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THE STEREOSELECTIVE PHARMACOKINETICS OF ETODOLAC IN HUMANS AND RATS

BY

DION R. BROCKS

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **Doctor of Philosophy**

IN

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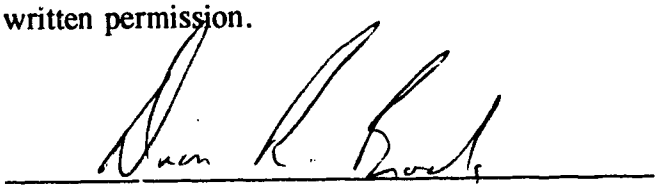

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **THE STEREOSELECTIVE PHARMACOKINETICS OF ETODOLAC IN HUMANS AND RATS** submitted by **Dion R. Brocks** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Pharmaceutical Sciences (Pharmacokinetics)**.

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Dec 11/1992

for Dr. M. Mayersohn

To my wife, Trish, and my daughter, Natasha.

ABSTRACT

The non-steroidal anti-inflammatory agent, etodolac, is chiral and marketed as the racemate. The pharmacokinetics of etodolac enantiomers were studied in humans and male Sprague-Dawley rats. In the rat, plasma concentrations of inactive *R*-etodolac were greater than active *S*-etodolac; the AUC of the *R* enantiomer was approximately 4 fold greater than that of *S*-etodolac. There was no interaction between the enantiomers. Enterohepatic recirculation was apparent in the plasma-time courses of *S*-etodolac. Bile duct-cannulation caused a reduction in the AUC of *S*-, but not *R*-etodolac. *R*-Etodolac was more highly bound to plasma proteins than *S*-etodolac. Complete recovery of *S*-etodolac as acyl-glucuronides was achieved in bile; only 33% of *R*-etodolac was likewise recovered. Urine was a minor route of elimination.

Unlike plasma, the *S*:*R* AUC ratios were near unity in rat tissues. The tissue distribution was better explained by binding of enantiomers to plasma than tissues. The Vmax for *in vitro* glucuronidation of *S*-etodolac by hepatic microsomal protein mirrored the biliary elimination. The *in vitro* oxidative metabolism by hepatic microsomes was greater for *R*- than *S*-etodolac.

In rats, phenobarbital caused significant reductions ($\approx$25%) in the AUC of both enantiomers. Glucuronidation was not altered, although the oxidative metabolism of both enantiomers was elevated. Cimetidine did not affect etodolac

pharmacokinetics.

In humans, plasma concentrations of *R*- greatly exceeded those of *S*-etodolac. Stereoselectivity was evident, with $R > S$ for C_{max} and AUC, and $S > R$ for Cl/F and Vd_β/F. Less than 25% of the dose of each enantiomer was excreted in the urine as acyl-glucuronides. Bile was a minor route of elimination. There were no differences between the young and elderly.

Concentrations of *S*-etodolac in synovial fluid were twice those in the plasma of arthritic patients. Conjugates were present in synovial fluid. The unbound fractions of the enantiomers were greater in synovial fluid than plasma, although *R*- was bound more than *S*-etodolac.

In both species, stereoselectivity was attributable to enantioselective protein binding and biotransformation. The results reflect the importance of considering stereoselectivity in evaluating the pharmacokinetics of etodolac.

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AUC	Area under the plasma or tissue concentration-time curve
AUC_{0-t}	Area under the plasma or tissue concentration-time curve from 0 hours to the last measured concentration
$AUC_{0-\infty}$	Area under the plasma or tissue concentration-time curve from 0 hours to infinity
AUC_b	Area under the plasma-time curve in bile duct-cannulated rats
AUC_c	Area under the plasma concentration-time curve of acyl-glucuronides
AUMC	Area under the first moment curve
bid	Twice daily administration
C	Substrate concentration
CL	Total body clearance
CL_b	Biliary clearance
CL_c	Clearance through acyl-glucuronidation
$CL_{(c)}$	Clearance corrected for enterohepatic recirculation
CL_{CR}	Creatinine clearance
CL/F	Body clearance after oral doses
CL_I	Intrinsic clearance
CL_R	Renal clearance
cm	Centimeters
C_{max}	Maximum plasma concentration after oral doses
F	Absolute bioavailability
F_b	Fraction of the dose excreted in bile
F_{ba}	Fraction of the dose excreted into bile which is reabsorbed
Fig.	Figure
g	Centrifugal force
g	Grams
h	Hours
HCl	Hydrochloric acid

HCT	Haematocrit
HPLC	High performance liquid chromatography
H_2SO_4	Sulfuric acid
ip	Intraperitoneal
iv	Intravenous
KCl	Potassium chloride
kg	Kilograms
Km	Michaelis-Menten constant
L	Litres
M	Molar
mg	Milligrams
$MgCl_2$	Magnesium chloride
min	Minutes
mL	Milliliters
mmol	Millimoles
mM	Millimolar
N	Normal
$NADP^+$	ß-Nicotinamide adenine dinucleotide phosphate, oxidized form
NaH_2PO_4	Sodium phosphate, monobasic
Na_2HPO_4	Sodium phosphate, dibasic
NaOH	Sodium hydroxide
NSAID	Non-steroidal anti-inflammatory drug
qd	Once daily administration
Q_H	Hepatic blood flow
r^2	Correlation coefficient
s	Seconds
SD	Standard deviation
SE	Standard error
SF	Synovial fluid
t_{max}	Time to maximal plasma concentration

$t_{½}$	Elimination half-life
U	Units
UDPGA	Uridine 5' diphosphoglucuronic acid
Vd	Volume of distribution
Vd_{β}	Volume of distribution (area)
Vd/F	Volume of distribution after oral doses
Vd_{ss}	Volume of distribution (steady state)
Vmax	Maximal reaction velocity
vs	Versus
y	Years
°C	Degrees celsius
α	Level of significance
ß	Terminal elimination rate constant
μg	Micrograms
μL	Microliters
μM	Micromolar
ΣX_b	Cumulative excretion in bile
ΣX_u	Cumulative urinary excretion
$\approx$	Approximately

1. Introduction

Etodolac (Lodine; Wyeth Ayerst, New York, NY) is a nonsteroidal antiinflammatory drug (NSAID) which was initially developed by Ayerst Laboratories in Montreal, Quebec, Canada, in the 1970's. Early studies indicated that etodolac was an effective NSAID, which had a favorable ratio of antiinflammatory activity to side-effects. The clinical evidence was sufficiently satisfactory to allow etodolac to be marketed in some parts of Europe, including the United Kingdom, Italy, France, and Switzerland, for the treatment of pain and inflammation associated with various forms of arthritis. The drug has more recently been given approval for marketing in the United States of America, although its official use is presently restricted to the treatment of osteoarthritis, and as a general purpose analgesic (Anonymous 1991).

The pharmacokinetics of etodolac have been studied in the past by the manufacturer, in a series of papers published in the 1980's. Over the same time frame, an awareness was being developed in clinical pharmacology regarding the possibility of stereoselectivity in the pharmacokinetics of chiral drugs (Ariens 1984; Hutt & Caldwell 1984; Drayer 1986; Ariens & Wuis 1987; Ariens *et al.* 1988). This is also an important consideration for etodolac, as it too is a chiral drug which is marketed as the racemate.

In 1986 a gas chromatographic assay method for the stereospecific analysis of etodolac enantiomers in biological samples was published by Singh *et al.* (1986). This was followed in 1988 by the development of an HPLC method for the analysis of

etodolac enantiomers (Jamali *et al.* 1988). In both of these studies preliminary results obtained from human volunteers indicated that the disposition of etodolac is strikingly stereoselective, with plasma concentrations of the inactive enantiomer greatly exceeding those of the active enantiomer.

The purpose of the studies to be presented in this thesis are to further explore the initial finding reported by Singh *et al.* (1986) and Jamali *et al.* (1988). The major objectives were to delineate the causes of the stereoselectivity observed in humans, and to explore the rat as an animal model whose study could allow for an examination of the mechanisms underlying stereoselective pharmacokinetics in humans.

Before describing the rationale and objectives for the stereoselective pharmacokinetic studies, a description of the chemistry, pharmacology, therapeutic usefulness, side effects, and nonstereospecific pharmacokinetics of etodolac, will be presented.

1.1. Etodolac

1.1.1. Chemistry of etodolac

In 1976, scientists at Ayerst Research Laboratories in Montreal, Quebec, Canada, reported on the chemistry and antiinflammatory activities of a series of 1-ethyl- and 1-*n*-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acid derivatives (Demerson *et al.* 1976). From this work the compound (±)-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-aceticacid), hereafter known as etodolac or etodolic acid, was generated (*Fig.* 1). Many of the synthesized compounds, which numbered 37 in total, possessed significant antiinflammatory

activity in the rat; however, etodolac had the highest antiinflammatory to ulcerogenic side-effects ratio of the generated compounds. Probably for this reason, further drug development of this class of compounds centred on etodolac.

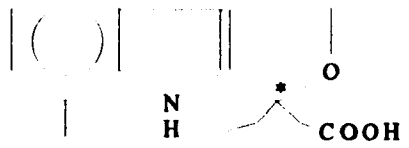

Figure 1. Structure of etodolac. Chiral centre is denoted by the asterisk.

Etodolac possesses a chiral centre (*Fig.* 1), and is marketed as the racemate. The separation of the enantiomers was first described by Demerson *et al.* (1983). Using the *S*-(-)- borneal ester of (-)-etodolac, the absolute configurations of the enantiomers were found to be *S*-(+) and *R*-(-) (Humber *et al.* 1986). This configuration is similar to most other NSAIDs, with the exception of ketorolac (Brocks & Jamali 1992).

1.1.2. Basic Pharmacology

1.1.2.1 Antiinflammatory activities

In the initial experiments performed in the rat as described by Demerson *et al.* (1976), etodolac was found to result in lowered paw swelling after *Mycobacterium*

butyricum injection, and inhibition of carrageenin-induced paw edema. In a more detailed set of experiments, Martel & Klicius (1976) found that etodolac was about 6.5 times more effective at preventing and treating established adjuvant arthritis in Wistar/Lewis and Charles River rats than phenylbutazone after oral doses. As compared to indomethacin, etodolac was about ¼ as effective in these activities. In terms of its ability to inhibit carrageenin paw edema, etodolac had equal activity to phenylbutazone.

Many more of the pharmacological properties of etodolac were examined, in a number of different species, in a more recent study (Inoue *et al.* 1991a). The authors also performed a more thorough comparison of the effects of etodolac, as its activity was compared to indomethacin, diclofenac, piroxicam, and ASA. Similar to the reference drugs tested, etodolac was found to have significant antiinflammatory activity in ultraviolet-induced erythema, carrageenin-induced edema, granuloma formation, and swelling caused by adjuvant arthritis.

Etodolac also caused a reduction in the release of lysosomal enzymes, as measured by ß-glucuronidase release, from guinea pig peritoneal leucocytes activated with the chemoattractant *f*met-leu-phe (FMLP), and from articular leucocytes of patients with rheumatoid arthritis (Inoue *et al.* 1991a). In guinea pig leucocytes, migration induced by FMLP, and superoxide formation were both inhibited by etodolac, indomethacin and by diclofenac. Prostaglandin E_2 formation in rabbit articular chondrocytes was inhibited by etodolac and indomethacin, with the inhibitory concentrations required for 50% reduction (IC_{50}) being 5.4×10^{-8} and 1.2×10^{-8} M,

respectively (Inoue *et al.* 1991a).

In another series of experiments by the same group (Inoue *et al.* 1991b), the mechanism of action of etodolac were more thoroughly investigated. Etodolac was found not to have direct antagonistic activity to inflammation induced by either histamine or bradykinin. Similar to prostaglandins, both of these autocoids play important roles in the inflammatory response. However, etodolac was capable, unlike indomethacin or diclofenac, of inhibiting inflammation induced by concanavalin A; the inflammation induced by this agent is primarily mediated by bradykinin. The authors suggested that etodolac inhibits the formation of bradykinin from high molecular weight kininogen, an activity that is unique amongst the NSAIDs tested for this activity. The authors also suggested that this activity may be beneficial in reducing the degree of articular damage in patients with rheumatoid arthritis, as bradykinin induces the release of factors from macrophages which can cause articular damage.

With respect to the relative antiinflammatory activity of etodolac in adjuvant injected rats, etodolac was more active than phenylbutazone, aspirin, fenbufen, naproxen, and sulindac, but less effective than piroxicam and indomethacin, on a mg/kg basis (Martel & Klicius 1982).

1.1.2.2. Analgesic activities

Martel & Klicius (1976) also examined the analgesic activity of etodolac by injecting paws of Charles River rats with brewers yeast suspension, followed by the

application of measured pressure to the injected area. Using this test. they found that etodolac significantly increased the threshold of pain in the rats. Etodolac was effective at increasing the pain threshold in the inflamed rat paw, but not in non-inflamed paws of control rats (Inoue *et al.* 1991a). Analgesic activity was also demonstrated in the acetic acid writhing test in mice (Inoue *et al.* 1991a).

1.1.2.3. Antipyretic activity

All of the reference drugs studied by Ionue *et al.* (1991a), including etodolac, had significant antipyretic activities in rats given brewers yeast subcutaneously; the drugs had no effect on the rectal temperature of control rats not given the injection.

1.1.2.4. Enantiospecific pharmacology

One important aspect of the pharmacology of etodolac involves the chiral nature of the drug (*Fig.* 1). Demerson *et al.* (1983) found that the (+) enantiomer of etodolac possessed almost all of the antiinflammatory activity, as measured by reduction in paw volume of rats with adjuvant polyarthritis, and prostaglandin synthetase inhibitory activity of the drug; no activity was discernable with the *R*-(-) enantiomer. The active enantiomer was later found to have the *S*- absolute configuration (Humber *et al.* 1986).

1.1.3. Clinical trials

There have been many clinical trials and postmarketing surveillance studies,

some involving thousands of patients (Sernie 1990; Benhamou 1990), which have examined the therapeutic benefits and side effects of etodolac.

1.1.3.1. Rheumatoid arthritis

Etodolac is an effective agent in the treatment of rheumatoid arthritis. Several studies, reviewed by Lynch & Brogden (1986), have shown etodolac to cause significant improvements over placebo in indices of inflammation such as the number of painful or swollen joints, articular index, and intensity of pain.

In one randomized, parallel-group, double-blinded comparative study by Jacob *et al.* (1986), patients with rheumatoid arthritis were divided into 5 groups. In three of the groups the patients received graded doses of etodolac of 50 (33 patients), 100 (32 patients), or 200 mg/day (32 patients). There was also a group of patients receiving placebo (27 patients) or aspirin 3900 mg/day (28 patients). Patients were enroled in the study for 6 weeks; medication had to be taken for at least 12 days for the patients to be included in the final analysis. Ten variables were used to assess the comparative benefits of placebo, aspirin, and etodolac.

Low doses of etodolac (50 mg/day) resulted in no significant clinical improvements compared to placebo. However, etodolac at 200 mg/day resulted in a significant improvement over placebo in 7 of the 10 categories. Patients receiving aspirin had significant improvement over placebo in 8 of the 10 categories; in none of the categories was aspirin significantly better than etodolac 200 mg/day. The number of patients withdrawing from the study due to side effects was significantly

higher for atients receiving aspirin than those receiving 200 mg/day of etodolac.

1.1.3.2. Side effects

The most common adverse effects of etodolac are related to the gastrointestinal tract. In a review of 2629 patients receiving long-term treatment with etodolac (Schattenkirchner 1990), 38% of the patients reported a gastrointestinal complaint associated with the therapy. Dyspepsia and diarrhea accounted for the majority of the complaints. There was only a slight increase in the incidence of complaints with an increase in dosage, from 200 (37%) to 1000 mg (44%) daily. The incidence of gastrointestinal ulceration has been estimated to be only 0.3% in 3302 patients. Abnormalities in liver, renal, and hematological function afflicted less than 1% of 3302 patients receiving the drug. In comparing the frequency of side effects in patients <65 y as compared to those ≥ 65 y, the only side effect that had a significantly higher frequency in the older group was dyspepsia (Schattenkirchner 1990).

1.2. Pharmacokinetic studies of etodolac performed using nonstereospecific assays

1.2.1. Absorption

Following orally administered doses of regular-release solid formulations, peak plasma levels of etodolac are achieved within 1-2 h (Table I). Etodolac is absorbed more rapidly from solutions than from solid dosage forms. The longest t_{max} values are

obtained (Table I) after administration of sustained-release doses (Ultradol SR; Wyeth Ayerst) of etodolac (Dey *et al.* 1989).

