozen at -18 °C until analysis.

Duodenum, jejunum and ileum were excised from each animal, weighed, transferred to 25 ml glass scintillation vial and immediately frozen at -18 °C until analysis.

5.4.2. ADAPTATION STUDY

Male rats were randomized into three flurbiprofen (FL) treatments: multiple dose of 2.5 mg/kg every 12 hours via intraperitoneal injection (n=8), multiple dose of 2.5 mg/kg each 12 hours via oral administration (n=8), and continuous intraperitoneal infusion of 5 mg/kg/day (n=8). For the IP groups FL was dissolved in PEG 400, for the oral group FL was suspended in 2% methyl cellulose. The total duration of treatments was 8 days.

A permeability test was performed on the first day of treatment, for all 3 groups, 3 hours after the first IP and oral dose (noon). Permeability was also measured on day 4 and 7 at noon.

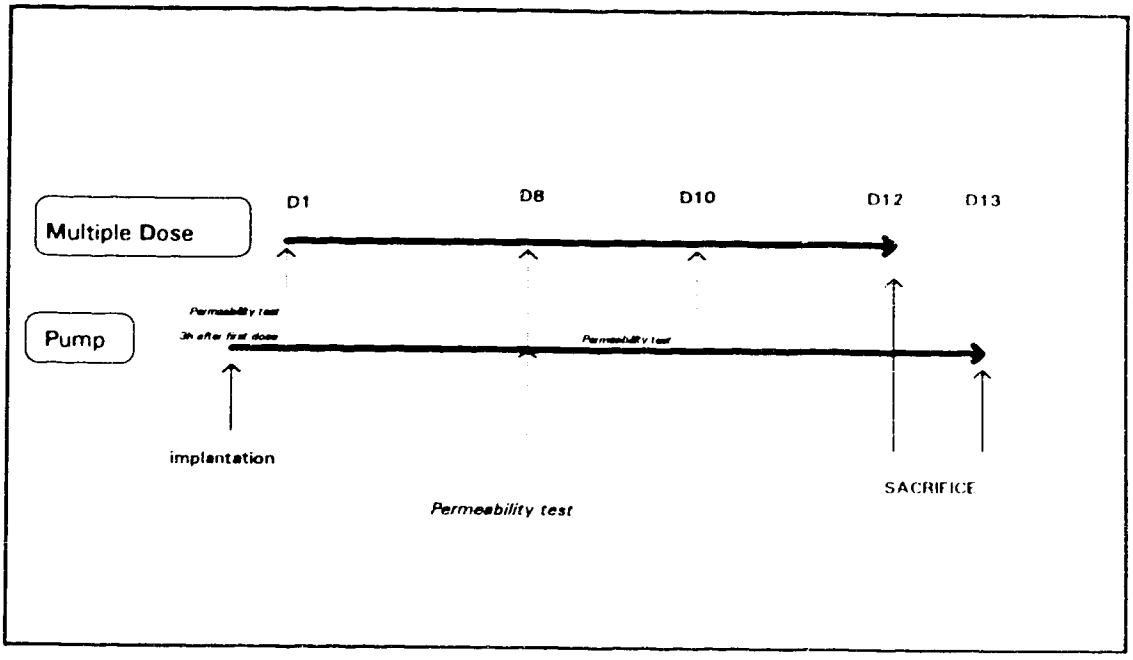


Figure 5.4-1: Design for the multiple dose / continuous infusion study.

5.5. GAMMA COUNTING ANALYSIS

Urine samples were counted in a Minaxi γ Auto-Gamma 5000 counter (Packard, Meriden, CT, USA) for 1 minute in a counting window scanning within a range of 0-2 Mev. Two standards of 100 µl of the administrated ^{51}Cr-EDTA solution (in 10 ml tap water + 1 ml H_2SO_4) as well as two blank samples (10 ml tap water + 1 ml H_2SO_4) were counted with each set of urine samples.

The relative permeability was determined by calculating the activity in each urine sample as a percentage of the administered dose after correction for the background radiation. Apparent permeability was reported as the percentage of dose excreted in urine from 0 to 8 hours.

5.6. DRUG ANALYSIS

5.6.1. FLURBIPROFEN ANALYSIS

Flurbiprofen was quantified in plasma, urine and tissue samples according to a method described previously (Berry & Jamali 1988). Tissue samples were ground and suspended into 4 ml of distilled water for duodenum, and 8 ml for jejunum and ileum. An aliquot of 1 ml of each suspension was taken for analysis.

The HPLC system used consisted of a Waters 6000A pump, a Waters 440 multiple-wavelength UV detector, WISP 712 auto sampler (Waters, Mississauga, Canada) and a Shimadzu CR501 integrator (Shimadzu, Columbia, MD, USA) operating at ambient temperature. The column was a 15 cm x 4.6 mm ID C_8 analytical column packed with 5 µm particles (CSC, Montreal, Canada), attached to a Waters Guard Pak pre column module containing a Nova-pak C_8 insert. The mobile phase consisted of phosphate buffer (pH=4.7)-acetonitrile-triethylamine (35:65:0.02 v/v) and the flow rate was 1ml/min.

5.6.2. TENOXICAM ANALYSIS

The HPLC system consisted of a Waters 6000A pump, a Waters 440 multiple-wavelength UV detector set at 360 nm, WISP 712 auto sampler (Waters, Mississauga, Canada) and a Shimadzu CR501 integrator (Shimadzu, Columbia, MD, USA) operating at ambient temperature. The column was a 25 cm x 4.6 mm ID C_8 analytical column packed with 5 µm particles (Phenomenex, Torrence, CA, USA) attached to a Waters Guard Pak precolumn module containing a Nova-pak C_8 insert. The mobile phase consisted of phosphate buffer (pH=4.7)-acetonitrile-triethylamine (70:30:0.02 v/v) with a flow rate of 1 ml/min.

5.6.2.1. Standard solutions

Stock solutions of TX (10 µg/ml) (solution I) and piroxicam (2 µg/ml) (IS) were prepared in 100 ml of water. The solutions were stored at 5°C in tinted bottles.

Two dilutions of TX solution I were prepared each day: a 1/10 dilution (solution II) and a 1/100 dilution (solution III). All standards were extracted in the same manner as described below for the plasma samples.

5.6.2.2. Sample preparation

Calibration curves using standard solutions were prepared as follows:

Final Concentration of Tenoxicam (μg/ml)	Tenoxicam added in 100 μl plasma volume
1	100 μl of solution III
5	50 μl of solution II
10	100 μl of solution II
20	20 μl of solution I
40	40 μl of solution I
60	60 μl of solution I

In 13x100 mm test tubes, to 100 μl of plasma (standards or samples) was added 50 μl of IS, 100 μl of 0.6 M H_2SO_4 and 3 ml of extracting solvent (diethyl ether). The tubes were vortex mixed for 30 seconds and then centrifuged for 5 minutes at 3000 rpm (1800 g) on a Clay-Adams centrifuge.

The top layer was transferred to a clean tube and this layer was evaporated to dryness using a SVC 100H Savant Speed Vac concentrator and refrigerated condensation trap (Emerston instruments, Scarborough, Canada). The residue was reconstituted in 200 μl of mobile phase. Different aliquots of this mixture (30-100 μl) were injected into the HPLC system for analysis.

5.7. PHARMACOKINETIC ANALYSIS

The plasma concentrations versus time were fit to a one compartment model using PCNONLIN version 4.0 (Metzler & Weiner 1992) and the elimination rate constant (K_E) was estimated. The quality of the fit was assessed by the Schwartz and Akaike criteria given, and by the distribution of the residuals. The area under the plasma curve (AUC) was calculated using the linear trapezoid rule from 0-48 h. $AUC_{48-\infty}$ was calculated as C_{48h} divided by K_E.

The apparent total body clearance (CL/F) was calculated by dividing the dose by $AUC_{0-\infty}$. The apparent volume of distribution (V_d/F) was estimated by dividing the apparent total body clearance by K_E.

5.8. PHARMACOLOGICAL ANALYSIS

Analysis of the dose-effect studies were performed using the sigmoidal E_{max} model with baseline effect available in PCNONLIN version 4.0 (Metzler & Weiner 1992):

$$E = E_0 + \frac{D^{\gamma}(E_{max} - E_0)}{D^{\gamma} + ED_{50}{}^{\gamma}}$$

where E is the measured effect, D is the dose, ED_{50} is the dose that produces 50% of the maximal response and γ is a number influencing the slope of the curve.

Quality of the fit was determined by the Schwartz and Akaike coefficients calculated by PCNONLIN as well as the distribution of the residuals.

5.9. STATISTICAL ANALYSIS

Linear regression was performed using the least-squares method for the correlation between 0-8 and 8-24 hours urine collection. The significance of the regression was tested by ANOVA, the significance of the slope and intercept were tested using the unpaired t-te..

The Student t-test for unpaired data was used to determine differences between groups, except for adaptation studies where Student t-test for paired data was chosen.

The level of significance was set at $\alpha = 0.05$. Results are presented as mean ± standard deviation (SD).

6. RESULTS

6.1. TENOXICAM HPLC ASSAY

TX and internal standard piroxicam were eluted at 5.4 and 7.2 min respectively. There were no interfering peaks from normal components of rat plasma.

Under our chromatographic conditions, the linear range of the assay was set from 0.5 to 60 μg/ml in plasma. The correlation coefficient (r^2) was at least equal to 0.999. In plasma the detection limit for TX was 250 ng/ml, the extraction yields were 75% and 82% for TX and piroxicam respectively (table IV).

The data demonstrating precision, calculated as the inter-day and intra-day variability, are shown in Table V. The coefficient of variation did not exceed 5% for low, medium and high concentrations. The accuracy of the assay was demonstrated by calculating the error on quality control samples. Error was below 10% for both low and high concentrations (Table VI).

Therefore, this assay was found suitable for determination of TX in rat plasma. An example of the chromatogram and calibration curve are displayed in Figure 6.1-1.

Table IV: Extraction yields for Tenoxicam and piroxicam in rat plasma.

	Extraction Yield (Mean ± SD)
TX 1µg/ml (n=3)	74 ± 2.64 %
TX 10µg/ml (n=3)	69 ± 1.53 %
TX 60 µg/ml (n=3)	75 ± 2.08 %
Piroxicam 50 µg/ml (n=9)	82 ± 2.27 %

Table V: Precision of the assay. Inter-day and intra-day variability for analysis of TX in rat plasma.

Concentration (µg/ml)	Ratio TX / IS (mean ± SD)	Coefficient of Variation (%)
Inter-day variability		
1 (n=8)	0.316 ± 0.016	5.0
10 (n=8)	3.184 ± 0.08	2.6
60 (n=8)	19.432 ± 0.9	4.8
Intra-day variability		
1 (n=3)	0.319 ± 0.013	4.03
10 (n=3)	3.163 ± 0.06	1.9
60 (n=3)	18.457 ± 0.3	1.63

Table VI : Accuracy of the assay

TX (µg/ml)	Calculated concentration (µg/ml) (mean ± SD)	Error % (mean ± SD)
0.5 (n=3)	0.520 ± 0.009	4.1 ± 1.87
20 (n=3)	21.481 ± 0.043	7.36 ± 0.23

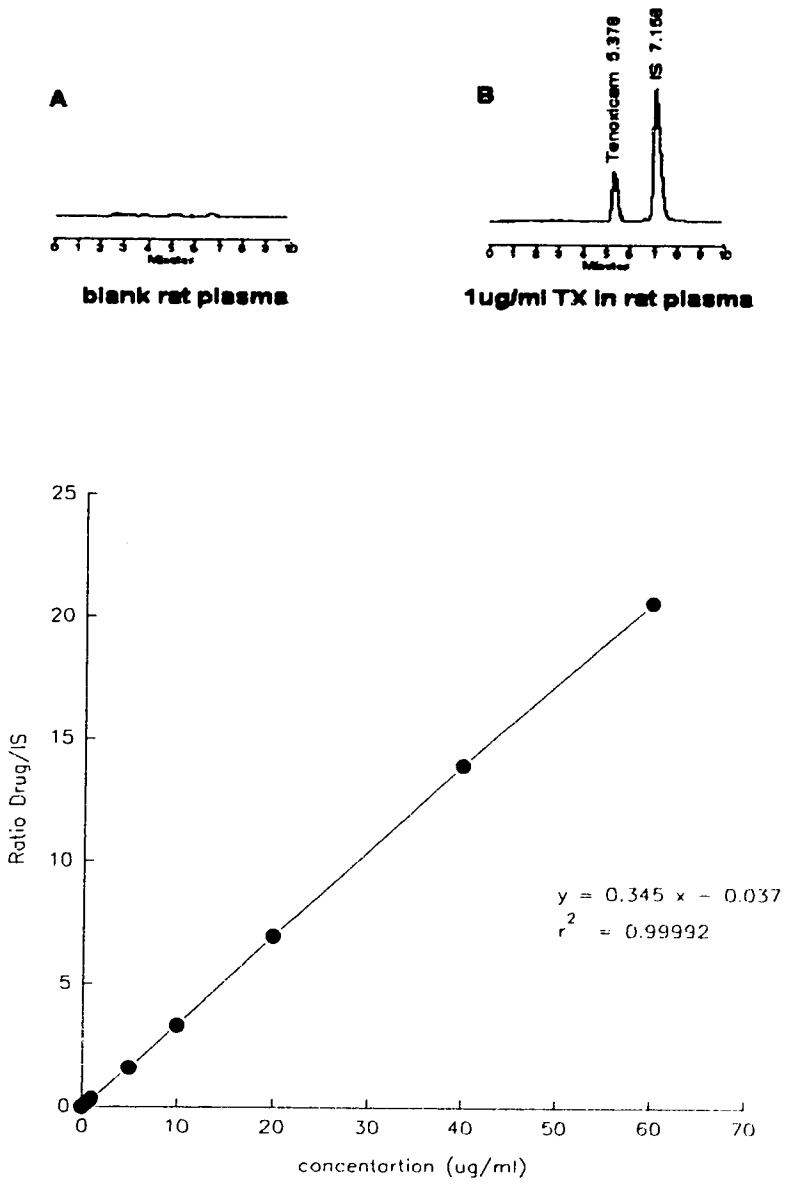


Figure 6.1-1: Example of chromatograms of blank rat plasma (A), tenoxicam and IS in rat plasma (B), and calibration curve in rat plasma (C).

6.2. TENOXICAM PHARMACOKINETIC STUDIES

The plasma concentration versus time profiles, after 5 mg/kg single oral dose of TX, are shown in Figure 6.2-1 and the pharmacokinetic parameters describing these profiles are presented in Table VII. C_{max} was 20.08 ± 4.96 µg/ml in males, and 28.89 ± 9.15 µg/ml in females; in males and females T_{max} was 0.33 ± 0.14 h and 1.24 ± 0.9 h respectively. $t_{½}$ was shorter in male rats compared to female rats (5.2 ± 1 h *vs* 16.88 ± 4 h). All the kinetic parameters were found to be significantly different between the sexes except V_d/F (p=0.06).

Table VII: Pharmacokinetic parameters after 5 mg/kg single oral dose of TX in male and female rats (n=3, mean ± SD).

	FEMALES	MALES
K_E (h^{-1})	0.043 ± 0.011	0.136 ± 0.027[a]
$AUC_{0-\infty}$ (mg.h/l)	587 ± 105	90.5 ± 21[a]
V_d/F (ml)	46.5 ± 18	111 ± 41
CL/F (ml/min)	0.029 ± 0.006	0.367 ± 0.09[a]

a: statistically significantly different from females (p<0.05).

Figure 6.2-1 and Table VIII illustrate the plasma concentration *vs* time profile after 20 mg/kg and pharmacokinetic parameters respectively. C_{max} was 85.2 ± 23.5 µg/ml (T_{max} = 2.66 ± 0.57 h) and 78.85 ± 7.9 µg/ml (T_{max} = 2.5 ± 0.86 h) in males and females respectively. The elimination half-life remains significantly longer in females with 21.06 ± 3 h *vs* 5.85 ± 0.95 h in males. The oral clearance in males was found to be significantly lower at this 20 mg/kg dose.

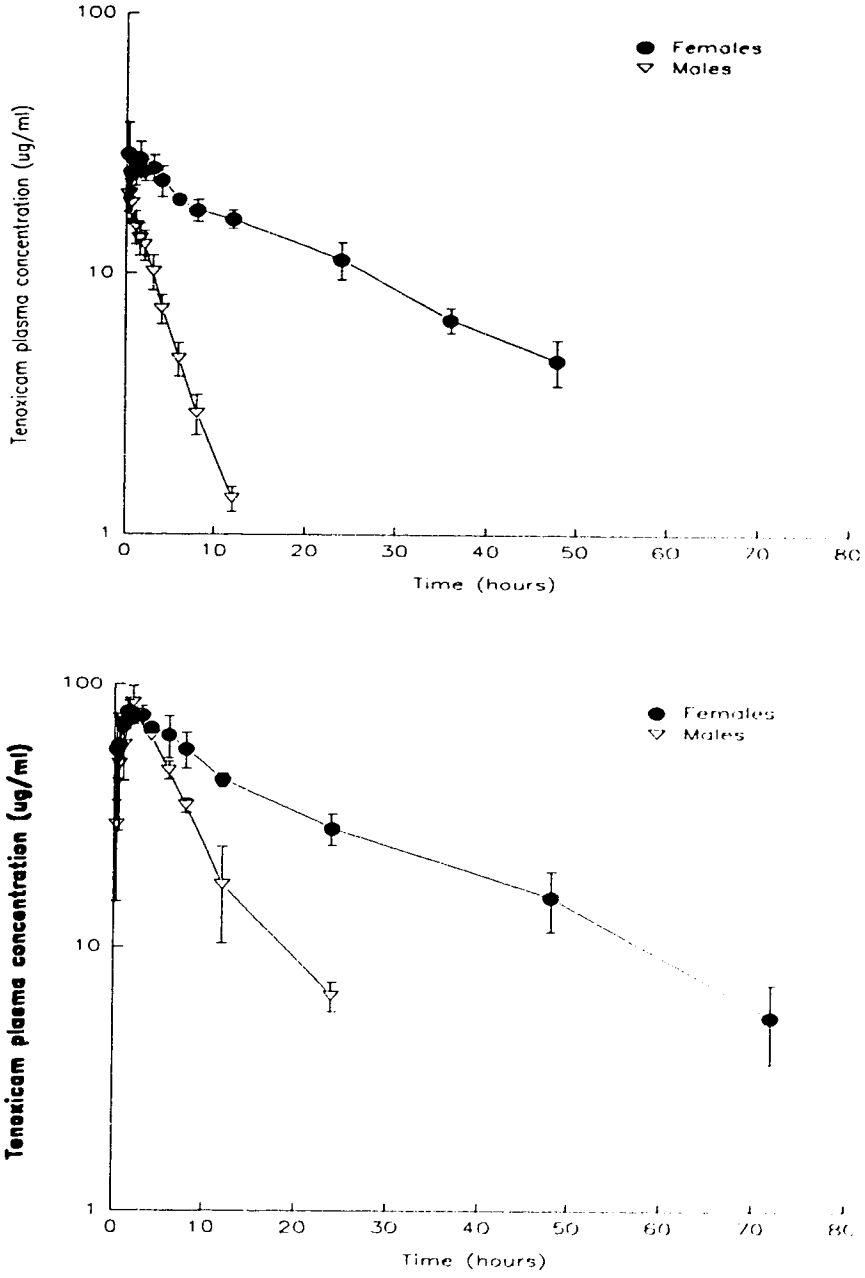


Figure 6.2-1: Time course of tenoxicam in male and female rats. Dose of 5mg/kg (upper panel), dose of 20mg/kg (lower panel). (n=3, mean ± SD).

Table VIII: Pharmacokinetic parameters after 20 mg/kg single oral dose of TX in male and female rats (n=3, mean ± SD).

	FEMALES	MALES
K_E (h^{-1})	0.043 ± 0.01	0.121 ± 0.02[a]
$AUC_{0-\infty}$ (mg.h/l)	2114 ± 238	734 ± 99[a]
V_d/F (ml)	70 ± 8.5	83 ± 4.6
CL/F (l/min)	0.037 ± 0.004	0.162 ± 0.02[ab]

a: statistically significantly different from females ($p<0.05$).

b: statistically significantly different from the 5 mg/kg dose ($p<0.05$).

6.3. TX PROTEIN BINDING

Protein binding was determined over the concentration range of 10 to 150 µg/ml. This range covers the plasma concentrations observed in male and female rats, after 5 or 20 mg/kg doses of TX. The free fraction in both sexes appears to be concentration dependent for plasma concentrations above 50 µg/ml (Figure 6.3-1). Except for the 10 µg/ml concentration, the free fraction was significantly lower in females over the concentration range studied.

6.4. TENOXICAM PERMEABILITY STUDIES

After administration of ^{51}Cr-EDTA, urine samples were collected from 0-4 h, 4-8 h and 8-24 h. The first two fractions were pooled since some rats produced very small volumes of urine in one of the time intervals.

Addition of the last fraction results did not improve the efficiency of the ^{51}Cr-EDTA test, hence, as indicated in Figure 6.4-1, results from 0-8 h and 8-24 h correlates well ($r=0.961$, ANOVA $p<0.05$). Therefore, all results are expressed as % dose of ^{51}Cr-EDTA excreted 0-8 h.

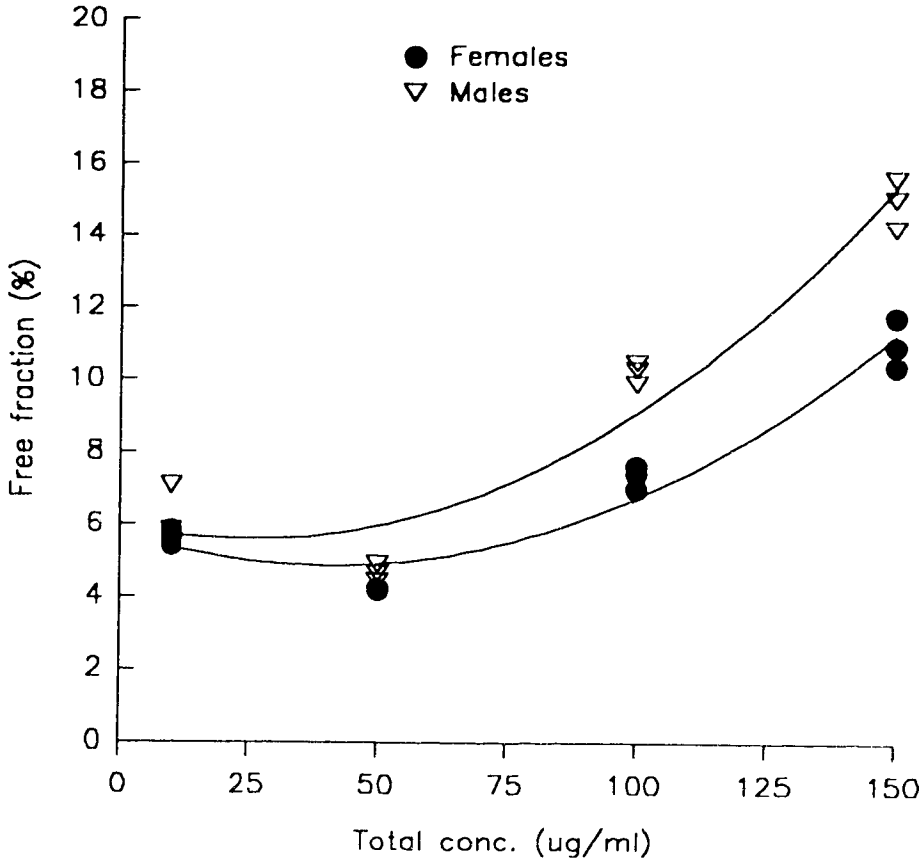


Figure 6.3-1: Free fraction of tenoxicam in male and female rat plasma (n=3).