The absolute bioavailability of etodolac has been assumed to be near 1 (Cayen *et al.* 1981). After giving 4 young healthy subjects doses of ^{14}C-etodolac, a total of 86.9% was recovered in urine and feces within 7 days (Ferdinandi *et al.* 1986). The bioavailability of sustained-release (SR) etodolac (Ultradol SR) is 78-84% of oral solutions (Dey *et al.* 1989). In rats and dogs, etodolac is nearly completely bioavailable. Cayen *et al.* (1981) found no significant difference between AUC after oral or *iv* administration ($F=0.94$) of etodolac to the rat. Similarly, in the dog the $AUC_{0\text{-}48}$ of oral doses compared to equal *iv* doses is 0.96 (Cayen *et al.* 1981).

After dosing 4 subjects with 200 mg of ^{14}C-etodolac, Ferdinandi *et al.* (1986) found a range in C_{max} from 9.5-22 mg/L. Interestingly, they suggested that this spread was due to interindividual differences in first-pass metabolism, even though most NSAIDs are drugs which possess a low hepatic extraction ratio. Other plausible explanations not offered were interindividual differences in absorption or the Vd of the drug.

The effects of particle size, repetitive dosing, and dosing regimen on the pharmacokinetics of etodolac were studied by Kraml *et al.* (1984) in human volunteers (Table I). The C_{max} attained with tablet or capsule solid dosage forms were 13-20% lower than after aqueous solution ($p<0.05$); the corresponding t_{max} after solutions were significantly lower than after the solid dosage forms. However, there was no difference between the solid and solution dosage forms in AUC or in $t_{½}$, indicating

that the extent of absorption was not dependent on the dosage form used. The authors suggested that the bioavailabilities of the solid dosage forms were complete. After giving capsules containing a smaller particle size of etodolac (micronized), the C_{max} was significantly higher than that of a regular tablet; this result was similar to that seen in the dog experiments. However, there was no difference between the regular tablet and the micronized dosage form in t_{max}, AUC_{0-48}, or $t_{½}$. After repeated doses of etodolac as 200 mg *qd* or 100 mg *bid*, there were no differences in the AUC_{t-24} of etodolac between the first dose and after 7 days of dosing; this suggested that there was no accumulation of etodolac with repeated doses. Twice daily administration yielded a significantly lower C_{max} but higher C_{min} than with equivalent daily doses given once daily. As the authors stated, the lack of accumulation with repeated doses was unexpected based on etodolac's $t_{½}$ of over 8 h. No explanation was offered for this unexpected finding. One possibility is that the volunteers did not comply fully in taking their tablets of etodolac during the course of the study; such an event could have resulted in a lack of drug accumulation.

Dey *et al.* (1989) studied the bioavailability of sustained release (SR) etodolac in humans (Table I). The subjects were male young healthy volunteers. Two different SR formulations of etodolac (Ultradol SR) were studied; after 14 h, 64 and 73% of the etodolac in the respective formulations was released in an *in vitro* dissolution test. The formulation giving the higher dissolution also gave a higher bioavailability *in vivo* (83% *vs* 78%). Both SR formulations had a significantly lower absorption than did an aqueous solution.

After solution, the clearance (D•F/AUC) calculated by Dey *et al.* (1989), assuming an F of 0.82, was lower than calculated for tablets (34.7 *vs* 42.8 L/h). Dey *et al.* (1989) compared their findings with those of Cayen *et al.* (1981), in which F was assumed to be 1, and an oral CL value of 40.8 mL/h/kg for tablets was reported. When an F of 1 was assumed in the calculation of CL, Dey *et al.* (1989) found that their calculated oral CL was 42.4 mL/h/kg, a value similar to that reported by Cayen *et al.* (1981).

Dey *et al.* (1989) stated that the value used for F (0.82) was based on the recovery of urinary radioactivity reported by Cayen *et al.* (1981). However, a careful review of the paper by Cayen *et al.* (1981) shows that such an experiment was not performed in humans. Ferdinandi *et al.* (1986) did report the urinary excretion of radiolabelled etodolac, but the mean amount eliminated in human urine was 73.1%; the mean total cumulative amount eliminated in 7 days in human feces and urine was 86.9%. Therefore, the value of 0.82 used by Dey *et al.* (1989) may be an underestimate of the true value.

Etodolac displays linear pharmacokinetics (*Fig.* 2) in humans from 200-600 mg as single dosages, or as repeated doses of 200-600 mg/day in a sustained-release formulation (Dey *et al.* 1989). The pharmacokinetics of etodolac were also linear in the rat (*Fig.* 2) over the range of 2-10 mg/kg; in rats single oral doses of 2, 5 and 10 mg/kg gave corresponding mean AUC of 68, 171, & 357 mg•h/L, respectively (Cayen *et al.* 1981).

In addition to their studies in humans, Kraml *et al.* (1984) also studied the

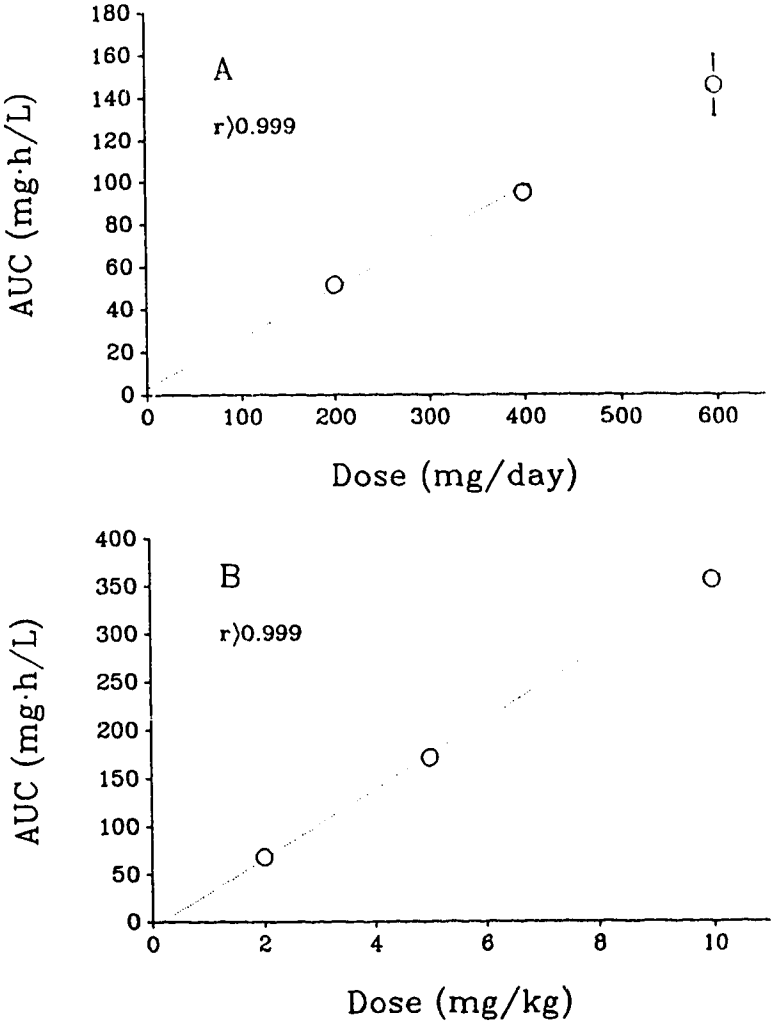

Figure 2 Relationship between dose of (±)etodolac and AUC. Top panel: After multiple dose administration of a sustained release formulation to healthy male volunteers (Dey et al. 1989). Bottom panel: After single oral doses given to rats (Cayen et al. 1981). Graphs were plotted using data presented in the respective papers.

effects of particle size, food, and antiulcer agents on etodolac pharmacokinetics in the dog. Capsules containing a smaller particle size of etodolac (micronized) produced a significantly higher C_{max} than regular capsules, but no difference in t_{max} or AUC. The presence of food caused a delayed absorption of micronized etodolac from capsules, but no change in overall bioavailability, as compared to fasted dogs. The effects of two antiulcer agents were also studied. The antacid magaldrate had no effect on the pharmacokinetics of etodolac, although the cytoprotective agent sucralfate caused a significantly reduced C_{max} (39 *vs* 48 mg/L in controls), but no change in t_{max} or AUC. No explanation was offered as to why the C_{max} was different, but not the t_{max}.

Table I: Mean pharmacokinetic parameters of etodolac in humans given single oral doses. Data generated using non-stereospecific assays.

Subjects	Sex	n	Age y	Wt kg	Dose mg	C_{max} mg/L	t_{max} h	AUC_{0-t} mg • h/L	$t_{1/2}$ h	CL mL/h/kg	Vd L/kg	Reference
Healthy	M	28	26	78	200, tab	17.4	1.2	70.7[a]	6.0			Kraml *et al.* 1984
	M	28	26	78	200, cap	15.9	1.4	71.6[a]	5.9			
	M	28	26	78	200, sol	20.0	0.5	67.9[a]	5.9			
Healthy	M	18	26	69	400, mic	28.6	1.4	115[b]	6.9			Kraml *et al.* 1984
	M	18	26	69	400, tab	21.0	1.6	115[b]	6.5			
Healthy	M	4					7	40.8			0.41	Cayen *et al.* 1981
Healthy	M	14	27		400, sol	36.8	0.55	133[c]	6.0	36.3	0.31	Dey *et al.* 1989
	M	37	27	71	200, cap				8.4	38.5	0.47	
	M	37	27	71	200, SR	4.6	7.2	51.5[a]				
	M	37	27	71	400, SR	7.5	7.9	104[a]				
	M	37	27	71	600, SR	11.9	7.8	146[a]				
Healthy	M	20	27		200	15.9	1.2	71.8[c]	6.0			Scatina *et al.* 1986
Healthy	M	24	76		200	15.3	1.2	63.0[c]	6.1			
Osteo-arthritis	M	20	75		200	16.7	1.3	74.6[c]	6.5			
Healthy	M	10			200	15.4	1.4	63.9[c]	5.7			Lasseter *et al.* 1988
Cirrhosis	M	10			200	17.4	1.1	67.4[c]	6.0			
Healthy	M	4	23	68	200	14.5	1.9	84[c]	6.1			Ferdinandi *et al.* 1986

Abbreviations: tab, tablet; sol, solution; cap, capsule; mic, micronized; SR, sustained release a. $t=24$ h b. $t=48$ h c. $t=\infty$

1.2.2. Distribution

1.2.2.1. Volume of distribution

The mean volume of distribution of etodolac calculated from oral doses is 0.3 to 0.5 L/kg (Table I). This value is higher than that of most other NSAIDs (Lin *et al.* 1987). Cayen *et al.* (1981) suggested that the high Vd was due to the unbound fraction of etodolac in drug-spiked plasma (3.6-4.7%), which was apparently higher (Table II) than that of other NSAIDs (Lin *et al.* 1987). However, the concentrations of etodolac used to spike the plasma were somewhat higher than those encountered clinically. Later studies (Ferdinandi *et al.* 1986; Scatina *et al.* 1986) showed that the earlier reported value (Cayen *et al.* 1981) for unbound fraction was indeed higher than that seen at the concentrations encountered *in vivo* (see Protein binding, below).

1.2.2.2. Protein binding

Cayen *et al.* (1981) first reported the protein binding of etodolac in humans, dogs, and rats, in serum samples spiked with either 20 or 100 mg of the racemate (Table II). In humans the unbound fraction was higher than that in rat and dog serum. The binding was reduced in all 3 species with increases in concentration; the unbound fractions at 100 mg/L were 1.3, 2.5, and 3.25-fold greater than at 20 mg/L in the human, dog, and rat serum, respectively.

Using ^{14}C-etodolac, Ferdinandi *et al.* (1986) found that the binding of etodolac to human serum was extensive. Over a concentration range of 6 to 33 mg/L, the unbound fraction of etodolac in serum was 0.66-1.04% (Table II); the extent of

binding of etodolac to proteins was independent of etodolac concentration within the range of drug concentrations studied. The authors discounted the unbound fraction of etodolac reported by Cayen *et al.* (1981), but did not offer an explanation for the discrepancy. It was likely that the higher unbound fraction reported by Cayen *et al.* (1981) was at least partly due to the higher concentrations (100 mg/L) which were used. Similar to Ferdinandi *et al.* (1986), Scatina *et al.* (1986) found that the mean unbound fraction of etodolac in the serum of young subjects and elderly patients with osteoarthritis ranged between 0.91 to 1.02% after single or multiple doses of 200 mg etodolac; there were no differences between the young and elderly groups of subjects in albumin concentration or total protein.

The binding of etodolac to serum and synovial fluid (Table II) after repeated doses was studied in patients with rheumatoid arthritis by Kraml *et al.* (1988). Samples were spiked with ^{14}C-etodolac and the binding was assessed using equilibrium dialysis. Etodolac was bound more extensively in serum than in synovial fluid. There were significant inverse correlations of moderate strength between the unbound fraction and total protein and albumin concentrations. Despite this finding and the lower concentration of albumin in the synovial fluid, the authors suggested the possibility of an active process for intrasynovial transport of etodolac as an explanation for the higher concentrations of unbound etodolac in synovial fluid.

Table II: The protein binding of (±)-etodolac in serum and synovial fluid.

Specimen	Concentration (mg/L)	Unbound fraction (%)			Notes	References
		Rat	Dog	Man		
Serum	20	0.68	1.7	3.6	Drug spiked samples; healthy human subjects	Cayen *et al.* 1981
Serum	100	2.2	4.4	4.7	Drug-spiked samples; healthy human subjects	Cayen *et al.* 1981
Serum	6-33	-	-	0.66-1.04	Healthy human subjects	Ferdinandi *et al.* 1986
Serum	2-15	-	-	0.97	Elderly osteoarthritic patients	Scatina *et al.* 1986
Serum	2-15	-	-	1.02	Young healthy subjects	Scatina *et al.* 1986
Serum	0.1-20	-	-	0.93	Arthritic patients	Kraml *et al.* 1988
Synovial fluid	0.1-3	-	-	2.5	Arthritic patients	Kraml *et al.* 1988

1.2.2.3. Tissue distribution

Using whole body autoradiograms of the rat, orally administered ^{14}C-etodolac (10 mg/kg) was observed to rapidly distribute to the tissues (Cayen *et al.* 1981). In the same study, another group of rats was killed at various times after oral administration of labelled drug, and a wide selection of tissues was removed and the radioactivity content measured. The levels of radioactivity were highest in blood vessels, connective tissues, and highly perfused organs such as kidney, liver and heart. The serum contained higher concentrations of etodolac than any of the tissues, and the rate of elimination from tissues paralleled that in the serum.

After giving ^{14}C-etodolac, Ferdinandi *et al.* (1986) found that the blood clots remaining after separation of human serum contained almost no radioactivity; hence studies using plasma should yield similar results to those using serum.

1.2.2.4. Distribution into the synovial fluid

Kraml *et al.* (1988) studied the time-course of etodolac concentrations in the synovial fluid of 5 patients with rheumatoid arthritis (age 35-71 y; 2 female, 3 male). The patients were given repeated doses of 200 mg twice daily for 7 days. On day 8 a dose of etodolac 200 mg was given, and serial serum and synovial fluid samples were collected for 32 h.

The mean C_{max} in the synovial fluid was lower (2.6 *vs* 15.6 mg/L), and the t_{max} was greater (3.2 *vs* 1.2 h), than in the serum, thus indicating a delayed entry of etodolac into the synovial fluid. The AUC_{0-24} of total (bound + unbound) drug in

serum was 67% greater than that in synovial fluid (33.6 *vs* 49.8 mg h/L, $p<0.05$), although the decline in concentrations in both fluids was equivalent as reflected in the $t_{½}$, which was not different (6 h).

The AUC_{0-24} of unbound drug was 72% higher in serum than in synovial fluid ($p<0.05$). There was no difference between the $t_{½}$ of unbound etodolac in synovial fluid and serum (6 h), or between the $t_{½}$ of unbound and total drug. The authors found that the relative total drug AUC ratio of [synovial fluid:serum] was considerably lower (67%) than that of the unbound drug (172%).

1.2.3. Elimination

In humans the $t_{½}$ of etodolac is of moderate duration, with mean values between 7-8 h (Table I). In other species, etodolac is eliminated somewhat more slowly. In the rat, the $t_{½}$ were calculated to be 16.8 and 16.6 h after oral and *iv* dosing, respectively (Cayen *et al.* 1981). In dogs, the mean $t_{½}$ is about 9.7 h. In both rats and dogs the elimination phases of the plasma concentration-time profiles are accompanied by fluctuations, which are indicative of enterohepatic recycling (Cayen *et al.* 1981). This explains to some extent the reported prolonged $t_{½}$ in these species.

1.2.3.1. Metabolism

The major metabolites of etodolac are depicted in *Fig.* 3. Cayen *et al.* (1981) studied the metabolism of etodolac in the rat, using ^{14}C-etodolac. Most of the radiolabelled material that was recovered was found to be unchanged drug. In the rat

bile, where most of the drug was recovered, 10% of the material was separated by thin layer chromatography and subsequently identified as being hydroxylated metabolite. Of the four hydroxylated metabolites identified, two were phenolic, while the other two were identified by mass spectroscopy as being 3- and 4-hydroxyetodolac. The authors could not find glucuronidated drug in urine or bile. In the dog, both unchanged and glucuronidated etodolac were seen in the bile.

In humans most of a dose of ^{14}C-etodolac was found in the urine over 24 h (Ferdinandi *et al.* 1986). Using thin layer chromatography for isolation, and mass spectroscopy and nuclear magnetic resonance for identification, the metabolites of etodolac were characterized. The acyl-glucuronide of etodolac comprised 20% of the urinary recovery. Hydroxylated metabolites were also found, and these accounted for 46% of the urinary recovery (11-24% each); these metabolites included 6- and 7-hydroxyetodolac, and 8-(1'-hydroxyethyl)etodolac. The metabolites were predominately found in urine as their respective glucuronide conjugates.

In human serum 90% of radiolabelled drug was present as unconjugated material (Ferdinandi *et al.* 1986). Of this, 70-80% was present as unchanged etodolac, 10% as 7-hydroxyetodolac, and 1-2% as 6-hydroxyetodolac.

An unusual metabolite of etodolac, 4-ureidoetodolac (*Fig.* 3), was found in the urine of humans, rats, dogs, and mice (Ferdinandi *et al.* 1987). In humans and rats this metabolite accounted for 8.1 and 53% of the radioactively labelled dose recovered

in the urine, respectively. Substrate/product stereoselectivity[1] (Jamali *et al.* 1989) did not appear to be present in the formation of this metabolite, because HPLC analysis of the urine extracts showed equal concentrations of the two 4-ureidoetodolac diastereomers. The formation of the ureide did not seem to require the initial metabolism of etodolac to 4-hydroxyetodolac, because the hydroxyl group of 4-hydroxyetodolac was only converted to the ureide under acidic, non-physiological conditions. Therefore, another intermediate was postulated to be involved in the formation of 4-ureidoetodolac.

The hydroxylated metabolites of etodolac, and ureidoetodolac, possess very little if any pharmacological activity, as determined using rat adjuvant arthritis, and prostaglandin production by chondrocytes (Humber *et al.* 1988).

Etodolac does not seem to affect oxidative drug metabolism, because no change was seen in the concentration of microsomal cytochrome P-450 in the livers of rats given 7 daily doses of 10 mg/kg etodolac (Cayen *et al.* 1981).

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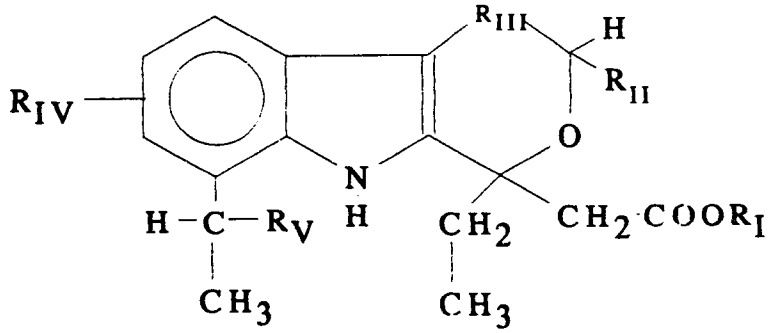
[1] When a prochiral carbon of an enantiomer is converted to a chiral carbon via biotransformation, and one isomer is preferentially formed, substrate/product stereoselectivity is said to have occurred.

Figure 3: The major metabolites of etodolac in humans and rats



Metabolite	R_I	R_{II}	R_{III}	R_{IV}	R_V	Species
6 or 7-hydroxyetodolac	H	H	CH_2	OH	H	humans, rats
Acyl-glucuronide	Glucuronic acid	H	CH_2	H	H	humans, rats
3-hydroxyetodolac	H	OH	CH_2	H	H	rats
4-hydroxyetodolac	H	H	CHOH	H	H	rats
8-1'-hydroxyetodolac	H	H	CH_2	H	OH	humans
4-Ureidoetodolac	H	H	$CHNHCONH_2$	H	H	humans, rats

1.2.3.2. Excretion

In humans etodolac and its metabolites are primarily excreted by way of the urine. Ferdinandi *et al.* (1986) found after giving ^{14}C-etodolac to four healthy black male volunteers, 61% was recovered in urine over 24 h; over 7 days 69-76% of the radioactivity was recovered. During a 7 day collection of urine and feces, 80-92% of the administered radioactivity was recovered. Ferdinandi *et al.* (1987), who studied the urinary excretion of etodolac in four species found that the total urinary recovery (% of dose) of ^{14}C-etodolac as a percent of the dose ranged between 6.2% (dog) to 65.8% (human); in rodents the urinary recovery was 11.4 (rat) and 52.7% (mouse).

Unlike humans, the bile rather than urine constituted the major route of excretion of etodolac in dogs and rats. In bile-duct cannulated rats, 92% of the cumulative radiolabelled dose was excreted into the bile. This elimination was quite rapid, 76% of the administered radioactivity being excreted in the bile within 12 h after dosing. Only 15% of the radiolabelled material was excreted into the urine, and of this, 10% was unchanged etodolac. Although the authors used ß-glucuronidase to evaluate the amount of glucuroconjugated etodolac, no conjugates were seen. Fluctuations in the plasma-concentration *vs* time profiles were evident in both species, and were attributed to extensive enterohepatic recirculation. In the rat this was confirmed, because after bile-duct cannulation the fluctuations disappeared, and the CL of etodolac was increased. The ultimate fate of most of the drug in these species was excretion into the feces; in a radiolabelled dose-recovery study, Cayen *et al.* (1981) found that 79 and 83% of the labelled dose was recovered in the feces of the

rat and the dog, respectively.

In the bile from dogs, both unchanged and glucuronidated etodolac were identified (Cayen *et al.* 1981). In the rat bile, however, most of the recovered radioactivity was as unchanged etodolac; no etodolac glucuronide conjugates were found. There was only a small amount of oxidized metabolites in the rat bile (Cayen *et al.* 1981).

The metabolites of etodolac appear to be quite rapidly eliminated from the plasma in rats and dogs. Specifically, in the rat, parent drug accounts for 95% of the radiolabelled drug found in the serum (Cayen *et al.* 1981). Similarly, in dogs, the ratio of total serum radioactivity/etodolac is 1.35 and 1.33 after oral and *iv* doses, respectively (Cayen *et al.* 1981).

1.2.4. Effect of pathophysiological changes on etodolac pharmacokinetics

1.2.4.1. Effect of aging

The pharmacokinetics of etodolac in elderly subjects were compared with young subjects by Scatina *et al.* (1986). In each group after repeated administration (every 12 h) of etodolac for 7 days the pharmacokinetic parameters were not significantly different between the young and the elderly subjects (Table I). In the elderly there was a significant increase of 13% in the $AUC_{0\text{-}12}$ between the first and the seventh day of dosing, although the authors felt that it would be of little clinical importance. It was concluded that dosage adjustments were probably not required in elderly patients.

1.2.4.2. Pharmacokinetics in osteoarthritis

In their study involving the elderly, Scatina *et al.* (1986) also included a group of 20 elderly patients with osteoarthritis (Table I); this is pertinent considering the presently approved indication for etodolac in the USA, which is for use as an analgesic, and as an antiinflammatory agent in the treatment of osteoarthritis (Anonymous 1991). The pharmacokinetics and serum protein binding of etodolac in this group of subjects were identical to those of the young subjects. However, after multiple dosing there was no detectable accumulation of drug in the osteoarthritic patients, unlike the healthy elderly subjects.

1.2.4.3. Pharmacokinetics in patients with hepatic cirrhosis

In a published abstract the pharmacokinetics of etodolac in patients with hepatic cirrhosis were reported (Lasseter *et al.* 1988). There were no differences between patients and a control group of young healthy volunteers in AUC, C_{max}, t_{max}, $t_{½}$, or serum protein binding. It was concluded that dosage adjustments in such patients are not necessary.

1.3. Rationale for the study of the enantioselective pharmacokinetics of etodolac

1.3.1. The basis for considering stereoselectivity in clinical pharmacology and pharmacokinetics

Most of the drugs that are used clinically are organic molecules, and are therefore primarily carbon based. When a carbon atom in a molecule is bonded to four different substituents, that carbon atom is termed a chiral, or an asymmetric, centre. There are two possible orientations of the substituents about the chiral centre, and therefore, there are 2 possible forms of the same molecule; these are called enantiomers. Enantiomers, which share the same chemical formula and structure, with the exception of the arrangement of the substituents about a chiral centre or plane, have virtually the same physical-chemical properties in an achiral environment (Wainer & Marcotte 1988). They do differ, however, in their abilities to rotate the plane of polarized light. In essence, if an enantiomer was dissolved in an achiral solvent, and a plane of polarized light were passed through the solution, then on the other side of the solution the emitted light would be bent in opposite directions in each of the two solutions. This is called optical activity, and is a characteristic property of molecules which contain chiral centres or planes. Depending on the absolute configurations of the substituents about a chiral centre, the enantiomers are most commonly termed *R* or *S*.

Although enantiomers possess the same physical-chemical properties, they

often differ in their biological properties. The basis for this difference lies in the chiral nature of biological systems. Many of the macromolecules that comprise cellular systems are themselves chiral, and have unique 3-dimensional structures. Furthermore, the basis of pharmacological activity of drugs lies in their ability to interact with specific receptor molecules, such as proteins, which are chiral macromolecules. Because the orientation of the substituents about one chiral centre in an enantiomer may permit a more favourable interaction with a receptor protein than with its corresponding enantiomer (antipode), the enantiomers may have different pharmacological properties.

There are chiral compounds in which the enantiomers differ in their pharmacological properties. For example, with the ß-adrenergic blocking drugs, the major pharmacological effects are attributable to the levorotatory enantiomers (Jamali *et al.* 1989). In another class of drugs, the chiral nonsteroidal antiinflammatory drugs (Table III), the *S* enantiomers possess many fold greater activity than the respective *R* enantiomers (Jamali 1988).

Sometimes enantiomers may possess distinct pharmacological properties. For example, the (-) enantiomer of the ß-adrenergic blocking drug, sotalol, possesses almost all of the ß-blocking activity. However, both of the (-) and (+) enantiomers possess class III antiarrhythmic activity (Jamali *et al.* 1989). In other cases one enantiomer may be responsible for activity, and its antipode for an untoward effect. There are many examples of differential activity of enantiomers, and the reader is referred to the review article by Jamali *et al.* (1989).

The processes involved in the determination of drug disposition, namely absorption, distribution, metabolism and excretion, are also governed to varying degrees by interactions of drugs with chiral macromolecules. For example, there may be transport proteins involved in absorption of drugs from the gastrointestinal tract. The distribution of drugs through the body may be restricted in some cases by binding of drugs to chiral proteins circulating in the bloodstream, including albumin and α_1-acid glycoprotein. The distribution of a drug may also be influenced by its binding to tissue proteins. Drug biotransformation is facilitated by metabolic proteins which can be located in several organs. Some excretory processes, such as renal tubular secretion, involve proteins. Due to the differential abilities of enantiomers to interact with proteins involved in any of these processes, the enantiomers of a chiral compound may possess different pharmacokinetic properties.

Many of the drugs which contain chiral centres are administered clinically as compounds containing equal proportions of the *R* and *S* enantiomers. Such a compound is called a racemate. Consequently, the therapeutic or toxicological activities of a racemic compound may be preferentially or equally attributed to one or both enantiomers.

The fundamental basis for studying the concentrations of drug in a biological sample, and therefore to establish pharmacokinetic indices, is that there is some measure of relationship between the concentration and the degree of pharmacological activity. In the past, most pharmacokinetic studies of racemic drug compounds were determined using nonstereospecific methodology. In essence, the sum of the

enantiomers was reflected in the concentration *vs* time curves, and in the derived pharmacokinetic parameters. Such an approach ignores the fact that enantiomers may differ in their pharmacological activity, and may render any interpretation of the pharmacokinetic data, in relationship to the observed pharmacodynamic parameters, either misleading or meaningless (Ariens 1984; Ariens & Wuis 1987; Evans *et al.* 1988; Jamali *et al.* 1989).

1.3.2. Stereospecific pharmacokinetics of nonsteroidal antiinflammatory drugs

1.3.2.1. Other NSAIDs

The nonsteroidal antiinflammatory drugs are widely used, as anti-arthritic agents, as antipyretics, and as analgesics in mild to moderate pain. A large number of the NSAIDs in existence today are chiral molecules (Table III). It is important to realize that for every chiral NSAID developed to date, almost all of the beneficial activities are possessed by the *S* enantiomer (Jamali 1988; Evans 1992); the respective *R* enantiomers are, for the most part, therapeutically irrelevant[1]. With the exception of naproxen, all of the chiral NSAIDs are, or have been, used clinically as the racemate. In the 1980's a number of papers (Wright & Jamali 1992) were published which described analytical methods for the determination of enantiomers of a number of nonsteroidal antiinflammatory drugs. Prior to these developments, pharmacokinetic data were generated based on non-stereospecific assays. Hence, the calculated

[1] Some evidence has been put forth which suggests that the R enantiomer of flurbiprofen possesses analgesic activity (Brune et al. 1991).

pharmacokinetic parameters were based on the sum of the *S* and *R* enantiomers.

Except for ketorolac (Guzman *et al.* 1986), the optical rotation of the dextrorotatory and levorotatory enantiomers of chiral NSAIDs have the *S* and *R* absolute configurations, respectively (Jamali 1988). The pharmacokinetics of the enantiomers of most chiral NSAIDs have been studied in humans and animals. With most of the chiral NSAIDs, the plasma concentrations of the active *S* enantiomer are either similar, or greater than, those of the *R* enantiomer (Table IV). For an important sub-class of NSAIDs, the 2-arylpropionic acids, part of the reason for greater plasma concentrations of the *S* enantiomer lies in the occurrence of a bioinversion of the *R* to the *S* enantiomer. This unique metabolic pathway is known to involve ibuprofen (Jamali *et al.* 1992), benoxaprofen (Bopp et al. 1979), fenoprofen (Rubin *et al.* 1985), and ketoprofen (Jamali & Brocks 1990) in humans (Jamali *et al.* 1990). In general, the nonsteroidal antiinflammatory drugs are extensively metabolized, have a low hepatic extraction ratio, and are extensively bound to albumin in plasma (Lin *et al.* 1987). Consequently, in cases where the plasma concentrations of the *S* enantiomer exceed those of their antipodes, differences in clearance might be explained by stereoselectivity in intrinsic metabolic clearance, and in plasma protein binding of the enantiomers. Where there is stereoselectivity in the volume of distribution, enantioselective plasma or tissue protein binding could be held responsible. For example, enantioselective plasma protein binding seems to be an important factor in determining the stereoselective pharmacokinetics of flurbiprofen (Berry & Jamali 1989).

Table III: Some nonsteroidal antiinflammatory drugs

i. Achiral	
Acetylsalicylic acid	Diclofenac
Diflunisal	Indomethacin
Mefenamic acid	Piroxicam
Tenoxicam	Tolmetin
Zomepirac	
ii. Chiral	
Benoxaprofen	Carprofen
Cicloprofen	Etodolac
Fenoprofen	Flobufen
Flunoxaprofen	Flurbiprofen
Ibuprofen	Indobufen
Indoprofen	Ketoprofen
Ketorolac	Loxoprofen
Metbufen	Naproxen
Pirprofen	Tiaprofenic acid
Suprofen	

Table IV: The *S:R* AUC ratios of some representative chiral NSAIDs in humans

Drug	*S:R* AUC ratio	References
Ibuprofen	1.4-2.5	Evans 1992
Ketoprofen	0.85-1.0	Evans 1992
Ketorolac	1.0	Brocks & Jamali 1992
Pirprofen	>1	Sioufi *et al.* 1987
Fenoprofen	>6	Rubin *et al.* 1985
Flurbiprofen	1.1	Jamali *et al.* 1988
Tiaprofenic acid	1.0	Singh *et al.* 1987
Indobufen	0.54	Benedetti *et al.* 1992
Suprofen	0.55-0.68	Shinohara *et al.* 1991
Etodolac	0.10	Jamali *et al.* 1988

1.3.2.2. Etodolac

The first report describing the stereospecific pharmacokinetics of etodolac was published in 1986 (Singh *et al.* 1986). In that report, a gas chromatographic method was described which involved a precolumn derivatization of etodolac with *S*-(+)-amphetamine. The etodolac-amphetamine amide diastereomers were separated using a fused-silica capillary column.