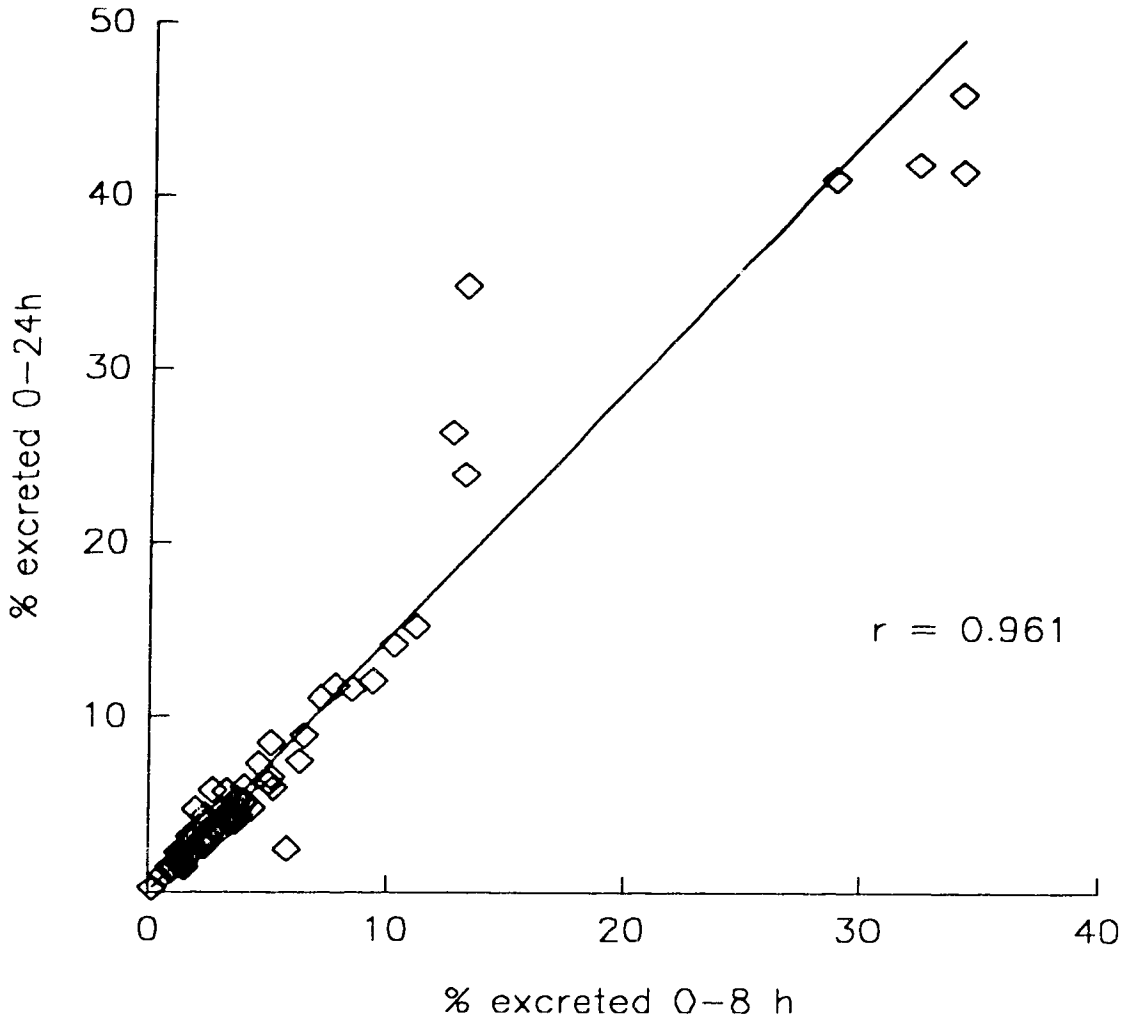


Figure 6.4-1: Correlation between percentage ^{51}Cr-EDTA excreted 0-8 hours and 0-24 hours, for control rats and rats receiving various oral doses of tenoxicam (n=94).

Base line values generated from untreated male (n=14) and female (n=13) rats in each and every experiment were pooled and their means (1.439 ± 0.249 for the males and 1.6 ± 0.167 for the females) were used as control.

6.4.1. TIME COURSE OF PERMEABILITY CHANGES

Changes in permeability over time in male and female rats receiving single 5 mg/kg oral dose of TX are presented in Figure 6.4-2. In both groups, a statistically significant increase in the apparent permeability was observed 1 hour after drug administration which persists until 6 hours. Due to a very large variability in the first two hours, the 3 hours (in the males, 4.48 ± 1.286 %) and 6 hours (in the females, 4.395 ± 1.487 %) post dose effects were considered as the maximum toxicity. Permeability returned gradually to base line value 24 hours following TX administration. No difference, at any time point, was found between sexes.

The changes in permeability over time at the 20 mg/kg are depicted for both males and females in figure 6.4-3. A more pronounced increase in permeability was observed at this dose, with maxima of 15.348 ± 2.279 % at 3 hours post dose in males and 19.948 ± 4.690 % at 6 hours post dose in females. From 6 to 72 hours, permeability in male rats remains significantly lower than in female rats. In males, permeability returns to base line values 36 hours after TX administration (2.716 ± 1.8 %). However, in females, permeability at 72 hours post dose was still significantly higher than the base line (3.461 ± 0.649 %).

6.4.2. DOSE-EFFECT RELATIONSHIP

Since the maximal permeability following oral TX occurred at 3 h post dose in the males and at 6 h post dose in the females, the dose-effect relationship was examined in each sex at these respective time points.

As shown in Figure 6.4-4, the dose-effect relationships were best described by a sigmoidal E_{max} model, with a plateau around 40 mg/kg in both males and females. The model parameters determined by PCNONLIN are presented in Table IX. No difference was found among sexes except for the 20 mg/kg dose where the effect was significantly higher in females (27.44 ± 2.75 *vs* 12.63 ± 1.19 %).

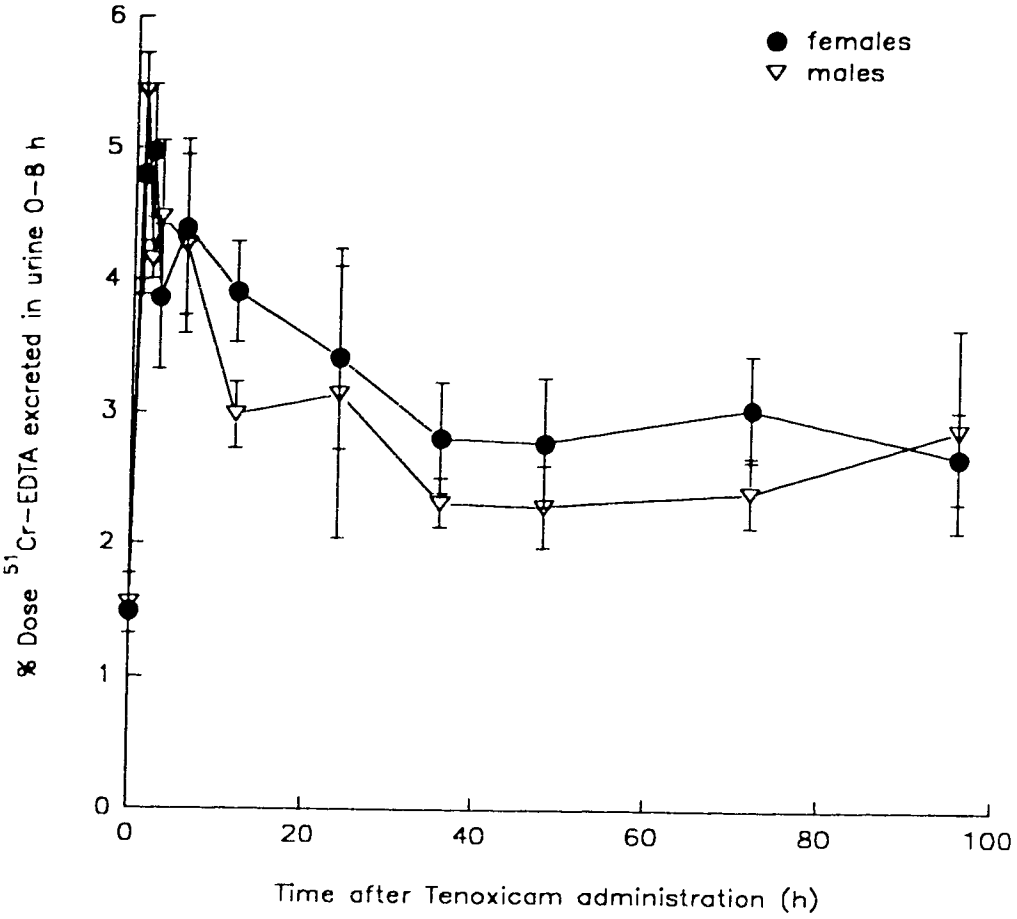


Figure 6.4-2; Time course of permeability changes induced by tenoxicam oral dose (5 mg/kg) in male and female rats (n=5, mean ± SD).

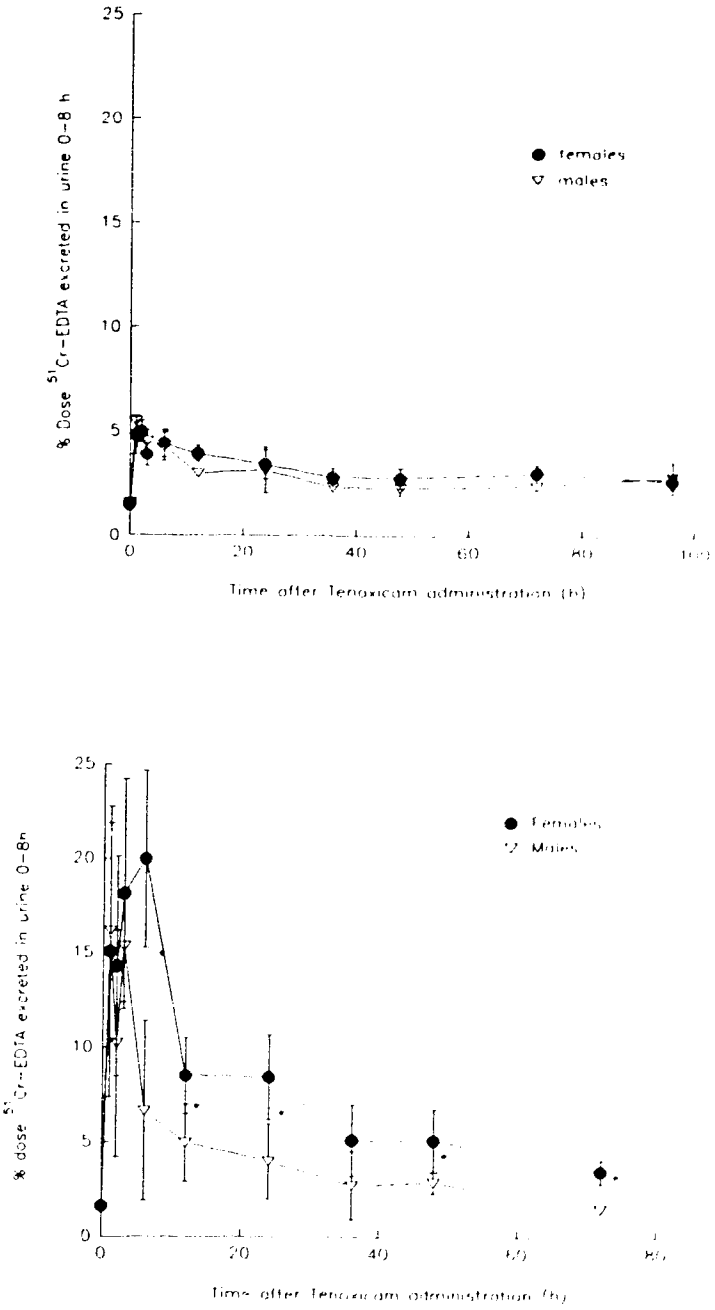


Figure 6.4-3: Time course of permeability changes after tenoxicam oral administration (5 mg/kg upper panel expanded y axis, 20 mg/kg lower panel) in male and female rats (n=5, mean ± SD).