The authors studied the plasma concentrations of orally administered etodolac in 2 subjects. One subject was a 56 y old female patient with unspecified arthritis, who had received 200 mg etodolac every 12 h for 1 month. The second subject was a healthy male volunteer (39 y) with no prior history of ingestion of etodolac. After dosing the subjects with a 200 mg dose of (±)-etodolac, blood samples were collected, and plasma was harvested. During the sample preparation, acyl-glucuronides of etodolac were purposefully hydrolysed; there was no attempt to distinguish between unchanged and acyl-glucuronidated etodolac enantiomers, in plasma and urine, in this particular study.

In both subjects, the plasma concentrations of *R*-(-)-etodolac substantially exceeded those of the active *S*-(+) enantiomer. The pharmacokinetic indices were not reported for the study subjects. Less than 10% of the dose of each enantiomer was excreted in the urine, as acyl-glucuronidated drug. The excretion of the *S*-(+) enantiomer in urine was higher than the *R*-(-) enantiomer in both subjects.

In 1988 a second assay was published for the analysis of etodolac enantiomers (Jamali *et al.* 1988). This was an HPLC assay, which involved a precolumn

derivatization *via* formation of a mixed anhydride intermediate with ethylchloroformate, followed by the formation of a diastereomeric amide with L-(-)-α phenylethylamine. Unlike the gas chromatographic assay, this procedure permitted for quantitation of both the unchanged etodolac and acyl-glucuronidated species.

The pharmacokinetics of etodolac were characterized in two healthy male subjects after 200 mg etodolac every 12 h, for 7 doses. Similar to the results of Singh *et al.* (1986), the plasma concentrations of the *S*-(+) enantiomer were much lower than those of the inactive *R*-(-) enantiomer. Furthermore, pharmacokinetic data were reported. The AUC were 12.3- and 8.9-fold higher for *R* than *S* etodolac, in the two volunteers, respectively. In both subjects the *S* enantiomer had a much greater distribution than its antipode, the Vd_β/F being 8.2 and 4.1 fold higher for *S* than *R* etodolac. In the urine, an average of 23 and 11% of the dose of the *S* and *R* enantiomers, respectively, were excreted. All of the drug recovered in urine was derived from acyl-glucuronidated etodolac.

These preliminary data were of importance, because the concentrations of the active *S* enantiomer represented only a small fraction of the total (*S*+*R*) plasma concentrations of etodolac. For this reason, the relevance of the bulk of the previous data generated on the pharmacokinetics of (*S*+*R*)-etodolac could be questioned. As stated above, the objective of performing a pharmacokinetic study should be to relate the drug concentrations in blood, serum, or plasma, to the pharmacological activity of the drug. If, in the place of the active entity, an inactive substance is measured, such an objective cannot be achieved. Therefore, it was necessary to further evaluate

the pharmacokinetics of etodolac, from a stereospecific perspective.

1.4. Hypotheses

1. The pharmacokinetic differences between the enantiomers of etodolac are due to enantioselectivity in:

   a. Metabolic clearance

   b. Plasma protein binding

2. The rat is a suitable animal to study the underlying mechanisms for the stereoselective pharmacokinetics of etodolac.

1.5. Specific objectives, and their rationale

1.5.1. Study the stereospecific pharmacokinetics of etodolac in the rat

Due to a limited availability of individual enantiomers of etodolac and ethical issues involved in administration of individual enantiomers to humans (De Camp 1989), we sought to develop an animal model for the study of the stereospecific pharmacokinetics of etodolac. For this purpose we chose to study the rat, and to investigate the basis for any differences which might be seen in the pharmacokinetics

of etodolac enantiomers. We also performed experiments to:

1.5.1.1. Determine if there is an interaction between the enantiomers of etodolac

Both individual enantiomers and racemic etodolac were administered to determine if the enantiomers influence the pharmacokinetics of one another. Such an interaction has been observed between the enantiomers of flurbiprofen (Berry & Jamali 1989).

1.5.1.2. Determine factors which might be involved in the distribution of etodolac enantiomers

The distribution of NSAIDs, including etodolac, is highly dependent on binding to plasma proteins (Lin *et al.* 1987). Therefore, the *in vitro* relative binding affinities of etodolac enantiomers to plasma and tissues, and the *in vivo* tissue distribution, were examined.

1.5.1.3. Determine if there is stereoselectivity in the excretion of etodolac enantiomers

In the rat, most of the dose of ^{14}C-etodolac has been reported to be excreted in the bile, as unchanged drug (Cayen *et al.* 1981). Consequently, the excretion of etodolac enantiomers was studied in rats which were cannulated at the common bile duct. Urinary excretion was also evaluated in rats with an intact or interrupted biliary tract.

1.5.1.4. Determine enantioselective aspects of biotransformation of etodolac

Most NSAIDs are extensively metabolised. Several metabolites of etodolac have been characterized, in humans and rats. Therefore, the *in vitro* microsomal metabolism of etodolac enantiomers was studied in the rat, to assess the relative oxidative metabolism and acyl-glucuronidation of the etodolac enantiomers.

1.5.1.5. Study the effects of enzyme inhibition and induction

Etodolac and other NSAIDs are used in the long term treatment of arthritis, which may subject patients to an increased risk of gastrointestinal disturbances (Taha *et al.* 1989). To treat these complications, H2 histamine blocking agents such as cimetidine are occasionally co-prescribed during therapy with NSAIDs (Verbeeck 1990). This could possibly influence the outcome of the treatment with etodolac, because cimetidine can competitively inhibit the cytochrome P-450 mediated metabolism of a number of drugs (Somogyi & Muirhead 1987). Alternatively, the co-administration of anticonvulsant drugs such as phenobarbital or phenytoin can increase the metabolism of NSAIDs, through their potent inducing effects on drug metabolism (Hansten & Horn 1989).

The effects of enzyme induction or inhibition on the pharmacokinetics of etodolac enantiomers have not been previously reported; hence, we sought to describe the effects of an enzyme inducer (phenobarbital) and an inhibitor (cimetidine) on the pharmacokinetics of etodolac enantiomers in the rat.

1.5.2. Study the pharmacokinetics of etodolac enantiomers in humans

In the human studies, we sought to:

1.5.2.1. Confirm and elaborate on the enantioselective pharmacokinetics of etodolac in humans

There is only limited data available which describe the pharmacokinetics of etodolac enantiomers in humans. What is known has been derived from only four subjects in two preliminary studies (Singh *et al.* 1986; Jamali *et al.* 1988). Therefore, the stereospecific pharmacokinetics of etodolac were studied in a group of young, healthy volunteers.

1.5.2.2. Determine the effect of aging on the stereoselective pharmacokinetics of etodolac

In humans, etodolac is extensively metabolized, almost no intact drug being recovered in the urine (Ferdinandi *et al.* 1986). Although most of a dose of etodolac is eliminated by oxidative metabolism, 10-20% is eliminated as acyl-glucuronides (Jamali *et al.* 1989; Ferdinandi *et al.* 1986). The plasma protein binding of etodolac is extensive (Ferdinandi *et al.* 1986). Because aging can affect both the metabolism and protein binding of drugs (Chapron 1988), it is possible that the pharmacokinetics of the enantiomers of etodolac may differ between young and elderly subjects.

1.5.2.3. Establish the extent of biliary excretion of etodolac

There is enterohepatic recirculation of etodolac in rats and dogs, because a large fraction of the dose is excreted in the bile (Cayen *et al.* 1981). Biliary excretion of etodolac enantiomers, which has not been previously studied in humans, is of clinical importance since it might play a significant role in their elimination and because it has been speculated that enterohepatic recirculation increases the intestinal toxicity of NSAIDs (Rainsford 1987).

1.5.2.4. Determine the relative concentrations of etodolac enantiomers in the synovial fluid

One of the ultimate goals of conducting a pharmacokinetic study is to yield information which can be used to establish a concentration-effect relationship. With respect to the NSAIDs in rheumatoid arthritis, this is perhaps best facilitated by relating concentrations in plasma to those at the site of action, namely the synovial fluid (Wallis & Simkin 1983). This information can then be used to better understand the plasma concentration *vs* antiinflammatory activity relationship.

The non-stereospecific disposition of etodolac in synovial fluid has previously been reported by Kraml *et al.* (1988). They found considerable concentrations of etodolac in synovial fluid following oral administration to arthritic patients. That study, however, did not provide insight into the concentrations of the pharmacologically active *S*-enantiomer (Humber *et al.* 1986) of etodolac in synovial

fluid. This is of particular concern, as it has been shown that plasma concentrations of inactive *R*-etodolac greatly exceed those of *S*-etodolac following administration of racemate (Singh *et al.* 1986; Jamali *et al.* 1988). Therefore, we sought to examine the uptake of etodolac in synovial fluid from a stereospecific perspective, and examine whether plasma concentrations of total (*S*+*R*) etodolac indeed reflects those of the active enantiomer at the site of action.

1.5.2.5. Determine the extent of protein binding of etodolac in plasma and synovial fluid

Because protein binding is an important factor in the disposition of NSAIDs, binding of enantiomers to both plasma and synovial fluid, and to hyaluronic acid, a major component of synovial fluid, were also assessed.

2. Experimental

2.1. Chemicals

Racemate and pure enantiomers of etodolac and internal standard [(±)-2-(4-benzoylphenyl)butyric acid], were kindly provided by Wyeth-Ayerst Laboratories (New York, NY), and Rhone-Poulenc (Montreal, Canada), respectively. Etodolac capsules (Lodine; Wyeth-Ayerst, New York, NY) were purchased commercially. For microsomal incubations, all reagents were purchased from Sigma Laboratories (St. Louis, MO). Injectable cimetidine (Tagamet, 150 mg/mL; SmithKline & French, Mississauga, Canada) was purchased from a hospital pharmacy. All reagents required for microsomal incubations, *S*-(-)-α-phenylethylamine, *R*-(+)-α-phenylethylamine, kits for the determination of albumin and total protein concentrations, human umbilical cord hyaluronic acid, and urethane, were purchased from Sigma Laboratories (St. Louis, MO, USA).

Isopropyl alcohol, ethylchloroformate, iso octane, chloroform, HPLC grade water, H_2SO_4, NaOH, NaH_2PO_4, Na_2HPO_4, NaCl, and diethyl ether were purchased from BDH (Edmonton, Canada). Methoxyflurane (Metofane) was obtained from Pitman-Moore Ltd (Mississauga, Canada), polyethylene glycol 400 from Fisher Scientific (Edmonton, Canada), heparin (Hepalean) from Organon Teknika (Toronto, Canada).

2.2 Assays

2.2.1. Etodolac enantiomers

Concentrations of intact etodolac and its conjugated metabolites were determined in plasma and urine using a normal-phase HPLC method (Jamali *et al.* 1988). Briefly, the method consisted of acidification of samples followed by extraction with isopropyl alcohol:iso-octane (5:95). After transferring the organic layer to clean tubes, the solvent was evaporated, and derivatization was performed using ethyl chloroformate and *S*(-)-α phenylethylamine. The resultant diastereomers were extracted into chloroform, which was then evaporated to dryness. The residue was dissolved in 0.2 mL of mobile phase, and injected into the HPLC. To measure conjugates, samples were hydrolysed with 1 M NaOH prior to extraction, then acidified and treated as described above. The difference in etodolac concentrations between hydrolysed and unhydrolysed samples was taken as being etodolac in its conjugated form (*Figs.* 4-6). Inadvertent hydrolysis of conjugated drug was minimized by pre-rinsing the storage vessels with 1M HCl. All samples were stored at -20°C until analyzed.

There was a change to the assay involving the use of the derivatizing reagent. In the published description of the assay method, and early in the course of the presently described studies, *S*(-)-α-phenylethylamine was used. However, when this reagent is used, the diastereomer of the active *S* enantiomer of etodolac elutes later than that of the *R* enantiomer (e.g., *Fig.* 4). Hence, the peak corresponding to the *S* enantiomer is wider than the *R* enantiomer, and sensitivity could be compromised at

lower concentrations. This is important, because the *S* enantiomer attains much lower concentrations in human plasma than the *R* enantiomer of etodolac. Consequently, to ensure for maximal assay sensitivity of *S*-etodolac, *R*-(+)-α-phenylethylamine was tried as a derivatizing reagent.

A minor change was made in the composition of the extraction solvent (3% isopropanol in iso-octane rather than 5%) when extracting etodolac from human bile. This resulted in larger peak areas for the etodolac enantiomers. The volume of human bile used for the analysis was 0.5 mL. For the assay of intact etodolac and its conjugated metabolites in human plasma and synovial fluid, sample volumes of 0.5 mL were used for analysis.

Some modification of the assay was required for extraction of etodolac from rat tissues. Two volumes of HPLC grade water were added to weighed tissue samples (wet wt = 0.5-1.5 g). The mixtures were then homogenized using Potter-Elvehjem tissue grinders driven by a T-Line Laboratory Stirrer (Talboys Engineering, Montrose, PA). Internal standard was placed into the glass mortar during the homogenization step. Samples were transferred to test tubes and acidified with 200 μL of 0.6 M H_2SO_4. Etodolac was extracted with 6 mL of isopropanol/iso-octane (5:95). After transfer of the organic layer to clean tubes, 6 mL of HPLC grade water was added and, following mixing and centrifugation, the organic layer was discarded. The aqueous layer was acidified with 0.6 M H_2SO_4, and chloroform was added. After mixing and centrifugation the aqueous layer was removed, and the remaining chloroform layer was evaporated to dryness. Derivatization was performed as for the

plasma samples.

2.2.2. Protein concentrations

The human plasma albumin concentration was determined using the bromocresol purple method with detection at 600 nm. The total protein concentrations were determined using the method of Lowry *et al.* (1951).

2.3. Human subjects

All experimentation involving human subjects was performed at the Clinical Investigational Unit at the University of Alberta Hospital, in accordance with the provisions of the Declaration of Helsinki. Signed informed consent was required of all participants before the study.

The subjects involved in the pharmacokinetic studies were six young and six elderly, ambulatory, non-arthritic, volunteers and three other patients who had undergone cholecystectomy for cholelithiasis. These patients had a T-tube inserted in the bile-duct for drainage and collection of bile. All subjects had normal renal and hepatic functions; their characteristics are shown in Table V. Three elderly subjects, and each of the cholecystectomy patients, took other drugs during the pharmacokinetic studies (Table V). None of these medications was expected to alter the pharmacokinetics of NSAIDs (Hansten & Horn 1989).

The disposition of etodolac into the synovial fluid was studied in six arthritic patients (62 ± 6 y)(Table VI) requiring synovial fluid aspiration; none had taken another NSAID for at least 24 h prior to ingesting etodolac.

Table V: Clinical indices of volunteers involved in the human pharmacokinetic studies.

Subject	Sex	Age y	Weight kg	Height cm	Actual CL_{CR} mL/min	Plasma albumin g/L	24 h urine output mL	24 h bile output[b] mL	Other medications
Young:									
1	M	32	80	186	126	41	1373		
2	M	27	81	183	141	43	3060		
3	F	25	50	164	93.5	40	1470		
4	M	26	78	176	137	40	3025		
5	M	33	67	175	99.1	53	1542		
6	F	27	55	152	98.1	70	1581		
Mean		**28[a]**	**69**	**173**	**116[a]**	**48**	**2010**		
SD		**3.3**	**13**	**12.7**	**21.3**	**12**	**804**		
Elderly:									
7	F	73	52	157	44.9	47	1700		captopril, doxepin
8	F	65	55	152	66.6	45	1330		
9	M	75	58	170	107	39	2200		gemfibrizol, propranolol, isosorbide dinitrate
10	M	66	87	174	93.8	56	3310		oxybutynin
11	M	77	93	178	97.7	52	1975		
12	M	80	98	180	95.1	39	1725		
Mean		**73**	**74**	**169**	**84.2**	**46**	**2040**		
SD		**6.0**	**21**	**11.5**	**23.5**	**7.0**	**687**		
After cholecystectomy:									
13	M	70	74	170	142	34	5725	211	meperidine, diazepam
14	F	55	55	155	76.0	34	780	189	dimenhydrinate, triazolam, oxycodone, acetaminophen
15	F	30	65	165	93.8	43	880	623	acetaminophen
Mean		**52**	**65**	**160**	**104**	**37**	**2460**	**341**	
SD		**20**	**9.5**	**7.9**	**34.1**	**5.4**	**2830**	**244**	

a. Significantly different from elderly subjects b. Bile not collected in young and elderly subjects

Table VI: Clinical indices of arthritic patients involved in the synovial fluid disposition studies.