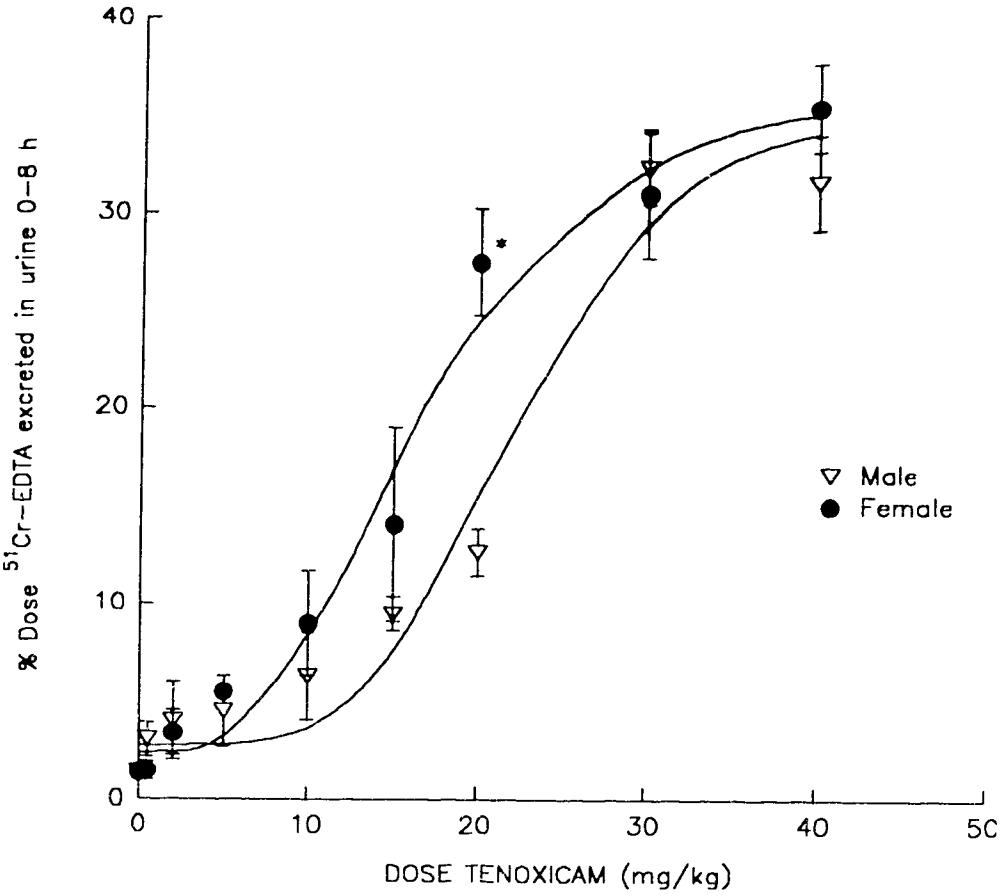


Figure 6.4-4: Dose-effect relationship of tenoxicam on intestinal permeability in male and female rats (n=3, mean ± SD).

Table IX: Pharmacodynamic parameters for TX in male and female rats. Estimation by PCNONLIN ± SD.

	FEMALES	MALES
E_{max} (%)	37.49 ± 2.78	36.28 ± 2.98
ED_{50} (mg/kg)	16.8 ± 1.33	22.24 ± 1.43
E_0 (%)	2.33 ± 0.72	2.73 ± 0.45
γ	3.02 ± 0.6	4.53 ± 0.88

6.4.3. MULTIPLE DOSE STUDY

Percent increase in intestinal permeability after multiple oral doses of TX (0.5 and 1 mg/kg) was compared to that observed after same single oral doses (Figure 6.4-5). These two doses were selected for their equivalence to human daily dose. No significant difference was found between the single dose and multiple dose treatments after both 3 or 48 h permeability measurements.

Both single and multiple dose regimen at 0.5 mg/kg did not increase significantly the intestinal permeability (3 and 48 h) from base line value. However, after single and multiple dose treatments at the 1 mg/kg dose, permeability was significantly higher than controls at 3 h.

6.5. FLURBIPROFEN STUDIES

6.5.1. MULTIPLE DOSE / INFUSION STUDY

The permeability changes induced after multiple IP injections are presented in Figure 6.5-1. The augmentation of permeability on day 1 (3 h after the first dose), day 8 or day 10 were significantly higher than base line. Furthermore, intestinal

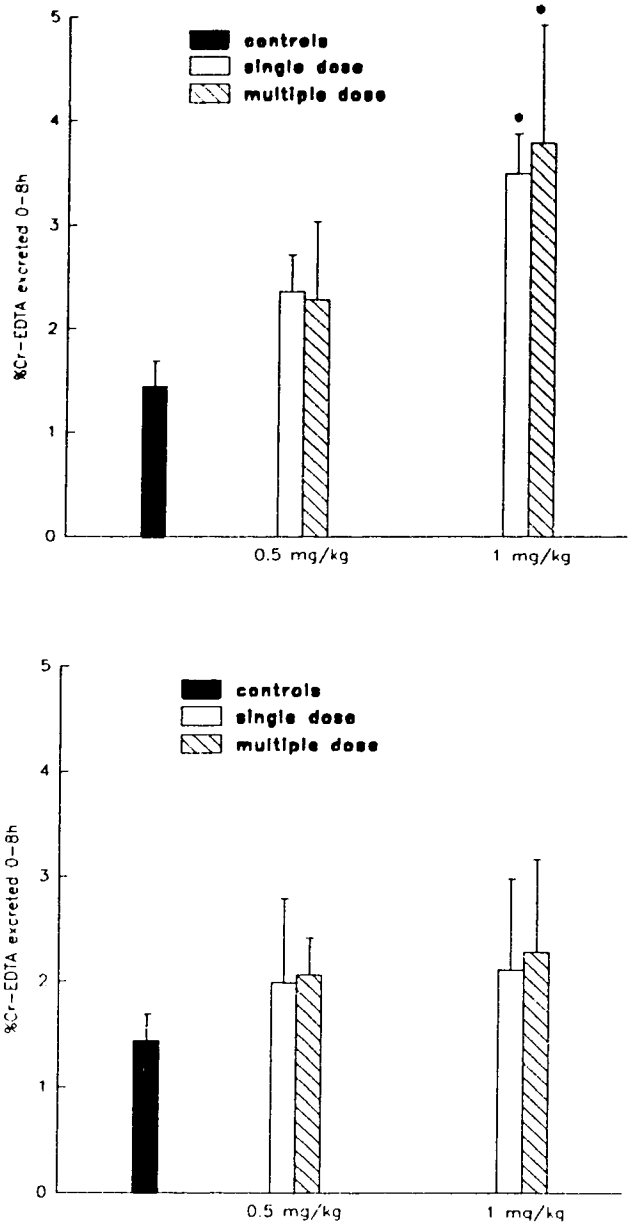


Figure 6.4-5: Effect of single and multiple oral doses of tenoxicam (0.5 and 1 mg/kg) on intestinal permeability. Measurement 3 hours after last dose (upper panel) and 48 hours after the last dose (lower panel). (n=4, mean ± SD).

*: significantly different from baseline

permeability significantly decreases on day 8 and day 10, but remains higher than controls. After continuous IP infusion, the permeability measured on day 8 (2.41 ± 0.478 %) was significantly above the base line value, but lower than that observed in the multiple dose group on the same day (4.06 ± 0.15 %) (Figure 6.5-2).

Animals in the multiple dose group were sacrificed 5 hours after the last IP administration. Rats in the infusion group were sacrificed at the same time as those in the multiple dose group. The plasma levels obtained on the day of sacrifice were not different from one another in the two treatments (Table X). Intestinal segments were excised, R and S-FL were quantified in each tissue for each group. Tissue levels of S-FL tended to be higher in the multiple dose group, but only S-FL concentration in duodenum reached statistical significance (Table X).

Table X: Plasma and intestinal tissue levels of flurbiprofen in multiple dose group and pump group (n=8, mean ± SD).

	PUMP GROUP		MULTIPLE DOSE GROUP	
	R-Flurbiprofen	*S-Flurbiprofen*	*R-Flurbiprofen*	*S-Flurbiprofen*
Plasma (µg/ml)	1.266 ± 0.1	3.67 ± 0.9	1.166 ± 0.7	4.55 ± 1.8
Duodenum (µg/g tissue)	2.27 ± 0.4	5.9 ± 1.8[a]	2.56 ± 1.8	9.70 ± 4.6
Jejunum (µg/g tissue)	1.92 ± 0.99	5.97 ± 1.8	1.89 ± 1.04	7.22 ± 1.7
Ileum (µg/g tissue)	2.65 ± 1.7	5.74 ± 2.1	3.62 ± 2.5	6.53 ± 2.3

a: statistically lower than multiple dose group ($p<0.05$).

6.5.2. ADAPTATION STUDY

The permeability changes (on day 1, 4 and 7) induced by the 3 different treatments are presented in Figure 6.5-3. Intestinal toxicity after oral treatment was consistently higher than the one induced by IP multiple dose treatment, however,

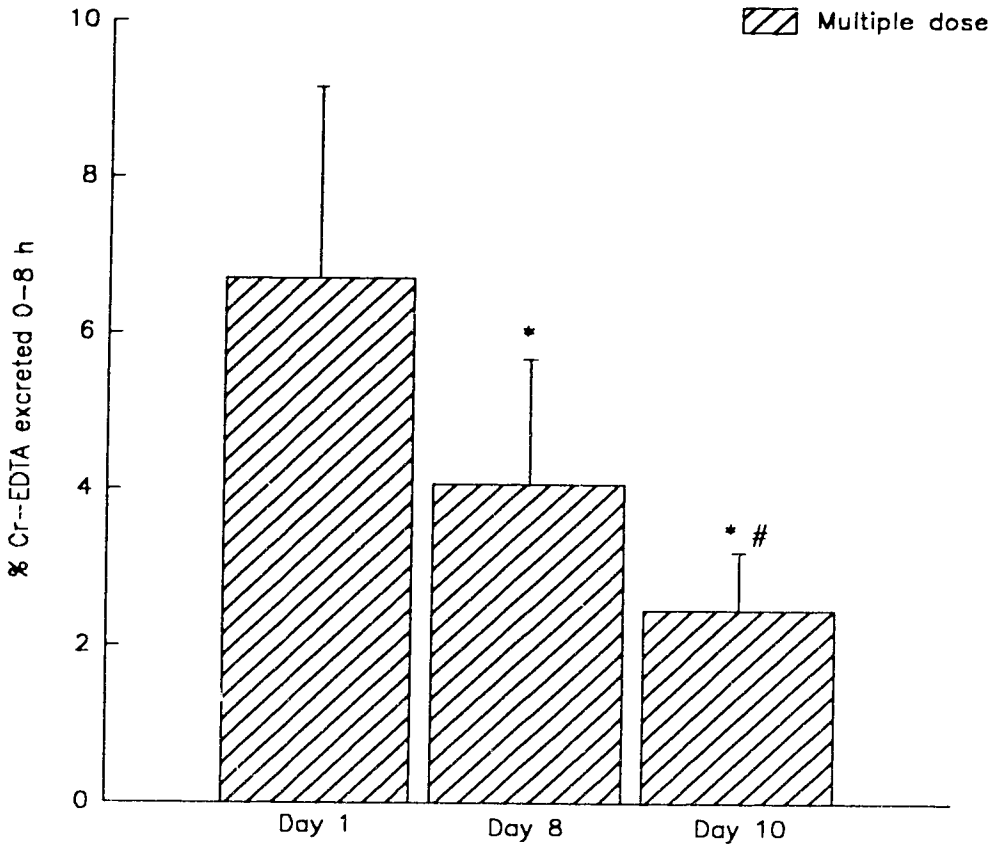


Figure 6.5-1: Permeability changes induced by multiple doses of flurbiprofen (5 mg/kg/day). (n=8, mean ± SD).

*: significantly different than day one,

#: significantly different than day 8.