Patient	Age	Sex	Diagnosis	Sampling time (h post-dose)	Total protein, g/L		Albumin, g/L		WBC[a], 10^9/L	
					Plasma	SF	Plasma	SF	Plasma	SF
1	61	F	Polymyalgia rheumatica	12	65.0	37.0	29.6	17.7	9.3	12.4
2	58	F	Rheumatoid arthritis	12	52.7	29.3	25.9	19.3	5.7	4.5
3	58	F	Chondrocalcinosis	2	68.2	35.1	38.2	25.3	10.3	17.1
4	71	M	Rheumatoid arthritis	12	77.5	50.4	46.3	25.9	11.5	ND
5	56	F	Rheumatoid arthritis	12	51.7	41.0	33.6	20.2	6.7	17.8
6	67	F	Rheumatoid arthritis	12	57.3	29.6	24.1	24.9	7.3	7.8
Mean	**62**				**62.1**	**37.1**	**33.0**	**22.2**	**8.47**	**11.9**
SD	**6**				**10**	**7.9**	**8.3**	**3.6**	**2.3**	**5.8**

a. - White blood celi count.

ND - Not determined

2.4 Dosing and sample collection

2.4.1. Human pharmacokinetic studies

All subjects fasted overnight before the study. On the study day a catheter was inserted into a forearm vein for blood sampling. The subjects then took a single 200 mg capsule of etodolac with 200 mL of water at 0800 h. The subjects remained in a sitting position for 2 h, then ate a breakfast of their choice. Blood (7 mL), from which plasma was harvested by centrifugation, was obtained at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, and 24 h after the dose, in heparinized collection tubes. Between collections, the catheters were flushed with 1 mL of a 100 U/mL solution of heparin. In the cholecystectomy patients blood sampling was conducted for 12 h. Each subject's urine was collected at intervals of 0-3, 3-6, 6-12, and 12-24 h after dosing. Bile was collected from the cholecystectomy patients at intervals of 0-2, 2-4, 4-6, 6-8, 8-12, and 12-24 h.

2.4.2. Human synovial fluid disposition

A single oral dose of 200 mg etodolac was taken by the patients from whom synovial fluid was withdrawn. Five patients took the drug 12 h before the procedure; a single patient took it 2 h before (Table VI). Blood, from which plasma was separated, and synovial fluid from the knee joint, were obtained concurrently.

2.4.3. Rat pharmacokinetic studies

2.4.3.1. Surgical procedures

A total of 21 male Sprague-Dawley rats were used. Body weight was 278±48 g (range 210 to 367 g). All rats were catheterized by insertion of silastic tubing (0.025" i.d. x 0.047" o.d.; Dow-Corning, Midland MI) into the right jugular vein. Three rats were also cannulated at the common bile duct using Tygon microbore tubing (0.01" i.d. x 0.03" o.d.). The procedure included passage of the bile duct-cannulation tubing from the abdominal cavity to the back of the neck through the skin. The distal end of the cannula was then placed into a collection vial which was attached to a harness fitted on each animal's back. All surgical procedures were performed with anaesthesia, using diethyl ether for induction, and methoxyflurane for maintenance. An overnight rest was allowed before dosing, and all animals were permitted freedom of movement for the duration of the experiments. Rats were housed in plastic metabolic cages during experiments, and food and water was allowed *ad libitum*.

2.4.3.2. Dosing and sample collection

Etodolac was dissolved in 100% polyethylene glycol 400 and administered as bolus doses into the jugular vein cannula. Three groups of 6 rats received either 5 mg/kg of racemate, or 2.5 mg/kg of pure *R*- or *S*-etodolac. Bile duct-cannulated rats received 5 mg/kg of racemic etodolac. Each cannula was flushed with 0.3 mL of 100 U/mL heparin after drug administration, and with 0.2 mL between blood sampling.

Mean body weights between groups were not significantly different (one-way ANOVA).

Blood (0.1-0.2 mL) was collected at 0, 0.25, 0.5, 1, 2, 3, 4, 6, 12, and 24 h after drug administration. Additional samples were obtained at 36, 48, 72, 96, and 120 h, when cannulae remained patent. Samples were taken post-dose at 24 h in 4 rats, 48 h in 10 rats, 72 h in 1 rat, 96 h in 1 rat, and 120 h in 2 rats. In bile duct-cannulated rats, samples were obtained up to 24 h post-dose. Immediately following collection, each blood sample was centrifuged using a Fisher model 235A microcentrifuge (Fisher Scientific, Edmonton, Canada) for 3 minutes. Urine was collected at 0-3, 3-6, 6-9, 9-12, 12-24, and 24-48 h post-dose. Total excreted bile was collected at intervals of 0-3, 3-6, 9-12, 12-24, 24-36, and 36-48 h post-dose.

2.4.3.3. Tissue distribution

Etodolac was dissolved in 100% polyethylene glycol 400 (20 mg racemate/mL) and administered as 10 mg racemate/kg bolus *iv* doses into the tail vein. A total of thirty-two male Sprague-Dawley rats (wt = 308±34 g) was killed by cervical dislocation at 1, 3, 6, 12, or 24 h after dosing. Six rats were included in the 1, 3 and 6 h groups, whereas 7 rats were in the 12 and 24 h groups. Blood was collected and plasma separated by centrifugation at 2600 rpm for 5 minutes. Selected tissues (liver, kidney, perinephric fat, heart, and brain) were immediately excised and frozen at -20°C until analyzed. Mean body weights between groups were not significantly different.

2.4.3.4. Drug interaction studies in the rat

A total of 25 male Sprague-Dawley rats, weighing between 265-321 g, was studied. All rats were catheterized at the right jugular vein as described above. An overnight rest was allowed before dosing, and all animals were permitted freedom of movement for the duration of the experiments. Rats were housed in plastic metabolic cages during experiments, and food and water was allowed *ad libitum*. All rats were subjected to a 12 h day/night cycle throughout the course of the experiments.

Rats were divided into 4 groups; control, phenobarbital pretreated, low-dose cimetidine (L-CIM), and high-dose cimetidine (H-CIM). Mean body weights between groups were not significantly different. Phenobarbital (80 mg/mL) was dissolved in 100% polyethylene glycol 400 (PEG 400), and administered as 75 mg/kg *ip* doses daily for 3 days prior to the experiment; all doses were given between 0900-1000 h. Control and cimetidine pretreated rats were given *ip* doses of PEG 400, equal to those received by phenobarbital rats, daily for 3 days prior to pharmacokinetic studies. The L-CIM rats were given 50 mg/kg cimetidine *iv* 30 min prior to the administration of etodolac. In the H-CIM group, 120 mg/kg of cimetidine were given *iv* followed 5 min later by etodolac, to increase the concentrations of cimetidine in plasma. To assess the effects of multiple doses of cimetidine (H-CIM-M), two rats were given 3 daily doses of 120 mg/kg/day *ip* of cimetidine in place of PEG-400, and otherwise treated as the H-CIM rats.

At 1000 h on the day following the surgery, etodolac (10 mg/mL in 100% PEG 400) was administered as 5 mg/kg of racemate into the jugular vein cannula.

Each cannula was flushed with 0.15 mL of 100 U/mL heparin after drug administration, and between blood sampling. Blood samples (0.15-0.2 mL) were collected at 0, 0.25, 0.5, 1, 2, 3, 4, 6, 12, and 24 h after administration of etodolac; sampling was limited to 24 h, to ensure patency of the cannulae. Immediately following collection, plasma was separated from blood by centrifugation for 3 min using a Fisher model 235A microcentrifuge. The 24 h urine output was collected and pooled. All samples were stored at -20°C until analyzed.

To study the influence of phenobarbital on the biliary excretion of acyl-glucuronidated etodolac enantiomers, four male rats given phenobarbital (75 mg/kg/day *ip* for 3 days) in PEG 400, and 4 control rats pretreated with PEG 400, were anesthetized with 1.2 g/kg urethane. A midline incision was made in the abdomen, and a cannula was inserted in the bile duct, followed by cannulation of the jugular vein, as described above. Under anaesthesia, each rat was then administered 5 mg/kg of racemic etodolac *iv*, and blood samples were collected at 0.25, 0.5, 0.75, 1, 1.5, 2, and 3 h. Bile samples were obtained during the intervals between the blood sample collection times. Samples were stored and analyzed as described above for etodolac and its conjugates.

2.4.3.5. Urinary excretion in female rats

Three female Sprague-Dawley rats (250-300 g) were given 3 mg of (±)etodolac dissolved in 100% PEG 400 (10 mg/mL), via oral gavage. The urine was collected for 24 h and stored at -20° until analyzed for etodolac enantiomers.

2.5. Plasma protein and tissue binding studies

2.5.1. Apparatus and technique

In all of the protein binding studies, dialysis was performed using a Spectrum (Los Angeles, CA, USA) apparatus, using Sigma Diagnostics (St. Louis, MO, USA) dialysis sacks (molecular weight cut off = 12,000 daltons). One side of each cell was filled with one mL of blank binding fluid, while the other contained one mL of isotonic Sorensen's phosphate buffer (pH 7.4) spiked with racemic etodolac. In all cases the dialysis was conducted at 37°, and in preliminary studies it was shown that a dialysis time of 4 h was sufficient for equilibrium; there were no significant differences between the unbound fractions when samples were dialysed for 4 or 6 h. In all studies, each side of the cell was analyzed for etodolac concentration after dialysis.

2.5.2. Binding studies in the rat

2.5.2.1. Rat plasma

Samples of blank rat plasma (1 mL) were subjected to equilibrium dialysis for 4 h. One side of each cell was filled with blank rat plasma, while the other contained 1 mL of buffer (pH 7.4) spiked with either 20 or 100 mg/L of (±)etodolac.

2.5.2.2. Rat tissue binding experiments.

The relative *in vitro* binding of etodolac enantiomers to rat liver, kidney, heart and brain tissues was assessed. Weighed tissue samples were first homogenized in 2

volumes of isotonic phosphate buffer (pH 7.4). One mL of homogenate was dialysed against an equal volume of isotonic phosphate buffer containing the equivalent of 20 mg/L (±)-etodolac. Drug losses were less than 10%, and no pertinent volume shifts were seen, during the dialysis procedure.

2.5.3. Binding studies in human fluids

2.5.3.1. Plasma and Synovial Fluid

Due to inadequate assay-sensitivity and the possibility of hydrolysis of conjugated etodolac during dialysis, it was not possible to determine the protein binding of actual patient samples. Therefore, the binding studies were conducted using spiked samples. Pooled specimens were obtained from young (44±12 y) rheumatoid arthritis patients (plasma, n=8; synovial fluid, n=4 subjects); these subjects had not taken an NSAID within the 24 h prior to sample collection. The buffer was spiked with racemic etodolac to provide for enantiomer concentrations of 5 or 50 mg/L.

The binding of etodolac was also studied in the plasma from healthy subjects (<45 y). The buffer was spiked with etodolac to provide for racemic concentrations of 20 mg/L.

The pH of both the plasma and synovial fluid before dialysis was >8.1. In order to achieve a post-dialysis pH of 7.4 to 7.5, the specimens had to be adjusted to pH 7.35 before dialysis with 1 M HCl. If left unadjusted for pH, the post-dialysis pH was 8.0-8.1.

In a competitive binding study (n=4 cells), plasma was dialysed *vs* synovial

fluid. The time required to achieve equilibrium was > 19 h if the drug was initially added to only one side. To reduce the equilibration time to 4 h, 65% and 35% of the drug was added to the plasma and synovial fluid sides, respectively. This allocation of drug was in accordance with the literature values for relative concentrations of albumin known to be in each fluid (McCarty 1989).

The volume shift was assessed by the Lowry method (Lowry *et al.* 1951) for determination of total protein concentration before and after dialysis.

2.5.3.2. Hyaluronic acid

Binding of etodolac to hyaluronic acid was assessed by dialysing one mL of a 3 mg/mL solution of hyaluronic acid (source, human umbilical cord) in isotonic Sorensen's phosphate buffer *vs* one mL of drug-spiked buffer for 4 h. The concentrations of (±)etodolac initially in the buffer side were 1 and 20 mg/L.

2.6. In vitro metabolism

2.6.1. Rat studies

2.6.1.1. Preparation of microsomes

Freshly obtained rat liver, kidney, lung, and jejunum were excised and immediately placed in ice-cold 1.15% KCl. Individual tissues from 4 rats were pooled (wt = 350-450 g). Tissues were homogenized in ice-cold 100 mM phosphate buffer (pH 7.4) containing 250 mM sucrose. Homogenates were centrifuged in a Beckman model L855 centrifuge (Beckman, Palo Alto, CA) at 10,000 *g* for 20 min. The

supernatant was then subjected to centrifugation at 105,000 *g* for 60 min. The resultant pellet was suspended in phosphate-sucrose buffer and again centrifuged at 105,000 *g* for 60 min. The final pellet was resuspended in 2 volumes of phosphate-sucrose buffer. Protein concentration was determined by the Lowry method (Lowry *et al.* 1951).

2.6.1.2. In vitro acyl-glucuronidation

The net acyl-glucuronidation of etodolac was determined in triplicate at racemic concentrations of 0.0017, 0.0087, 0.017, 0.085, 0.18, 0.44, and 0.65 mM; these concentrations encompass the range expected in plasma after *iv* doses of 2.5 mg enantiomer/kg. Reaction mixtures contained 15 mM uridine 5'diphosphoglucuronic acid (UDPGA), 5 mM $MgCl_2$, and 0.05% Triton X-100, in 100 mM phosphate/sucrose buffer (pH 7.4). Reactions were started by the addition of 1.1-1.2 mg of liver microsomal protein; total incubation volume was 1 mL. Incubations were run for 20 min at 37°. At the end of each incubation, aliquots (30-400 μL) were transferred to tubes containing either 150 μL 0.6N H_2SO_4 or 100 μL 2N NaOH. After 60 s, 300 μL of 0.6 N H_2SO_4 was added to the tubes containing NaOH. The difference between the acidified and basified/acidified tubes was taken as representing etodolac which had been metabolized to glucuronide conjugates.

2.6.1.3. In vitro microsomal oxidation

Microsomal oxidation reactions were carried out using freshly prepared kidney

and liver microsomes. Reaction mixtures contained 5 mM $MgCl_2$, 10 mM glucose 6-phosphate, 0.75 mM $NADP^+$, 2 U glucose 6-phosphate dehydrogenase, and either 4 mg of kidney or 8 mg of liver microsomes, in 0.05 M Tris-HCl buffer (pH 7.4). The total volume of the incubation mixture was 3 mL. Samples were spiked with 2 mg/L (7.0 μM) racemic etodolac, and incubated for 4 h at 37°. The reduction in the concentration of etodolac enantiomer following incubation with microsomes was determined; the rate of formation of oxidized metabolites was not studied, due to lack of purified metabolites.

2.6.1.4. Effects of phenobarbital on in vitro metabolism

Eight rats were given *ip* doses of either PEG-400 (n=4), or phenobarbital in PEG 400 (75 mg/kg/day; n=4). Rats weighed between 283-383 g. After three days, the animals were anethesitized with diethyl ether, and the livers were excised and immediately placed in ice-cold 1.15% KCl. The microsomal protein was then isolated as described above, and the protein concentration was determined by the Lowry method (Lowry *et al.* 1951).

The UDPGA-dependent net formation of glucuronidated etodolac enantiomers was studied in each liver sample at enantiomer concentrations of 0.016, 0.080, 0.16, 0.40, 0.60, and 0.8 mM. Reaction mixtures contained 12.7 mM UDPGA and 5 mM $MgCl_2$ in 100 mM phosphate/sucrose buffer (pH 7.4). Reactions were started by the addition of 1.1-1.51 mg of liver microsomal protein; total volume of each incubation was 1 mL. Incubations were run for 20 min at 37°C.