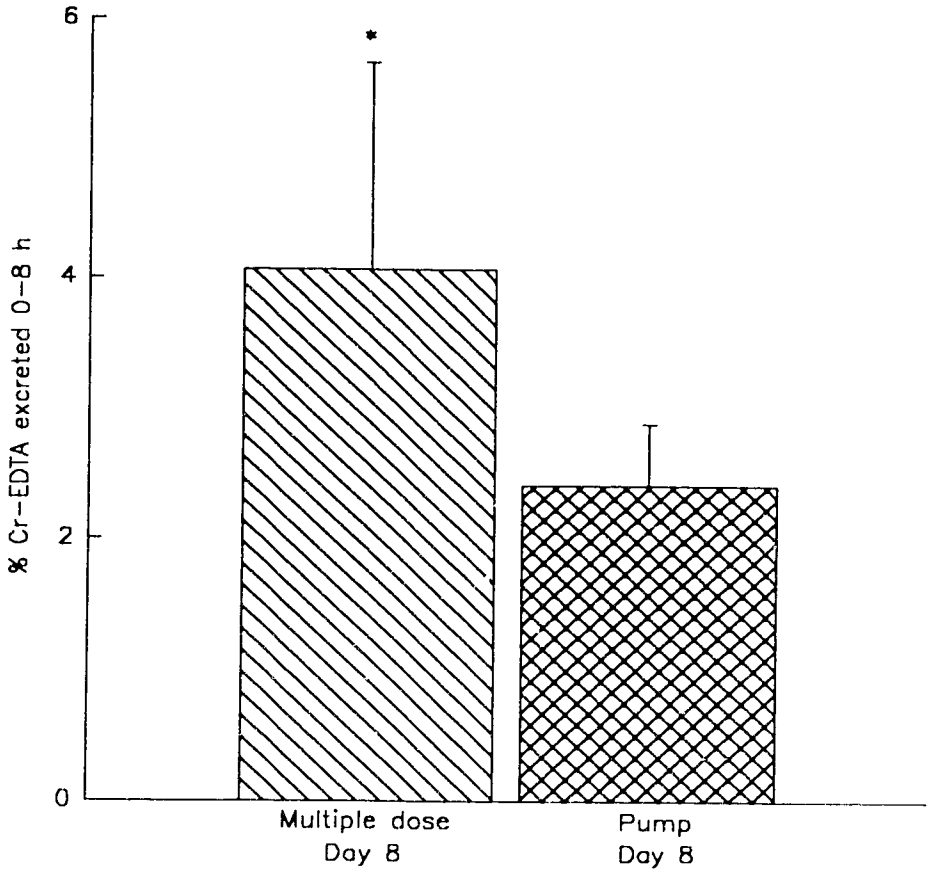


Figure 6.5-2: Comparison between continuous infusion and multiple dosing of flurbiprofen (5mg/kg/day). (n=8, mean ± SD).

*: significantly different from pump.

none of these differences reached statistical significance. Increase in intestinal permeability was lower after continuous IP infusion than after multiple IP injections ($p=0.059$ on day 1 and $p=0.02$ on day 4).

Adaptation was observed in all treatments. Toxicity significantly decreases on day 4 as compared to day 1 in both multiple dose treatments (IP and PO). On day 7 only the toxicity after multiple IP injections diminished significantly. Adaptation was less pronounced in the pump group due to a low toxicity profile of this treatment. The evolutions of intestinal permeability in each individual rat for each treatment are presented in Figure 6.5-4.

7. DISCUSSION

The possibility that long half-life NSAIDs are more toxic has been extensively discussed in the last ten years (Paulus 1985, Adams 1987, 1992, Furst 1992). It is believed that drugs with long serum $t_{½}$ maintain plasma concentrations throughout the dosing interval and thus may result in altered GI tract effects as compared with short-lived drugs (Furst 1992). However, this hypothesis do not consider the influence of intrinsic molecular toxicity of drugs, as a molecule with a shorter half-life may have a more (or less) potent effect than a molecule with a long half-life. TX is a new NSAID showing a half-life of 70 hours in humans. Interestingly, a noticeable sex difference was found in its disposition in the rats: $t_{½}$ in females was much longer than in males (16.88 ± 4 h *vs* 5.2 ± 1 h). A comparison of TX toxicity in male *vs* female provides a model to examine the influence of residence time on GI toxicity. Therefore, we could study the impact of increased residence time of a same drug in the body, without the obstacle of using different chemical compounds.

TX is described as a drug with high plasma protein binding (Day *et al.* 1988), a low volume of distribution and a very low clearance (Hartmann *et al.* 1987). The TX long $t_{½}$ permits administration of single daily doses which leads to improved compliance and efficacy in patients (Jacobs *et al.* 1988). Despite a number of reports examining the biological activity of TX in the rat, few studies have attempted to characterize the pharmacokinetics of the drug in this animal model (Lopez-Bustamante *et al.* 1992, 1993). They observed a significant decrease in the binding to plasma proteins when increasing the dose, and

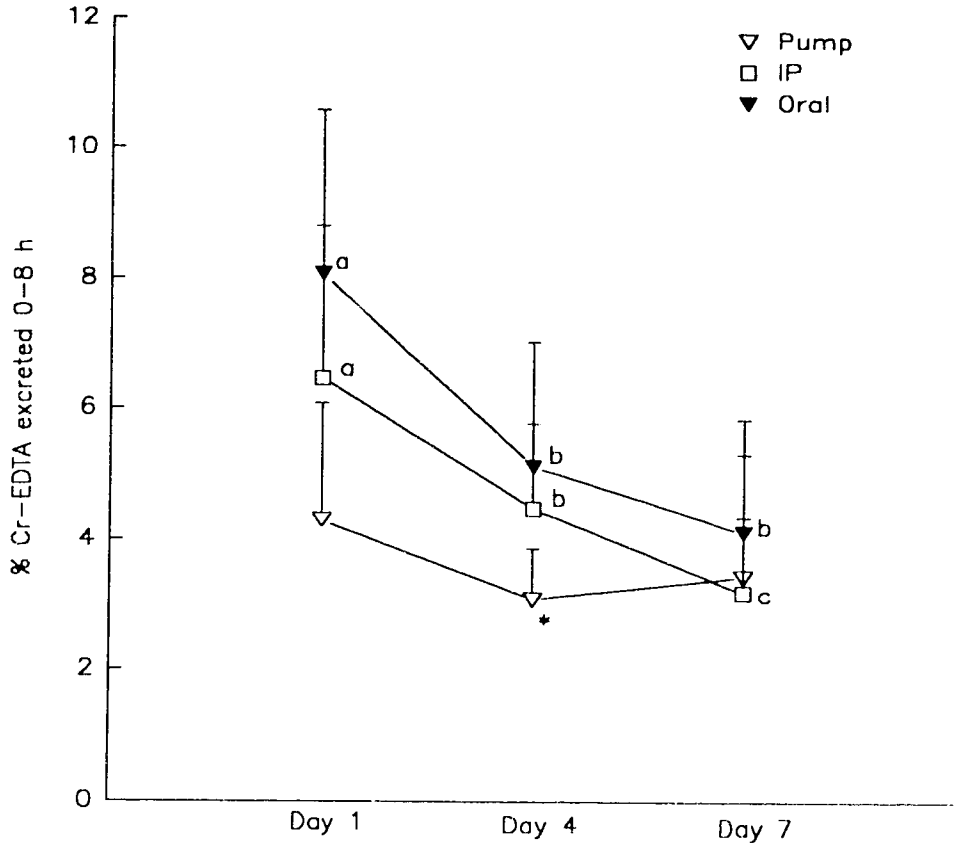


Figure 6.5-3: Permeability changes induced by oral and IP multiple dose and continuous IP infusion of flurbiprofen (5mg/kg/day). (n=8, mean ± SD).

Different letter in the same curve indicates significant difference,

*: significantly lower than IP.

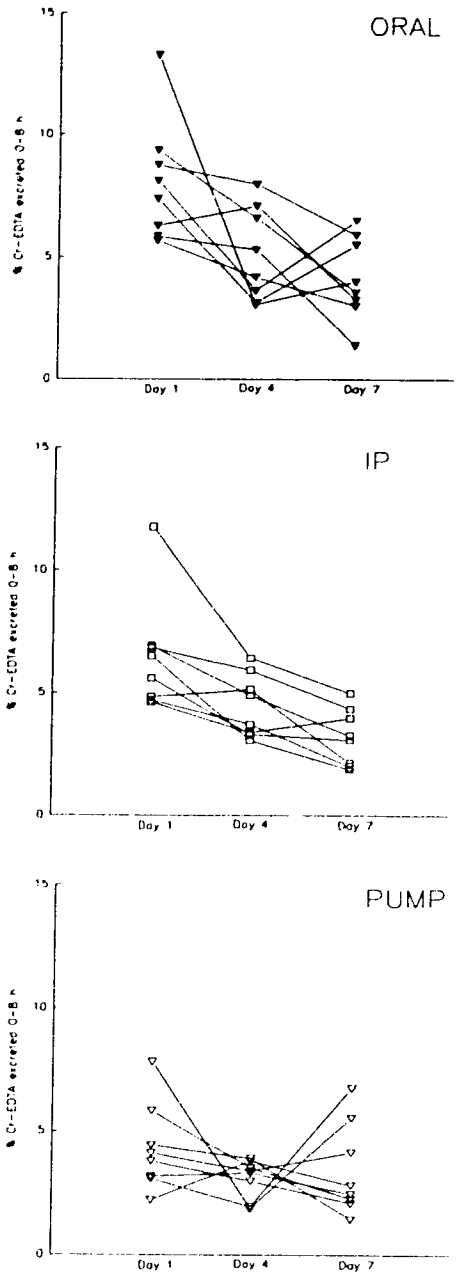


Figure 6.5-4: Evolution of intestinal permeability in each rat (n=8) for IP, PO multiple dose and IP infusion of flurbiprofen (5mg/kg/day).

suggested a nonlinear disposition of TX at high doses (10 mg/kg). Beside these results, the disposition pattern of TX in the rat remains largely undefined. Results from the pharmacokinetic study showed a relatively short elimination $t_{½}$ in male rats (5.2 ± 1 h). Previous studies have reported sex differences in pharmacokinetics of oxicam in rats, showing a lower clearance in females (Woolf & Radulovic 1989, Roskos & Boudinot 1990). The sex of the rat had a marked effect on TX pharmacokinetics with a mean oral clearance ten fold lower in females (0.029 ± 0.006 *vs* 0.367 ± 0.09 ml/min). The free fraction of TX in plasma was greater in male rats than in female rats. However, the magnitude of the difference in the free fraction was not as large as the difference in clearance and the protein binding only partially explains the sex dependent disposition of TX. Similar results have been observed with piroxicam (Roskos & Boudinot 1990).The other component would be a sex difference in the metabolism of TX, since TX is a low extraction drug and its clearance mainly depends on protein binding and enzyme activity (Lopez-Bustamante *et al.* 1992). Such disparities in metabolism between males and females have been shown with salicylates with higher toxicity in females (Scheresowa *et al.* 1975). These sexual differences might be primarily genetic in nature or rule by sex hormones (Bonate 1991). Most of the evidence for such a difference comes from animals, particularly rats (Sechserova *et al.* 1975, Roskos & Boudinot 1990) but some is also available from humans (Aarons *et al.* 1989, Greenblatt *et al.* 1986, Richardson *et al.* 1985). On the other hand, little is known about the effect of sex on the pharmacodynamics of drugs. Such a difference has been established for barbiturates (Hoffman & Levy 1989) showing a longer half life in females reflected by the much longer duration of action of effect in females. This marked difference in the disposition of TX provides insight as the effect of $t_{½}$ on lower GI toxicity. The sex difference of TX in the clearance in general and in half-life in particular was used to examine the influence of pharmacokinetic variability in the toxicity of NSAIDs. As a mean of toxicity measurement, ^{51}Cr-EDTA permeability was employed.

Since altered intestinal permeability after NSAIDs use precedes inflammatory damage, assessment of these early changes in mucosal permeability could be a reliable way to diagnose and to prevent GI complications. Rats have been successfully used as models for quantitative changes of intestinal permeability in enteropathies induced by ethanol, cetrimide and methotrexate, using the ^{51}Cr-EDTA test (Bjarnason *et al.* 1985). Recently, different studies from our laboratory have

examined increased intestinal permeability after NSAID administration using the same method (Davies *et al.* 1994). All the results suggest that the rat is a suitable model for measurement of intestinal permeability. Thus, the base line value of control rats (2.07 ± 1.181 %, from 0-24 h urine collection) is similar to that found in humans (2.45 %, CI = 2.11-2.86) (Aabakken 1989). Furthermore, as observed in our control rats, no sex difference has been observed in human base line permeability (Mielants *et al.* 1991). The small intestinal permeability changes in rats seem to parallel human ones. Indomethacin (Bjarnason *et al.* 1984a), naproxen (Bjarnason *et al.* 1984a, Aabakken 1989) and ibuprofen (Bjarnason *et al.* 1984a,1986) have shown a dose dependent effect on intestinal permeability; similar results have been observed in the rat.

Numerous studies in humans have demonstrated, using urinary excretion of ^{51}Cr-EDTA, increases in intestinal permeability caused by different NSAIDs like naproxen (Jenkins *et al.* 1987), indomethacin (Bjarnason *et al.* 1992, 1991), ibuprofen (Bjarnason *et al.* 1987,1984a) or nabumetone (Bjarnason *et al.* 1992, 1991). All these studies discuss NSAIDs' intestinal permeability, however none of them have examined the time course of these changes. Furthermore, no consideration is given to pharmacokinetics of these agents. Much has been published on the relationship between toxicity and elimination half-life (Adams 1987, 1992, Furst 1992). This hypothesis derived from study following the marketing of piroxicam, first NSAID with a long half-life in human (Collier & Pain 1985). Beside the limitations in some of the original data collection as mentioned later (Paulus 1985), this conclusion may over simplify a rather complex concentration-effect relationship and none of these studies clearly examined the relationship between prolonged residence time and lower intestinal toxicity. Other observations, however, show no correlation between different frequencies of NSAIDs' side effects and the corresponding half lives of the drugs (Albengres *et al.* 1988). A variety of approaches to reduce the risk of GI side effects have been considered such as controlled release formulation, enteric coating, suppositories, and pro-drugs (Vavra & Paulus 1992, Verbeeck 1992). However, they have had a little success and considered only the local component of these side effects. Hence, the prolonged-release form of indomethacin (Osmosin®) was briefly marketed in UK in 1983, and withdrawn due to increased in lower intestine toxicity (Day 1983). In this case, the toxicity was displaced further down the GI tract, and sustained release formulations might just delay the toxicity from the upper GI to the lower GI. The relative

importance of local and systemic effects may be unique to each drug and related to specific chemical and pharmacokinetic properties of the individual product. This has major implications for enteric coating of a drug and other measures taken to diminish local toxicity. Since an effective coating principle developed for one substance will not necessarily be advantageous for another (Aabakken 1992).

In this work, the 5 mg/kg dose was selected to achieve a high enough increase in intestinal permeability to measure its time course, since the dose therapeutically equivalent to human dose (0.5 mg/kg) produced a significant but small effect (3.04 ± 0.33 %). The time course of permeability changes induced by TX (5 mg/kg) was established in both male and female rats. Surprisingly, the large differences in the kinetics of TX between males and females were not reflected in the time courses of the effects which were found identical (Figure 6.3-2). Furthermore, no relationship was found between concentration and effect in either sex. This might be explained by the very low effect measured after the 5 mg/kg dose. Indeed, the dose used to established the time course of the effect was rather low, considering the dose-effect curve (Figure 6.4-4). The 0.5-10 mg/kg dose range produce a very small effect; therefore, it might be very difficult to detect any difference between sexes at this level of toxicity. Considering the very low effect of TX on intestinal permeability at this 5 mg/kg dose, we assessed the permeability after increasing doses (Figure 6.4-4). The dose-effect relationships were sigmoidal in both males and females. Although the E_{max}s were found identical, the increase of effect was faster in females (as shown by a lower ED_{50} in females), suggesting a greater sensitivity in the 15-30 mg/kg dose range. Except ED_{50}, all the other parmacodynamic parameters were identical (Table IX). As the 20 mg/kg dose induced a significantly higher effect on permeability in females, we decided to examine the time course of permeability at this particular dose. As expected, the male and female permeability profiles were statistically different (Figure 6.4-3). The maximum effect is significantly higher in females (19.948 ± 4.690 % *vs* 15.348 ± 1.332 %). Moreover, permeability remains above base line value 72 h post dose in females while it is back to normal value 24 h post dose in males. The difference was more important in term of magnitude of the effect compared to the duration of the effect, suggesting a minor influence of the prolonged residence of TX in females. In this study, we established the time course of TX-induced permeability in the rat at two different doses. Despite a longer elimination half life in females, the TX toxicity

profiles were identical at the 5 mg/kg dose. The toxicity, however, was significantly higher in females at the 20 mg/kg dose.

However, the toxicity profile of TX was not high enough to allow the detection of significant differences. In addition to the drug clearance, other sex related parameters may be involved in the difference noticed in the toxicity profile of TX in male and female rats. Alternatively, a comparison of short with long half-life drugs may be carried out in the same sex. This, however, may ignore intrinsic molecular differences between the two drugs. Therefore the same NSAID may be used as regular *vs* sustained release formulations (SR). However, SR also are not suitable for comparison with regular release since the presence of drug in the intestine (i.e. the site of toxic action) is prolonged rather than the systemic half-life or residence time. Hence, Alzet pumps were selected to mimic long half-life. Alzet pump are osmotic pumps, designed to release products at a constant rate. They have been successfully used to deliver ibuprofen and flurbiprofen intraperitonealy (Orita *et al.* 1987, Offenbacher *et al.* 1988). In our study, the IP implantation had the advantage to avoid continuous GI release as encountered after SR. In this regard, we selected flurbiprofen for its shorter half-life and the significant increase in permeability produced by a 5mg/kg dose ($\approx$ 10%). A comparison of pulse IP therapy *vs* Alzet pump provided informations on the effect of C_{max} concentrations on lower GI toxicity. A comparison of pulse IP therapy vs pulse PO therapy provided informations on repeated GI exposure impact on toxicity.

A lower toxicity profile was noticed after the pump therapy as compared to the two other treatments. These observations were consistent over time (on days 1, 4 and 7) suggesting that prolonged and constant systemic availability (simulated long half-life) improved the safety of FL. Secondly, the comparison between IP and PO therapies gave data on the local and systemic component of FL effect on intestinal permeability. Even though it did not reach statistical significance, the toxicity after IP pulse treatment was consistently lower than after PO. This suggests a presystemic component of the effect on intestinal permeability. Even though the achieved steady-state concentrations were equivalent, effects were different. Since pulse therapies were more toxic than infusion therapy, it appears that fluctuations in blood levels induce more damage. It might be due to the peak concentrations, causing damage at each administration. Pulse therapy also induced higher tissue concentrations in different parts of the small intestine (Table X). Therefore, local effects might be more important in repeated administrations.

An interesting observation subsequent to the multiple dose/continuous infusion study, was a progressive decrease of intestinal permeability over time, despite the continued administrations. Hence, in each pulse treatment (PO and IP) the effect significantly diminished from day 1 to day 4. Furthermore, the effect on day 7 permeability induced by IP pulse treatment was significantly lower than in day 4 (Figure 6.5-3). In the continuous infusion therapy, the same trend was noticed but due to the low toxicity profile of this treatment statistical significance was not achieved. This phenomenon of adaptation has been observed for upper GI tract side effects with repeated administrations of aspirin first in rat (St John *et al.* 1973) and then confirmed in humans (Graham *et al.* 1983, 1988). However, data of lower intestinal adaptation have not been reported up to now. There is abundant evidence that the gastric mucosa can increase its resistance to injury when challenged repeatedly with potentially harmful agents. It has been proven with many agents including ethanol, alkali, acid, bile hyperosmolar solutions and NSAIDs (Deregnacourt 1979, Lacy 1985, Kuwayama 1986). It has been proposed that adaptive cytoprotection is due to stimulation of endogenous prostaglandin synthesis (Robert 1983, Konturek & Konturek 1994). Thus, some mild irritants such as low concentration solutions of alkali, or ethanol can stimulate prostaglandins synthesis and do not show any toxicity (Robert 1983). If given prior more toxic products, these mild irritants can also prevent their damage. However, some studies have shown that adaptive cytoprotection still occur after doses of indomethacin sufficient to cause a profound depression of PGE2 release (Hawkey *et al.* 1988). Therefore, the stimulation of endogenous production of prostaglandin could not be the sole mechanism of adaptation. The hypothesis that adaptation could be due to formation of a protective covering of surface debris has been brought up but needs further investigation. Beside PG other factors such as epidermal growth factor are essential in the protection of the gastric mucosa (Konturek & Konturek 1994). Furthermore, evidence of increase in the rate of proliferation in gastric and duodenal epithelium by NSAIDs may form one of the mechanisms underlying gastric and duodenal adaptation (Levi *et al.* 1992). All these studies involve oral administration of ulcerogenic substances and the emphasis is made on the local adaptation to the presystemic effect. In our work, adaptation of the lower intestine has been observed after oral and IP multiple administration. Therefore the presence of a systemic mechanism of cytoprotection should be envisaged. These observations of adaptation are, however, in contradiction with the highest incidence of damage

during long term NSAID therapy (Bjarnason & Peters, 1989). This discrepancy may be explained by the fact that during the long term therapy more frequent doses are used. This may increase the chances of causing injury, i.e. the frequency of exposure rather than the drug accumulation might be the cause of higher toxicity after repeated doses.

8. CONCLUSION

Clearance of TX is lower in female than in male rats. Thus, making a sex comparison of toxicity provides a valid measure of the influence of pharmacokinetic variables on the toxicity of NSAIDs. The results, however, were not conclusive mainly due to the lack of substantial toxic effect of TX and also to the possibility of interference of other sex-related factors.

Flurbiprofen, a potent intestinal permeability enhancer, with short half-life when given as immediate release formulation, mimicked characteristics of a long half-life drug when it was administered IP with osmotic pumps. The enhanced intestinal permeability was less after administration via IP pump than that after IP or PO pulse therapy. This suggests attainment of higher safety profile when the frequency of administration is reduced, i.e. fewer concentration fluctuations and less frequent exposure to the intestinal mucosa.

Prolonged administration of NSAIDs resulted in gradual reduction in the effect on intestinal permeability, suggestive of adaptation. Therefore, the higher incidence of toxicity reported after long term NSAID therapy could be the result of frequent exposure of the GI mucosa rather than drug accumulation.

REFERENCES

Aabaken L. *Non Steroidal Antiinflammatory Drugs-The extending scope of gastrointestinal side effects.* Aliment Pharmacol Ther. 6: 143-162. (1992).

Aabakken L. *^{51}Cr-ethylenediamintetraacetic acid absorption test. Methodologic aspects.* Scand J Gastroenterol. 24: 351-358. (1989)

Aarons L, Hopkins K, Rowland M, Brossel and Thiercelin JF. *Route of administration and sex differences in the pharmacokinetics of aspirin, administered as its lysine salt.* Pharm Res. 6: 660-666. (1989)

Abramson SB and Weissmann G. *The mechanism of action of Non Steroidal Antiinflammatory Drugs.* Arthritis Rheum. 32: 1-9. (1989)

Adams SS. *Non Steroidal Antiinflammatory Drugs, plasma half-life and adverse reactions.* Lancet. 2: 1204-1205. (1987)

Adams SS. *NSAIDs, plasma half life and adverse reactions.* Lancet 1: 653-654. (1988).