Over the 20 min incubation period there was no spontaneous hydrolysis of the glucuronide conjugates in the reaction mixture; this was confirmed by adding 75 μL of human urine containing glucuronidated etodolac to the reaction mixture in the absence of microsomes. The concentrations of *S*- and *R*-etodolac in the incubation mixtures were 21.3 and 13.2 mg of conjugated etodolac equivalents. This experiment was repeated in the presence of microsomal protein with or without UDPGA, to assess the presence of enzymatic hydrolysis of the acyl-glucuronides.

At the end of each incubation, aliquots (30-400 μL) were transferred to tubes containing either 150 μL 0.6N H_2SO_4 or 100 μL 2N NaOH. After 60 s 300 μL of 0.6 N H_2SO_4 was added to the tubes containing NaOH. The difference between the acidified and basified/acidified tubes was taken as representing etodolac which had been metabolized to acyl-glucuronide conjugates.

Microsomal oxidation was studied using freshly prepared liver microsomes. The reaction mixtures (3 mL) contained 5 mM $MgCl_2$, 10 mM glucose 6-phosphate, 0.75 mM $NADP^+$, 2 U glucose 6-phosphate dehydrogenase, and 2.8-3.8 mg of liver microsomes, in 0.05 M Tris-HCl buffer (pH 7.4). Samples were spiked with racemic etodolac to provide enantiomer concentrations of 3.2 μM, and incubated for 4 h at 37°. The reduction in the concentration of etodolac enantiomer following incubation with microsomes was determined.

2.6.2. Glucuronidation in human synovial membrane and fluid

Synovial membrane (7 g) was obtained from a man (66 y) with rheumatoid

arthritis who had undergone synovectomy. The tissue was placed in ice cold 1.15% KCl, then homogenized in ice-cold 100 mM phosphate buffer containing 250 mM sucrose. Microsomal protein was isolated by centrifugation as described above.

Racemic etodolac (2 mg/L) was incubated with 7.5 mM UDPGA, 250 mM sucrose, 5 mM $MgCl_2$, 0.05% Triton X-100, and 1 mg microsomal protein, in 100 mM phosphate buffer. Total incubation volume was 1 mL; incubations were run for 1 h at 37°. The difference in concentration between acidified and basified samples was taken as representing conjugated etodolac.

In order to assess for glucuroconjugative activity in synovial fluid, samples of freshly obtained human synovial fluid were spiked with 10 mM of UDPGA and incubated for 4 h at 37°.

2.7. Data analysis

2.7.1. Pharmacokinetic indices

The half-lives ($t_{½}$) of the terminal portion of the log plasma concentration *vs* time curves of *R*-etodolac were measured by linear regression analysis. The terminal elimination rate constant (ß) was calculated by $0.693/t_{½}$. The areas under the plasma concentration *vs* time curves (AUC_{0-t}) of unchanged and conjugated etodolac enantiomers were determined using the linear trapezoidal rule for 0 h to the time of the last measured plasma concentration (C_{last}). The $AUC_{0-\infty}$ (AUC) was calculated by adding the AUC_{0-t} and $C_{last}/ß$. Total body clearance (CL) was determined by CL = Dose/AUC. The volume of distribution at steady-state was calculated by Vd_{ss} =

dose · AUMC/AUC², where AUMC is the area under the concentration x time *vs* time curve from 0 h to infinity. The area volume of distribution (Vd_β) was calculated by CL/β (Gibaldi & Perrier 1982).

In humans, the renal clearances (CL_R) of conjugated enantiomers were calculated by dividing the cumulative urinary excretion (ΣXu_{0-t}) by the AUC_{0-t} of conjugated enantiomer. The clearance of etodolac through conjugation was calculated using the expression $CL_c = \Sigma Xu_{0-t}$ of conjugates/AUC. Because urine and plasma were collected for less than 5-7 $t_{½}$, CL_c was corrected for the amount of conjugates remaining to be excreted by multiplying the expression by $AUC_c/AUC_{0-t,c}$, where AUC_c is the area under the concentration *vs* time curve of conjugates. This calculation assumes the renal pathway to be the main route of elimination of conjugates.

In the rat, the biliary clearance of etodolac as acyl-glucuronides (CL_b) was calculated as the cumulative amount of drug excreted in bile from time [0-t] divided by AUC_{0-t} of unchanged etodolac. The fraction of the drug excreted in bile as acyl-glucuronides which was reabsorbed (F_{ba}) was calculated by (Tse *et al.* 1982):

$$F_{ba} = (1\text{-}AUC_b/AUC)/F_b:$$

where AUC_b is the value for bile duct-cannulated rats, and F_b is the fraction of the dose excreted in bile. Correction of CL and Vd_{ss} ($CL_{(c)}$ and $Vd_{ss(c)}$) was accomplished by substitution of the expression [dose x $(1+(F_{ba} \times F_b))$] for dose in the respective calculations (Kochak *et al.* 1985).

In the interaction studies in the rat, the $AUC_{0-\infty}$, CL, and Vd_β of *S*-etodolac were not determined because of the difficulty, owing to the fluctuations in plasma

concentrations caused by enterohepatic recirculation, in estimating $t_{1/4}$. In the tissue distribution studies, the areas under the plasma and tissue drug concentration *vs* time curves from 0 to 24 h (AUC_{0-24}) were estimated using the linear trapezoidal rule.

The Vmax and Km values of the net glucuronidation of etodolac enantiomers by rat hepatic microsomes were estimated by fitting the data to the Michaelis-Menten equation, $V = (Vmax \cdot C)/(Km+C)$, using a nonlinear least squares data-fitting computer program (Yamaoka *et al.* 1981). The apparent microsomal oxidative activity was expressed as the decline in the concentration of etodolac enantiomer, per concentration of microsomal protein.

2.7.2. Statistical analysis

Statistical significance was evaluated using Student's paired or unpaired t test, by one-way ANOVA, and by Duncan's Multiple Range Test, as deemed appropriate. Linear regression analysis and Pearson's correlation coefficient was used to assess the relationship between plasma and synovial fluid concentrations of unchanged and conjugated etodolac enantiomers. The level of significance was set at $\alpha=0.05$. Unless otherwise indicated, values are expressed as mean$\pm$SD.

3. Results

3.1. Assays

The only notable difference between using *S*-(-)-α- and *R*-(+)-α-phenylethylamine as derivatizing reagents was in the elution order of the diastereomers of the etodolac enantiomers, which was reversed (e.g., *Figs.* 6 & 7). Furthermore, the chromatographic peak corresponding to the diastereomer of the *S* enantiomer, owing to its earlier elution time, was slightly sharper. This probably resulted in an improved sensitivity for the *S* enantiomer. Therefore, for most of the analyses described in this thesis, *R*-(+)-α-phenylethylamine was used.

Standard calibration curves of etodolac extracted from human bile (*Fig.* 5) yielded r^2 values of over 0.99. Chromatograms of human and rat bile samples are depicted in *Figs.* 5 and 6, respectively. The interday coefficients of variation in human plasma at 0.05 mg/L and 0.1 mg/L of *S*-etodolac were 18.7 and 15.1%, and the mean measured concentrations were 0.052 and 0.098 mg/L, respectively. The interday coefficients of variation at concentrations equal to and higher than 0.2 mg/L of both enantiomers were less than 10% in human plasma, urine, and bile. The calibration curves prepared using plasma were identical to those of synovial fluid. Excellent linearity ($r^2 > 0.995$) was observed between the peak area ratios (drug:internal standard) in plasma and synovial fluid.

Calibration curves prepared using rat plasma (0.1 mL), urine (0.5 mL), and bile (0.1 mL), were identical to those of human specimens. No peaks were seen in the

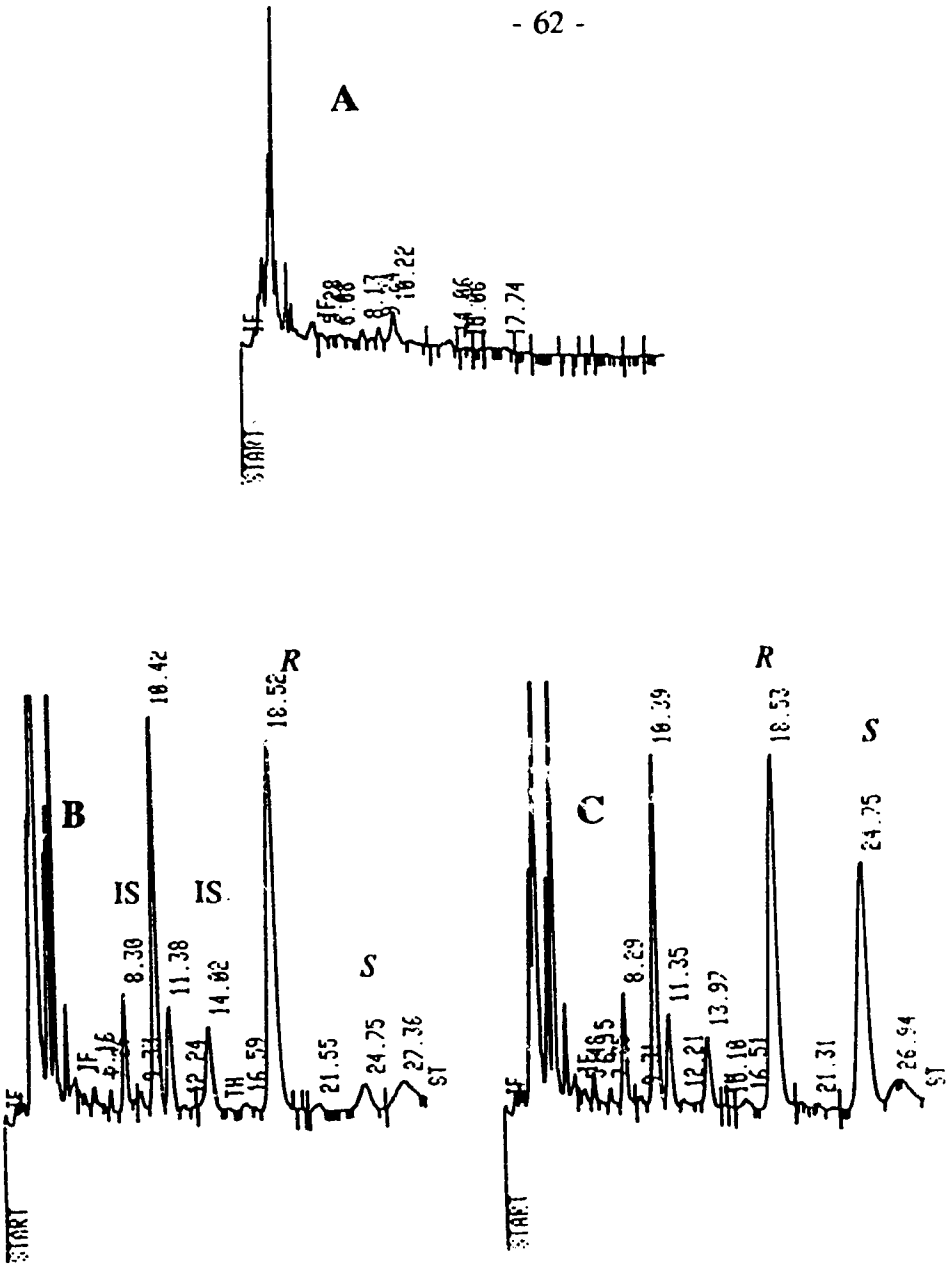


Figure 4 Chromatograms of etodolac enantiomers in synovial fluid. Key: A, blank synovial fluid; B, synovial fluid from a patient 12 h after 200 mg (±)-etodolac; C, same as B, except alkali hydrolysed. Samples were derivatized using *S*-(-)-α-phenylethylamine.

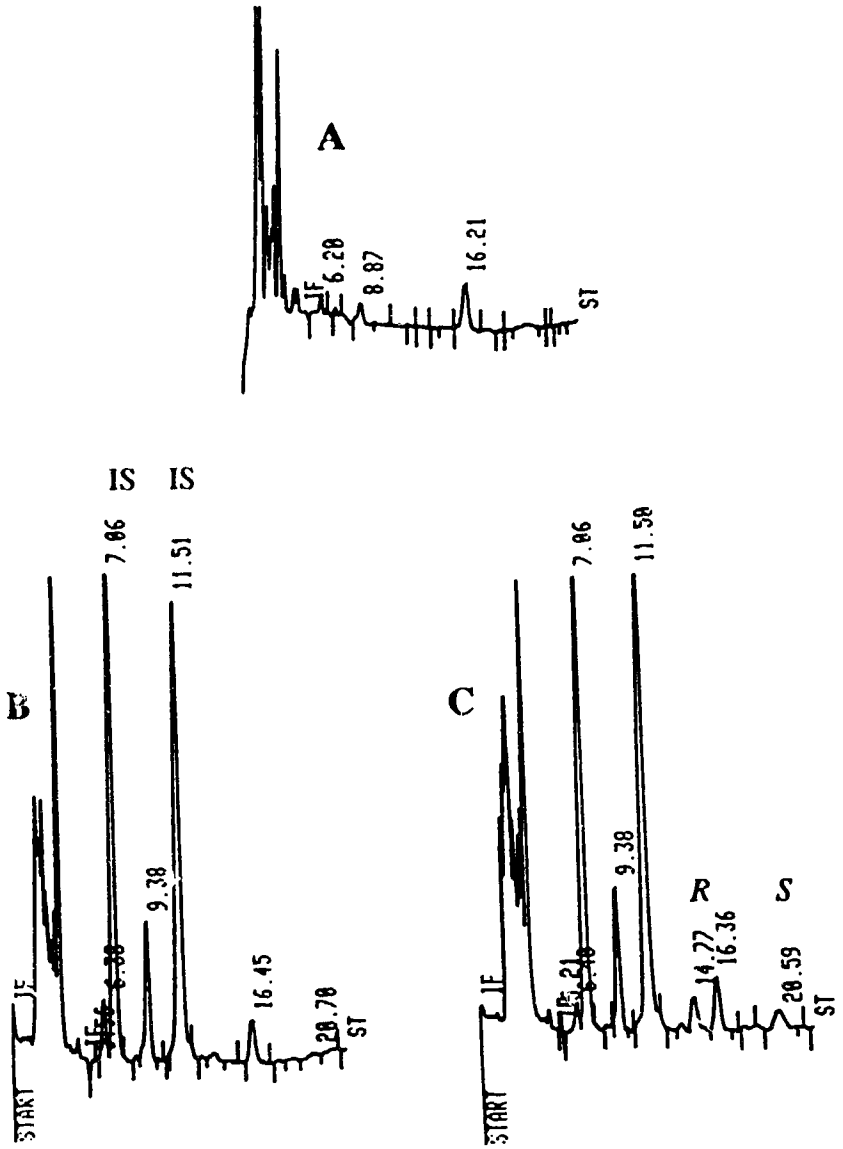


Figure 5 Chromatograms of etodolac enantiomers in human bile collected from a cholecystectomy patient , 0 h, or 10-12 h, after a single oral 200 mg dose of (±)-etodolac. Key: A, 0 h bile sample; B, 10-12 h cumulative bile sample; C, 10-12 h cumulative bile sample, alkali hydrolysed. Samples were derivatized using *S*-(-)-α-phenylethylamine.