Adams SS. *The propionic acids: a personal perspective.* J Clin Pharmacol. 32: 317-323. (1992)

Al-Ghamdi MS, Al-Mohana FA, Al-Mustafa ZH and Al-Saeed IS. *The effect of haemodialysis on the pharmacokinetics of tenoxicam in patients with end-stage renal disease.* EUR J Clin Pharmacol. 43: 197-199. (1992)

Al-Ghamdi MS, Dissanyake AS, Cader ZA and Jain S. *Tenoxicam induced gastropathy in the rat: a comparison of piroxicam and diclofenac sodium, and the inhibitory effects of ranitidine and sucralfate.* J Int Med Res. 19: 242-248. (1991)

Albengres E, Pinquier JL, Riant P, Bree F, Urien S, Barre J and Tillement JP. *Pharmacological criteria for risk-benefit evaluation of NSAIDs.* Scand J Rheumatology. (suppl 73): 3-15. (1988)

Appelton I, Tomlinson A and Willoughby DA. *Inducible cyclooxygenase (COX-2): a safer therapeutic target ?* Br J Rheumatol. 33: 410-412. (1994)

Berry BW and Jamali F. *Stereospecific high performance liquid chromatographic (HPLC) assay of flurbiprofen in biological specimens.* Pharm Res. 5: 123-125. (1988)

Bird HA, Allen JG, Dixon JS and Wright V. *Pharmacokinetic comparison of tenoxicam in plasma and synovial fluid.* Br J Rheumatol. 24: 351-356. (1985)

Bird HA, Pickup ME, Taylor P, Lowe JR, McEvoy M, Galloway DB and Wright V. *Gastro-intestinal blood loss with high dose of Tilcotil® (Ro 12-0068) and aspirin: an open crossover clinical trial and pharmacokinetic assessment in normal volunteers.* Curr Med Res Op. 8: 412-416. (1983)

Bjarnason I and Peters TJ. *Intestinal permeability, Non Steroidal Antiinflammatory Drug enteropathy and inflammatory bowel disease.* Gut Festschrift. 22-28. (1989)

Bjarnason I, Fehilly B, Smethurst P, Menzies IS and Levi AJ. *Importance of local versus systemic effect of Non Steroidal Antiinflammatory Drugs in increasing small intestinal permeability in man.* Gut. 32: 275-277. (1991)

Bjarnason I, Hayllar J, MacPherson AJ and Russel AS. *Side effect of Non Steroidal Antiinflammatory Drugs on the small and large intestine in humans.* Gastroenterology. 104: 1832-1847. (1993)

Bjarnason I, Hayllar J, Smethurst P, Price A and Gumpel MJ. *Metronidazole reduces intestinal inflammation and blood loss in Non Steroidal Antiinflammatory Drug induced enteropathy.* Gut. 33: 1204-1208. (1992)

Bjarnason I, Marsh MN, Price A, Levi AJ and Peters TJ. *Intestinal permeability in patients with coeliac disease and dermatitis herpetiformis.* Gut. 26: 1214-1219. (1985)

Bjarnason I, O'Morain C, Levi AJ and Peters TJ. *Absorption of ^{51}Chromium-labelled ethylene diamine tetracetic acid in inflammatory bowel disease.* Gastroenterology. 85: 318-322. (1983b)

Bjarnason I, Peters TJ and Levi AJ. *Intestinal permeability: clinical correlates.* Am J Dig Dis. 4: 83-92. (1986)

Bjarnason I, Peters TJ and Veall N. *A persistent defect in intestinal permeability in coeliac disease demonstrated by a ^{51}Cr-EDTA absorption test.* Lancet. i:323-325. (1983a)

Bjarnason I, Prouse P, Smith T, Gumpel MJ, Zanelli G, Smethurst P, Levi S and Levi JA. *Blood and protein loss via small-intestinal inflammation induced by Non Steroidal Antiinflammatory Drugs.* Lancet. 711-714. (1987)

Bjarnason I, Smethurst P, Fenn CG, Lee CE, Menzies IS and Levi J. *Misoprostol reduces indomethacin-induced changes in humal small intestinal permeability.* Dig Dis Sci. 34: 407-411. (1989)

Bjarnason I, Smethurst P, Levi AJ and Peters TJ. *Intestinal permeability to ^{51}Cr-EDTA in rats with experimentally induced enteropathy.* Gut. 26: 579-585. (1985)

Bjarnason I, Smethurst P, MacPherson A, Walker F, MacElney JC, Passmore AP and Menzies IS. *Glucose and citrate reduce the permeability changes caused by indomethacin in humans.* Gastroenterology. 102: 1546-1550. (1992)

Bjarnason I, So A, Levi AJ, Peters TJ, Williams P, Zanelli GD, Gumpel JM and Ansell B. *Intestinal permeability and inflammation in rheumatoid arthritis: effect of Non Steroidal Antiinflammatory Drugs.* Lancet. 1171-1173. (1984a)

Bjarnason I, Ward K and Peters TJ. *The leaky gut of alcoholism. Possible route of entry of toxic compounds.* Lancet. 1: 179-182. (1984b)

Bjarnason I, Zanelli G, Prouse P, Gumpel MJ and Levi AJ. *Effect of Non Steroidal Antiinflammatory Drugs on the human small intestine.* Drugs. 32 (suppl. 1): 35-41. (1986)

Bjarnason I, Zanelli G, Smith T, Prouse P, De Lacey G, Gumpel MJ and Levi AJ. *Non Steroidal Antiinflammatory Drug induced inflammation in humans.* Gastroenterology. 93: 480-489. (1987)

Bonate PL. *Gender related differences in xenobiotic metabolism.* J Clin Pharmacol. 31: 684-690. (1991)

Brogden RN. *Non Steroidal Antiinflammatory analgesics other than salicylates.* Drugs. 32: 27-45. (1986)

Brooks PM and Day RO. *Non Steroidal Antiinflammatory Drugs-Differences and similarities.* N Engl J Med. 324: 1716-1725. (1991)

Carson JL, Strom BL, Morse ML, West SL, Soper KA, Stolley PD and Jones JK. *The relative gastrointestinal toxicity of Non Steroidal Antiniflammatory Drugs.* Arch Intern Med. 147: 1054-1059. (1987)

Clive DM and Stoff JS. *Renal syndrome associated with Non Steroidal Antiinflammatory Drugs.* N Engl J Med. 310: 563-572. (1984)

Collier DSJ and Pain JA. *Non Steroidal Antiinflammatory Drugs and hospital admission for perforated peptic ulcer.* Lancet 2: 380-382. (1985)

Committee on Safety of Medicine. *Non Steroidal Antiinflammatory Drugs and serious adverse reactions (II).* Br Med J. 292: 1190-1191. (1986)

Crevoisier C, Zaugg PY, Heizmann P and Meyer J. *Influence of liver cirrhosis upon the pharmacokinetics of tenoxicam.* Int J Clin Pharmacol Res.9: 327-334. (1989)

Csaky TZ. *Intestinal permeation and permeability, an overview.* Handbook Exp Pharmacology. 70: 51-59. (1987)

Davenport HW. *Gastric mucosal injury by fatty and acetyl salicylic acids.* Gastroenterology. 46: 245-253. (1964)

Davies GR and Rampton DS. *The pro-drug sulindac may reduce the risk of intestinal damage associated with the use of Non Steroidal Antiinflammatory Drugs.* Aliment. Pharmaco. Therap. 5: 593-598. (1991)

Davies GR, Wilkie ME and Rampton DS. *Effects of metronidazole and misoprostol on indomethacin-induced changes in intestinal permeability.* Dig Dis Sci. 38: 417-425. (1993)

Davies NM, Wright MR and Jamali F. *Antiinflammatory drugs-induced small intestinal permeability: the rat a suitable model.* J Pharm Sci. in press (1994)

Day RO, Paulli P, Lam S, Swanson BR, Williams KM and Wade DN. *The effect of concurrent aspirin upon plasma concentrations of tenoxicam.* Br J Clin Pharmacol. 26: 455-462. (1988)

Day RO, Williams KM, Graham S and Handel M. *The parmacokinetics of total and unbound concentrations of tenoxicam in synovial fluid and plasma.* Arthritis Rheum. 34: 751-760. (1991)

Day TK. *Intestinal perforation associated with osmotic slow release of indomethacin capsule.* Br J Med. 287: 1671. (1983)

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

Delahunty T and Hollander D. *A comparison of intestinal permeability between humans and three common laboratory animals.* Comp Biochem Physiol. 86A: 565-567. (1987)

Deregnacourt J and Code CF. *Increased resistance of the gastric mucosa to barrier breakers in the rat.* Gastroenterology. 77: 309-312. (1979)

Diffey BL, Daymond TJ and Fairgreaves H. *Phototoxic reactions to piroxicam, naproxen and tiaprofenic acid.* Br J Rheumatol. 22: 239-242. (1983)

Ejstrup I, Knudsen JV and Petersen L. *A randomised double-blind multicentre trial comparing tenoxicam and ketoprofen in osteoarthritis.* Scand J Rheumatology. (suppl. 80): 48-53. (1989)

Elia M, Behrens R, Northrop C, Wraight P and Neale G. *Evaluation of mannitol, lactulose and ^{51}Cr-labelled ethylenediamine tetra-acetate as markers of intestinal permeability in man.* Clin Sc. 73: 197-204. (1987)

Fenner H. *Comparative biochemical pharmacology of the oxicams.* Scand J Rheumatology. Suppl. 80: 54-58. (1989)

Fenner H. *Non Steroidal Antiinflammatory Drugs: Benefit/risk evaluation in rheumatic diseases.* J Rheumatol. 19(suppl 32): 98-99. (1992)

Forget P, Sodoyez-Goffraux F and Zappitelli A. *Permeability of the small intestine to ^{51}Cr-EDTA in children with acute gastroenteritis or eczema.* J Pediatr Gastroenterol Nutr. 4: 393-396. (1985)

Fries JF, Williams CA and Bloch DA. *The relative toxicity of Non Steroidal Antiinflammatory Drugs.* Arthritis Rheum. 34: 1353-1361. (1991)

Furst DE. *Are there differences among Non Steroidal Antiinflammatory Drugs?* Arthritis Rheum. 37: 1-9. (1994)

Furst DE. *Clinical significance of long versus short serum half-lives in NSAIDs.* Famaey and Paulus (Eds). Therapeutic Application of NSAIDs. Subpopulation and New Formulations. 359-372. Marcel Dekker Inc. NY. (1992)

Graham DY, Smith JL and Dobbs SM. *Gastric adaptation occurs with aspirin administration in man.* Dig Dis Sci. 28: 1-6. (1983)

Graham DY, Smith JL, Spjut HJ and Torres E. *Gastric adaptation: Studies in humans during continuous aspirin administration.* Gastroenterology. 95: 327-333. (1988)

Greenblatt DJ, Abernethy DR, Boxenbaum HG, Maltis R, Ochs HR, Harmatz JS and Schader RI. *Influence of age, gender and obesity on salicylate kinetics following single doses of aspirin.* Arthritis Rheum. 29: 971-980. (1986)

Guentert TW, Heintz RC and Joly R. *Overview of the pharmacokinetics of tenoxicam.* Eur J Rheumatol Infl. 9: 15-25. (1987)

Halter F. *Mechanisms of gastrointestinal toxicity of NSAIDs.* Scand J Rheumatol. Suppl 73: 16-21. (1988)

Hartmann D, Kleinbloesem CH, Lucker PW and Vetter G. *Study on the possible interaction between tenoxicam and furosemide.* Arzneim Forsch. 37: 1072-1076. (1987)

Hawkey CJ, Kemp RT, Walt RP, Bhaskar NK, Davies J and Filipowicz B. *Evidence that adaptative cytoprotection in rats is not mediated by prostaglandins.* Gastroenterology. 94: 948-954. (1988)

Hayllar JA, MacPherson A, Bjarnarson I. *Gastroprotection and Non Steroidal Antiinflammatory Drugs (NSAIDs).* Drug Safety. 7: 86-105. (1992).