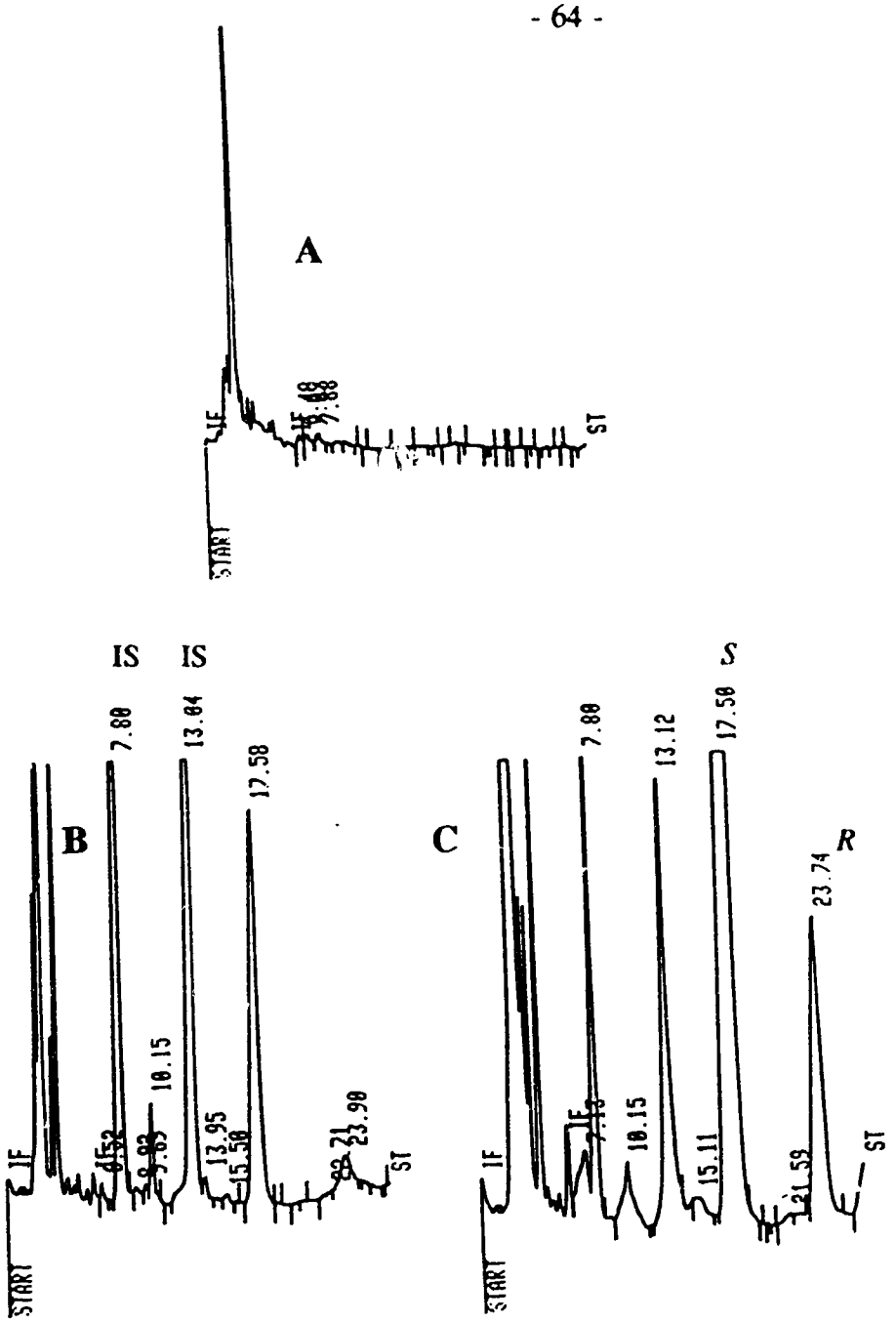


Figure 6 Chromatograms of etodolac enantiomers in the bile of a rat given 5 mg/kg (±)-etodolac. Key: A, pre-dose rat bile; B, 0-2 h post-dose rat bile; C, 0-2 h bile sample, alkali hydrolysed. Samples were derivatized using *R*-(+)-α-phenylethylamine.

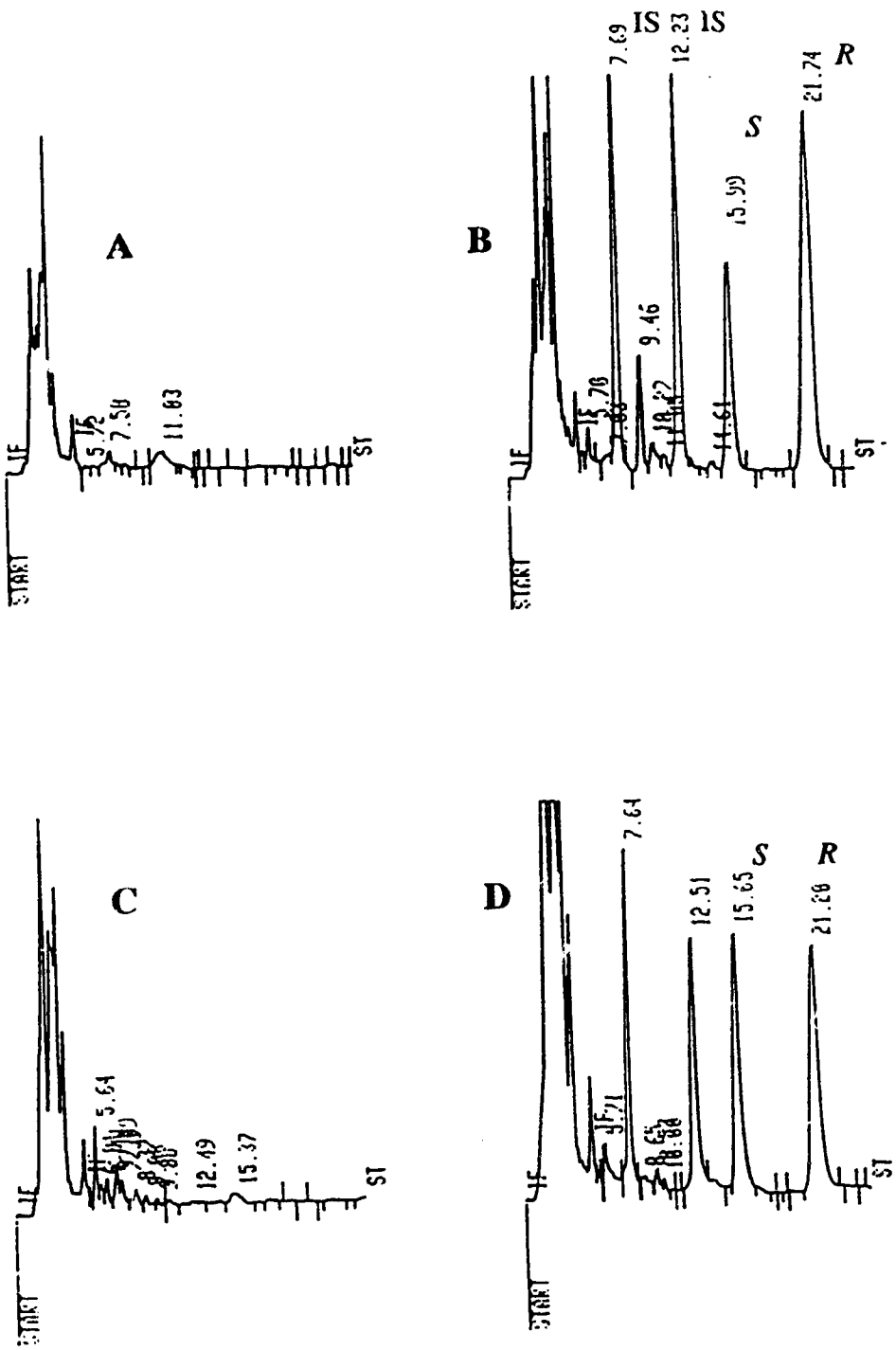


Figure 7 Chromatograms of rat specimens, before, and after 10 mg/kg iv doses of (±)-etodolac. Key: A, pre-dose rat plasma; B, 24 h post-dose rat plasma; C, pre-dose rat heart; D, 24 h post-dose rat heart. Samples derivatized using *R*-(+)-α-phenylethylamine.

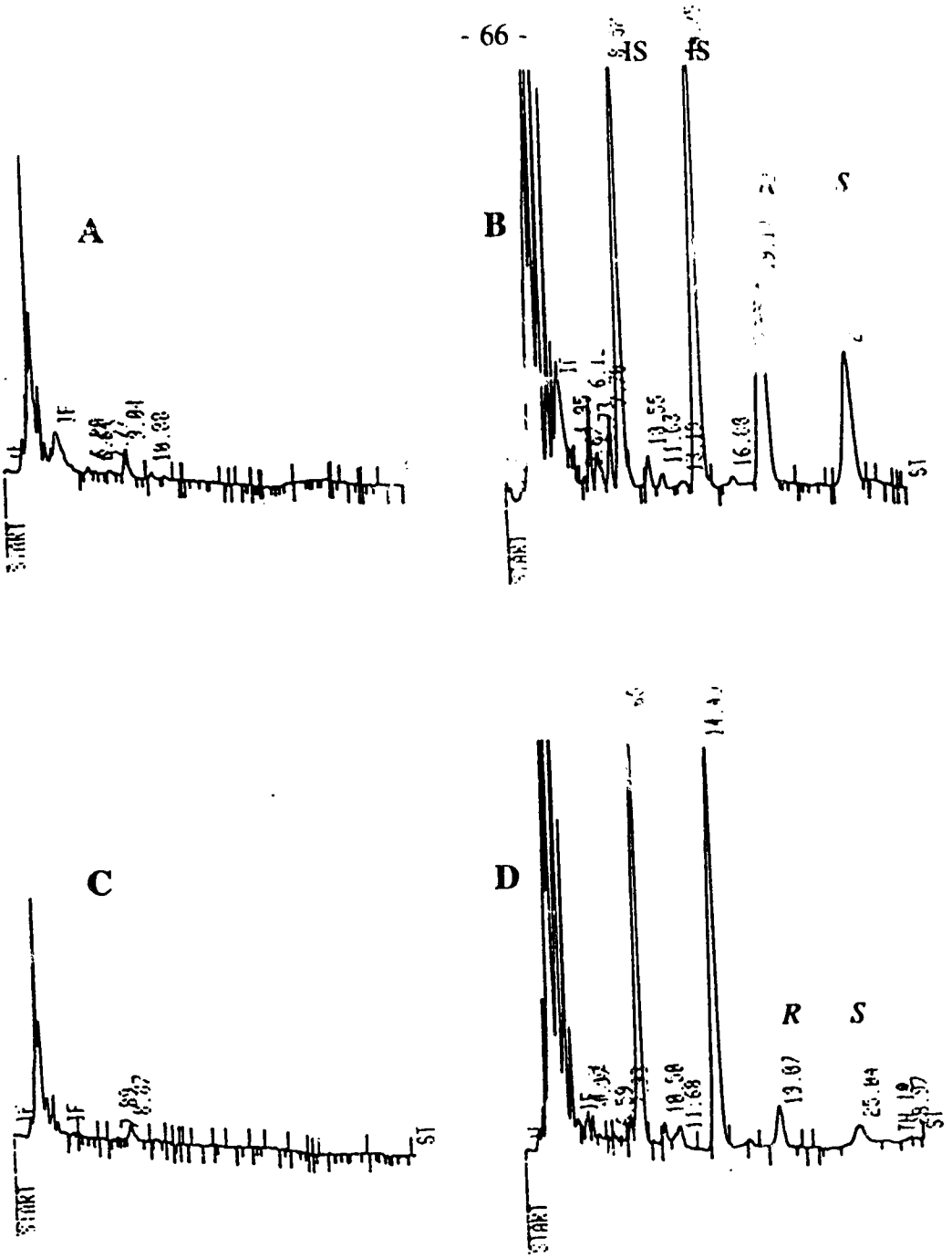


Figure 8 Chromatograms of etodolac enantiomers in rat tissues, before and after 10 mg/kg iv doses of (±)-etodolac. Key: A, pre-dose rat kidney; B, 24 h post-dose rat kidney; C, pre-dose rat brain; D, 24 h post-dose rat brain. Samples derivatized using *S*-(-)-α-phenylethylamine.

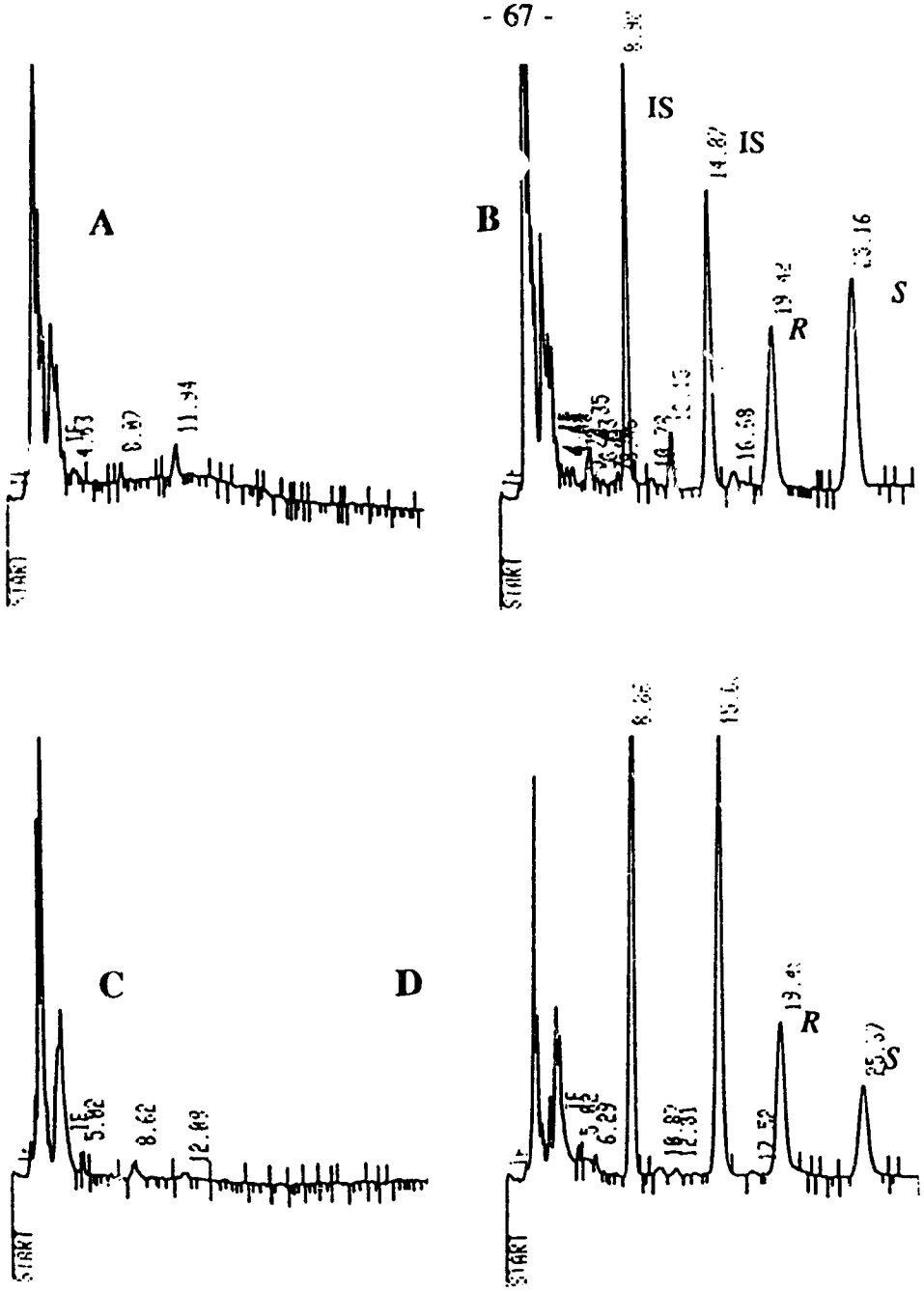


Figure 9 Chromatograms of rat specimens, before, and after 10 mg/kg iv doses of (±)-etodolac. Key: A, pre-dose rat liver; B, 24 h post-dose rat liver; C, pre-dose rat perinephric fat; D, 24 h post-dose rat perinephric fat. Samples derivatized using *S*-(-)-α-phenylethylamine.

blank rat plasma (*Fig.* 7), bile (*Fig.* 6) and urine samples which could interfere with etodolac or the internal standard. Excellent linearity ($r^2 > 0.993$) was observed between the peak area ratios (drug:internal standard) and corresponding plasma, bile, and urine concentrations of etodolac.

Calibration curves prepared using rat plasma and tissues yielded excellent linearity ($r^2 > 0.99$) between the peak area ratios (drug:internal standard) and corresponding rat plasma and tissue concentrations of etodolac. The intra-day coefficients of variation at 1 μg/g of tissue ranged from 3.9-10.2%; for brain and fat, where lower concentrations were observed *in vivo*, at 0.25 μg/g of tissue the coefficients of variation were 8.1 and 5.8%, respectively. No interfering peaks were present in the chromatograms of the derivatized tissue extracts (*Figs.* 7-9).