Henry DA. *Side-effects of Non Steroidal Antiinflammatory Drugs.* Bailliere's Clinical Rheumatology. Vol. 2, No. 2. 425-455. (1988)

Hochberg MC. *Association of Non Steroidal Antiinflammatory Drugs with upper gastrointestinal diseases: epidemiology and economic considerations.* J Rheumatol. 16 (suppl. 36): 63-67. (1992)

Hoffman A and Levy G. *Gender differences in the pharmacodynamics of barbiturates in rats.* Pharm Res. 6: 976-981. (1989)

Hollander D, Ricketts D and Boyd CAR. *Importance of 'probe' molecular geometry in determining intestinal permeability.* Can J Gastroenterol. 2(suppl. A): 35A-38A. (1988)

Holt S, Rigoglioso V, Malwinder S, Irshad M, Howden CW and Mainero M. *Non Steroidal Antiinflammatory Drugs and lower gastrointestinal bleeding.* Dig Dis Sci. 38: 1619-1623. (1993)

Ichihara S, Tsuyuki Y, Tomisawa H, Fukasawa H, Nakayama N and Tateishi M. *Metabolism of tenoxicam in rats.* Xenobiotica. 14: 727-739. (1984)

Jacobs J, Goldstein AG, Kelly ME and Bloom BS. *NSAID dosing schedule and compliance.* Drug Intell Clin Pharm. 22: 727-728. (1988)

Jenkins AP, Trew DR, Crump BJ, Nukajam WS, Foley JA, Menzies IS and Creamer B. *Do Non Steroidal Antiinflammatory Drugs increase colonic permeability?* Gut. 32: 66-69. (1991)

Jenkins RT, Rooney PJ, Jones DB, Bienenstock J and Goodacre RL. *Increased intestinal permeability in patients with rheumatoid arthitis: a side effect of oral Non Steroidal Antiinflammatory Drug therapy?* Br J Rheumatol. 26: 103-107. (1987)

Kent TH, Cardelli RM and Stamler FW. *Small intestinal ulcers and intestinal flora in rats given indomethacin.* Am J Pathol. 54: 237-245. (1969)

Konturek SJ and Konturek JW. *Gastric adaptation: Basic and clinical aspects.* Digestion. 55: 131-138. (1994)

Kuwayama J, Eastwood G, Miyaka S and Furukawa H. *Adaptation of gastric mucosa to repeated indomethacin administration in the rat.* Gastroenterology. 90: 1506. (1986)

Lacy ER. *Gastric mucosal resistance to repeated ethanol insults.* Scand J Gastroenterol. 20 (suppl. 110): 63-72. (1985)

Leclercq-Foucart J, Forget P, Sodoyez-Goffraux F and Zappitelli A. *Intestinal permeability to ^{51}Cr-EDTA in children with cystic fibrosis.* J Pediatr Gastroenterol Nutr. 5: 384-387. (1986)

Levi S, Goolad RA, Lee CY, Walport MJ, Wright NA and Hodgson HJF. *Effect of non steroidal antiinflammatory drugs and misoprostol on gastroduodenal epithelial proliferation in arthritis.* Gastroenterology. 102: 1605-1611. (1992)

Lewis JH. *Hepatic toxicity of Non Steroidal Antiinflammatory Drugs.* Clin Pharmacol. 3: 128-138. (1984)

Ligumsky M, Grossman MI and Kauffman. *Endogenous gastric mucosa prostaglandins: their role in mucosal integrity.* Am J Physiol. 242: G337-G341. (1982)

Ligumsky M, Siesteri M, Karmeli F, Zimmerman J, Okon E and Rachimilewitz D. *Rectal administration of Non Steroidal Antiinflammatory Drugs. Effect on rat gastric ulcerogenicity and prostaglandin E_2 synthesis.* Gastroenterology. 98: 1245-1249. (1990)

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

Lopez-Bustamante LG, Troconiz JIF and Fos D. *Effect of uremia and anephric state on the pharmacokinetics of tenoxicam in the rat.* J Pharm Pharmacol. 44: 898-901. (1992)

Lopez-Bustamante LG, Troconiz JIF and Fos D. *Tenoxicam: Acute dose-dependent disposition studies in rats.* J Pharm Sci. 82: 851-853. (1993)

Meade EA, Smith WL and De Witt DL. *Differential inhibition of prostaglandin endoperoxide synthetase (cyclooxygenase) isoenzymes by aspirin and other Non Steroidal Antiinflammatory Drugs.* J Biol Chem. 268: 6610-6614. (1993)

Merlie JP, Fagan D, Mudd J and needelman P. *Isolation and characterization of the complementary DNA for the sheep seminal vesicle prostaglandin endoperoxide synthetase (cyclooxygenase).* J Biol Chem. 263: 3550-3553. (1988)

Metzler MC and Weiner DL. *PCNONLIN-Version 4.1. Statistical Consultants Inc.* Lexington, KY. (1992)

Mielants H, Goemaere S, De Vos M, Schelstraete K, Goethals K, Maertens M, Ackerman C and Veys EM. *Intestinal mucosal permeability in inflammatory rheumatic diseases. I. Role of antiinflammatory drugs.* J Rheumatol. 18: 389-393. (1991)

Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ and Vane JR. *Selectivity of Non Steroidal Antiinflammatory Drugs as inhibitors of constitutive and inducible cyclooxygenase.* Proc Natl Acad Sci. 90: 11693-11697. (1993)

Morris AJ, Madhok R, Sturrock RD, Cappel HA and MacKenzie JF. *Enterosopic diagnosis of small bowel ulceration in patients receiving Non Steroidal Antiinflammatory Drugs.* Lancet. 337: 520. (1991)

Nilsen OG. *Clinical pharmacokinetics of tenoxicam.* Clin Pharmacokinet. 26(1): 16-43. (1994)

O'Brien WM and Bagby GF. *Rare adverse reactions to Non Steroidal Antiinflammatory Drugs.* J Rheumatol. 12 : 785-790. (1985a)

O'Brien WM and Bagby GF. *Rare adverse reactions to Non Steroidal Antiinflammatory Drugs.* J Rheumatol. 12 : 13-20. (1985b)

O'Mahony CP, Stevens FM and Bourke M. *^{51}Cr-EDTA test for coeliac disease.* Lancet. 1: 354-355. (1984)

Offenbacher S, Odie BM, Braswell LD, Johnson HG, Hall CM, Mc Clure H, Orkin JL, Strobert EA and Green MD. *Changes in cyclooxygenase metabolities in experimental periodontis in macaca mulata.* J Periodont Res. 24: 63-74. (1989)

Olkkola KT, Brunetto AV and Mattila MJ. *Pharmacokinetics of oxicam Non Steroidal Antiinflammatory agents.* Clin Pharmacokinet. 26(2): 107-120. (1994)

Orita H, Girgis W and Di Zerega GS. *Prevention of postsurgical peritoneal adhesion formation by intraperitoneal administration of ibuprofen.* Drug Dev Res. 10: 97-105. (1987)

Paulus HE. *Postmarketing surveillance of Non Steroidal Antiinflammatory Drugs.* Arthritis Rheum. 28: 1168-1169. (1986)

Peled Y, Watz C and Gilat T. *Measurement of intestinal permeability using ^{51}Cr-EDTA.* Am J Gastroenterol. 80: 770-773. (1984)

Rack-Masden J. *The role of eicosanoids in the gastrointestinal tract.* Scand J Gastroenterol. 22(suppl 127): 7-19. (1987)

Radack K and Deck C. *Do Non Steroidal Antiinflammatory Drugs interfere with blood pressure control in hypertensive patients?* J Gen Intern Med. 2:108-112. (1987)

Rainsford KD and Withehouse MW. *Biochemical gastroprotection from acute ulceration induced by aspirin and related drugs.* Biochem Pharmacol. 29: 1281-1289. (1980)

Rainsford KD. *Leukotrienes in the pathogenesis of NSAID-induced gastric and intestinal mucosal damage.* Agents Actions (sp. conf. issue). (1993)

Rainsford KD. *Mechanisms of gastrointestinal toxicity of Non Steroidal Antiinflammatory Drugs.* Scand J Gastroenterol. 24(suppl 163): 9-16. (1989)

Rainsford KD. *Mucosal lesions induced in the rat intestinal tract by the anti-inflammatory drug Wy-41,770, a weak inhibitor of prostaglandin synthesis, contrasted with those from the potent prostaglandin inhibitor, indomethacin.* Toxicol Pathol. 16: 366-375. (1988)

Rampton DS. *Non Steroidal Antiinflammatory Drugs and the lower gastrointestinal tract.* Scand J Gastroenterol. 22: 1-4. (1987)

Richardson CJ, Blocka KLN, Ross SG and Verbeeck RK. *Effect of age and sex on piroxicam disposition.* Clin Pharmacol Ther. 37: 13-18. (1985)

Robert A and Asano T. *Resistance of germ-free rats to indomethacin-induced intestinal lesions.* Prostaglandins. 14: 333-341. (1977)

Robert A, Nezamis JE, Lancaster C, Davies JP, Field SO and Hanchar AJ. *Mild irritants prevent gastric necrosis through "adaptative cytoprotection" mediated by prostaglandins.* Am J Physiol. 245: G113-121. (1983)

Rooney PJ, Jenkins RT and Buchanan WW. *A short review of the relationship between intestinal permeability and inflammatory joint disease.* Clin Exp Rheumatol. 8: 75-83. (1990)

Rooney PJ, Jenkins RT and Hunt RH. *Increased bowel permeability to 51Cr-EDTA in controls caused by naproxen is not prevented by cytoprotection.* Arthritis Rheum. 31(suppl. 1): R11. (1988)

Roskos LK and Boudinot FD. *Effect of dose and sex on the phrmacokinetics of piroxicam in the rat.* Biopharm Drug Disp. 11:215-225. (1990)

Rowe PH, Starlinger MJ, Kasdon E, Hollands MJ and Silen W. *Parenteral aspirin and sodium salicylate are equally injurious to the rat gastric mucosa.* Gastroenterology. 93: 863-871. (1987)

Satoh H, Guth PH and Grossman MI. *Role of bacteria in gastric ulceration produced by indomethacin in the rat: cytoprotective action of antibiotics.* Gastroenterology. 84: 483-489. (1983)

Schmitt M and Guenter TW. *Effect of age on the pharmacokinetics of tenoxicam in comparison to other Non Steroidal Antiinflammatory Drugs (NSAIDs).* Scand J Rheumatol. (suppl. 80): 86-89. (1989)

Schneider TH, Neurnberg B, Dietzel K and Brune K. *Biliary excretion of Non Steroidal Antiinflammatory Drugs in patients.* Br J Clin Pharmacol. 29: 127-131. (1990)

Selby P, McElwain TJ, Crofts M, Lopes N and Mundy J. *^{51}Cr-EDTA test for intestinal permeability.* Lancet i: 38-39. (1984)

Skeith KJ, Wright M and Davis P. *Differences in NSAID tolerability profiles. Fact or fiction?* Drug Safety. 10: 183-195. (1994)

St John DJB, Yeomans ND, McDermott FT and De Boer WG. *Adaptation of the gastric mucosa to repeated administration of aspirin in the rat.* Am J Dig Dis. 18: 881-887. (1973)

Vane JR and Botting RM. *The mode of action of anti-inflammatory drugs.* Postgrad Med J. 66(suppl 4): S2-S17. (1990)

Vane JR, Mitchell JA, Appleton I, Tomlinson A, Bishop-Bailey, Croxtall J and Willoughby DA. *Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation.* Proc Natl Acad Sci. 91: 2046-2050. (1994)

Vavra I and Paulus H. *Controlled-release and enteric-coated formulations of NSAIDs.* Famaey and Paulus (Eds). Therapeutic Application of NSAIDs. Subpopulation and New Formulations. 373-384. Marcel Dekker Inc. NY. (1992)

Verbeeck RK. *Rectal administration of NSAIDs suppositories.* Therapeutic Application of NSAIDs. Subpopulation and New Formulations. 447-460. Marcel Dekker Inc. NY. (1992)

Walker WA and Isselbacher KJ. *Uptake and transport of macromolecules by the intestine: possible role of clinical disorders.* Gastroenterology. 67: 531-550. (1974)

Whitehouse MW. *Oxicams:relative safety and anti-injury effects in rats.*Br J Clin Pharmacol. 22: 111S-116S. (1986)

Wilson K. *Sex-related differences in drug disposition in man.* Clin Pharmacokin. 9: 189-202. (1984)

Wisher TL. *Efficacy and tolerabilty of tenoxicam. An overview.* Eur J Rheumatol Infl. 9: 51-57. (1987)

Wittle BJR. *Gastric vasoconstictor actions of leukotriene C4 and* $PGF_{2\alpha}$ *and thromboxane mimetic U-44619 on rat submucosal microcirculation in vivo.* Am J Physio. 248: G580-586. (1985)

Woolf TF and Radulovic LL. *Oxicams: metabolic disposition in man and animals.* Drug Metab Rev. 21(2): 255-276. (1989)

Yoshikawa T, Naito Y, Ueda S, Oyamada H, Takemura T, Yoshida N, Sugino S and Kondo M. *Role of oxygen-derived free radicals in the pathogenesis of gastric mucosal lesions in the rat.* J Clin Gastroenterol 12(suppl 1): 565-571. (1990)