3.2. Studies in the rat

3.2.1. Pharmacokinetic studies

Plasma concentration-time profiles (*Figs.* 10 and 11) indicate that marked qualitative and quantitative differences exist between *S*- and *R*-etodolac. Concentrations of inactive *R*-etodolac were much greater than those of *S*-etodolac (Tables VII, VIII, IX). In all rats given racemate or *S*-etodolac, plasma concentrations of *S*-etodolac fell for 3-4 h, at which time a secondary peak developed between 3-6 h post-dose (*Fig.* 10). This was followed by a slow terminal elimination phase, which was interrupted by sporadic increases in plasma concentrations. These pronounced fluctuations were not seen in bile duct-cannulated rats (*Fig.* 11). Indeed, when

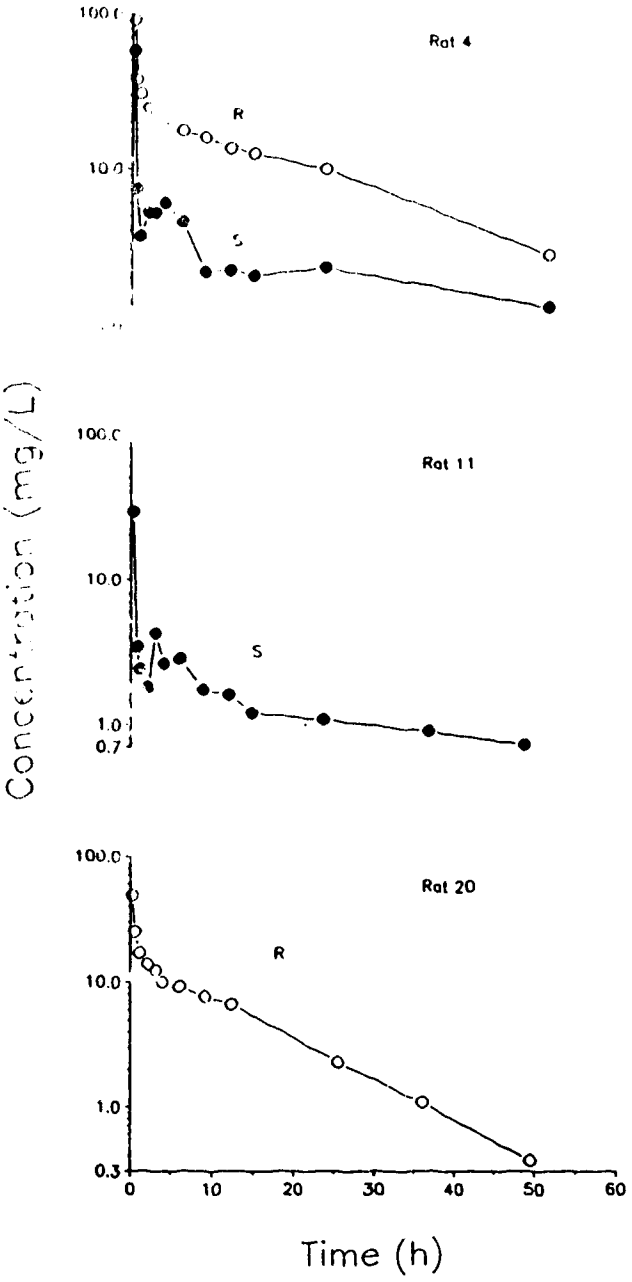


Figure 10 Plasma concentration-time profiles (see Table VII) of representative rats given etodolac enantiomers *iv* as 5 mg/kg racemate (rat 4), or 2.5 mg/kg of *S*- (rat 11) or *R*-etodolac (rat 20).

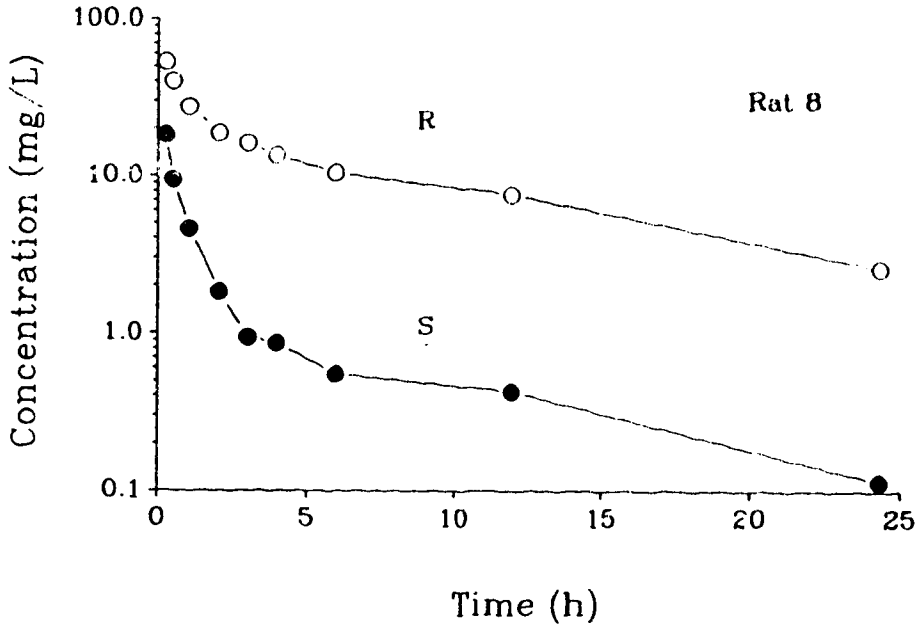


Figure 11 Plasma concentration-time profile of rat 8 (bile duct-cannulated; Table VIII) given *iv* racemic etodolac (5 mg/kg).

Table VII: Pharmacokinetic parameters of etodolac enantiomers after administration of 5 mg/kg racemic etodolac *iv* to rats.

	$t_{1/2}$[a], h		AUC, mg • h/L				CL[a], mL/min/kg				Vd_{ss}[a], L/kg			
			0-24 h		0-∞		CL		$CL_{(c)}$[b]		Vd_{ss}		$Vd_{ss(c)}$[b]	
	S	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*
1	c	17.2	57	261	c	398	c	0.105	c	0.132	c	0.091	c	0.113
2	11.1	18.5	70	264	85	403	0.492	0.103	0.837	0.130	0.231	0.090	0.393	0.113
3	26.5	27.1	64	252	134	497	0.310	0.084	0.528	0.105	0.473	0.123	0.805	0.155
4	32.7	17.0	88	391	197	633	0.211	0.066	0.359	0.083	0.336	0.066	0.571	0.083
5	37.2	10.0	62	168	137	197	0.303	0.211	0.517	0.266	0.536	0.142	0.912	0.178
6	42.9	19.4	89	345	23[c]	594	0.175	0.070	0.297	0.088	0.383	0.078	0.650	0.098
Mean	**30.1**[d]	**18.[illegible]**[e]	**72**[d]	**280**[e]	**158**[d]	**454**[e]	**0.298**[d]	**0.106**[d,e]	**0.507**[d]	**0.134**[e]	**0.392**	**0.098**[e]	**0.66[illegible]**	**0.123**[e]
SD	**12.2**	**5.5**	**14**	**78**	**60**	**158**	**0.121**	**0.053**	**0.209**	**0.068**	**0.119**	**0.029**	**0.202**	**0.036**

a. Due to considerable enterohepatic recycling, this calculation provides only a rough [illegible]e of the $t_{1/2}$ of *S*-etodolac; this is also true for $AUC_{0-\infty}$, CL and Vd_{ss}, which are derived from ß.

b. CL and Vd_{ss}, corrected for enterohepatic recycling.

c. Could not be determined due to excessive fluctuation in plasma concentrations.

d. Significantly different from bile duct-cannulated rats (Table VIII).

e. Significantly different from corresponding *S* enantiomer.

Table VIII: **Pharmacokinetic parameters of etodolac enantiomers after administration of 5 mg/kg racemic etodolac *iv* to bile duct-cannulated rats.**

	$t_{½}$, h		AUC, mg·h/L				CL, mL/min/kg		Vd_{ss}, L/kg		CL_b[a] mL/min/kg	
			0-24 h		0-∞							
Rat	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*
7	6.62	5.28	21.3	186	22.6	192	1.90	0.221	0.261	0.079	2.09	0.091
8	6.56	7.98	22.4	232	23.5	262	1.80	0.162	0.482	0.075	2.01	0.021
9	9.63	9.00	20.4	207	22.3	235	1.87	0.178	0.494	0.086	1.67	0.039
Mean	**7.60**	**7.42**	**21.4**	**208**[b]	**22.8**	**230**[b]	**1.86**	**0.187**[b]	**0.412**	**0.080**	**1.92**	**0.050**[b]
SD	**1.8**	**1.9**	**1.0**	**23**	**0.67**	**35**	**0.051**	**0.031**	**0.13**	**0.006**	**0.22**	**0.036**

a. Biliary clearance of (conjugated+unchanged) etodolac.
b. *Significantly different from corresponding S enantiomer.*

Table IX: Pharmacokinetic parameters of etodolac enantiomers after *iv* administration of 2.5 mg/kg of individual enantiomers to rats.

Rat	$t_{½}$[a], h	AUC, mg·h/L 0-24	AUC, mg·h/L 0-∞	CL[a], mL/min/kg CL	CL[a], mL/min/kg $CL_{(c)}$[b]	Vd_{ss}[a], L/kg Vd_{ss}	Vd_{ss}[a], L/kg $Vd_{ss(c)}$[b]
				***S*-etodolac**[c]			
10	27.1	61	135	0.310	0.531	0.614	1.05
11	46.0	53	124	0.338	0.576	0.724	1.23
12	14.8	102	156	0.267	0.455	0.252	0.43
13	23.0	60	84	0.497	0.847	0.621	1.06
14	25.4	45	76	0.551	0.937	0.637	1.08
15	d	91	d	d	d	d	d
Mean	**27.3**[e,f]	**69**[e,f]	**115**[e,f]	**0.393**[e,f]	**0.669**[e]	**0.570**[e]	**0.97**[e]
SD	**11.5**	**23**	**34**	**0.124**	**0.210**	**0.183**	**0.31**
				***R*-etodolac**[c]			
16	17.9	315	509	0.088	0.105	0.087	0.111
17	17.3	223	329	0.125	0.158	0.149	0.190
18	14.9	272	392	0.105	0.133	0.116	0.147
19	16.3	369	606	0.068	0.087	0.074	0.094
20	8.9	196	229	0.182	0.231	0.140	0.155
21	7.2	234	255	0.163	0.207	0.090	0.115
Mean	**13.8**	**268**	**387**	**0.122**	**0.154**	**0.109**	**0.135**[f]
SD	**4.6**	**64**	**147**	**0.044**	**0.057**	**0.031**	**0.035**

a. Due to considerable enterohepatic recycling, this calculation provides only a rough estimate of the $t_{½}$ of *S*-etodolac; this is also true of $AUC_{0-\infty}$, CL, and Vd_{ss}, which are derived from ß.
b. CL and Vd_{ss} corrected for enterohepatic recycling.
c. No significant differences from enantiomer when given as racemate (Table VII).
d. Could not be determined due to fluctuation in plasma concentrations.
e. Significantly different from *R*-etodolac.
f. Significantly different from bile duct-cannulated rats (Table VIII).

recirculation was prevented by cannulation of the bile duct (Tables VII & VIII), the AUC and $t_{½}$ of *S*-etodolac were significantly reduced, and CL was significantly increased. The pattern for *R*-etodolac was different, as the rapid decline in concentration was not followed by the substantial secondary peaks evident for *S*-etodolac (*Fig.* 11), and the AUC and CL were not significantly changed after bile duct-cannulation (Tables VII & VIII).

The calculated pharmacokinetic parameters further reflect the marked differences between the enantiomers. When administered separately, *S* and *R*-etodolac significantly differed in all parameters, with higher $t_{½}$, CL, Vd_{ss}, and lower ß and AUC for *S*-etodolac than *R*-etodolac (Table IX). The mean *S*:mean *R* ratio for $AUC_{0\text{-}24}$ in these rats was 0.26. Similarly, in rats dosed with racemate, significant differences between enantiomers were seen in $AUC_{0\text{-}24}$ (mean *S*:*R* ratio = 0.26±0.05). In two rats the final plasma concentrations coincided with a period of upward trend in concentration, which prevented estimation of terminal phase $t_{½}$ and derived values. Even in those rats where the final plasma concentrations did allow for an estimate of $t_{½}$, some degree of error is inherent due to enterohepatic recirculation, particularly for *S*-etodolac.

Using a non-linear least squares analysis computer program (Multi) (Yamaoka *et al.* 1981), the patterns of both enantiomers in bile duct-cannulated rats could best be described by a three-compartment open model. This was not assessed in other rats due to the fluctuations which were present in the plasma time courses.

The excreted bile contained 106.0±11% of the dose of *S*-etodolac and only

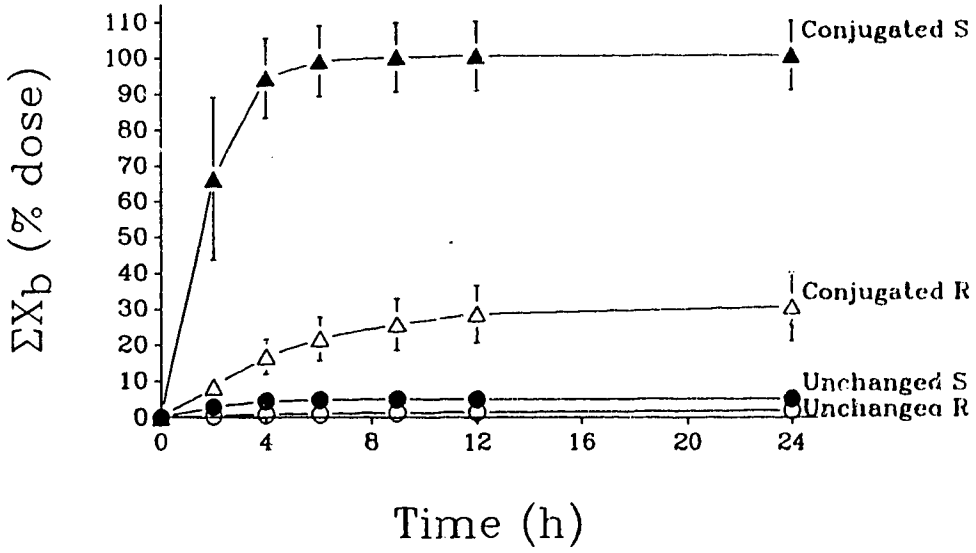


Figure 12 Mean (±SD) cumulative excretion (% dose) of etodolac in rat bile (n=3).

33.2±9.8% of the dose of *R*-etodolac (*Fig.* 12). Of this, hydrolysable conjugates accounted for 95.1±0.5 and 93.6±0.9% of the *S* and *R* enantiomers, respectively. The small amount of unchanged drug found in bile could have been generated from hydrolysis of conjugates during storage and handling. The fractions of *S* and *R*-etodolac in bile which were reabsorbed (F_{ba}) were 0.70 and 0.73, respectively. Biliary clearances (conjugated + unchanged drug) of *S*- and *R*-etodolac were 1.79±0.45 and 0.050±0.036 mL/min/kg, respectively (Table VIII). Although conjugates of etodolac were extensively excreted in bile, none could be detected in plasma. Less than 1% of the dose was found in urine, as conjugated etodolac.

With respect to plasma protein binding of etodolac (Table X), there were 3.1 and 5.1 fold greater free fractions of *S* than *R*-etodolac at low and high concentrations, respectively. In addition, for *S*, but not *R*-etodolac, the differences between the unbound fractions were significantly greater at 100 mg/L than at 20 mg/L of (±)-etodolac.

3.2.2. Tissue distribution and binding

The concentrations of both etodolac enantiomers were greater in plasma than in any of the tissues studied (*Fig.* 13, Table XI). The *S*:*R* ratio of $AUC_{0\text{-}24}$ in plasma (0.31) was similar to that seen in rats given 5 mg/kg of racemate (0.26; Table VII). In tissues, the *S*:*R* $AUC_{0\text{-}24}$ ratios were higher than in plasma, and were somewhat tissue specific (Table XI). The *S*:*R* concentration ratios in liver, kidney, heart, and fat were significantly different from those in plasma at all time points (*Fig.* 13). Brain

Table X: Etodolac protein binding in rat plasma.

Enantiomer concentration, mg/L	% Unbound *S*	% Unbound *R*	% Unbound *S*:*R*
10 (n=13)	1.16±0.44[a,b]	0.39±0.12	3.12±1.13[b]
50 (n=12)	2.22±1.04[a]	0.52±0.35	5.06±1.69

a. Significantly different from *R*-etodolac.

b. Significantly different from value at 50 mg/L.

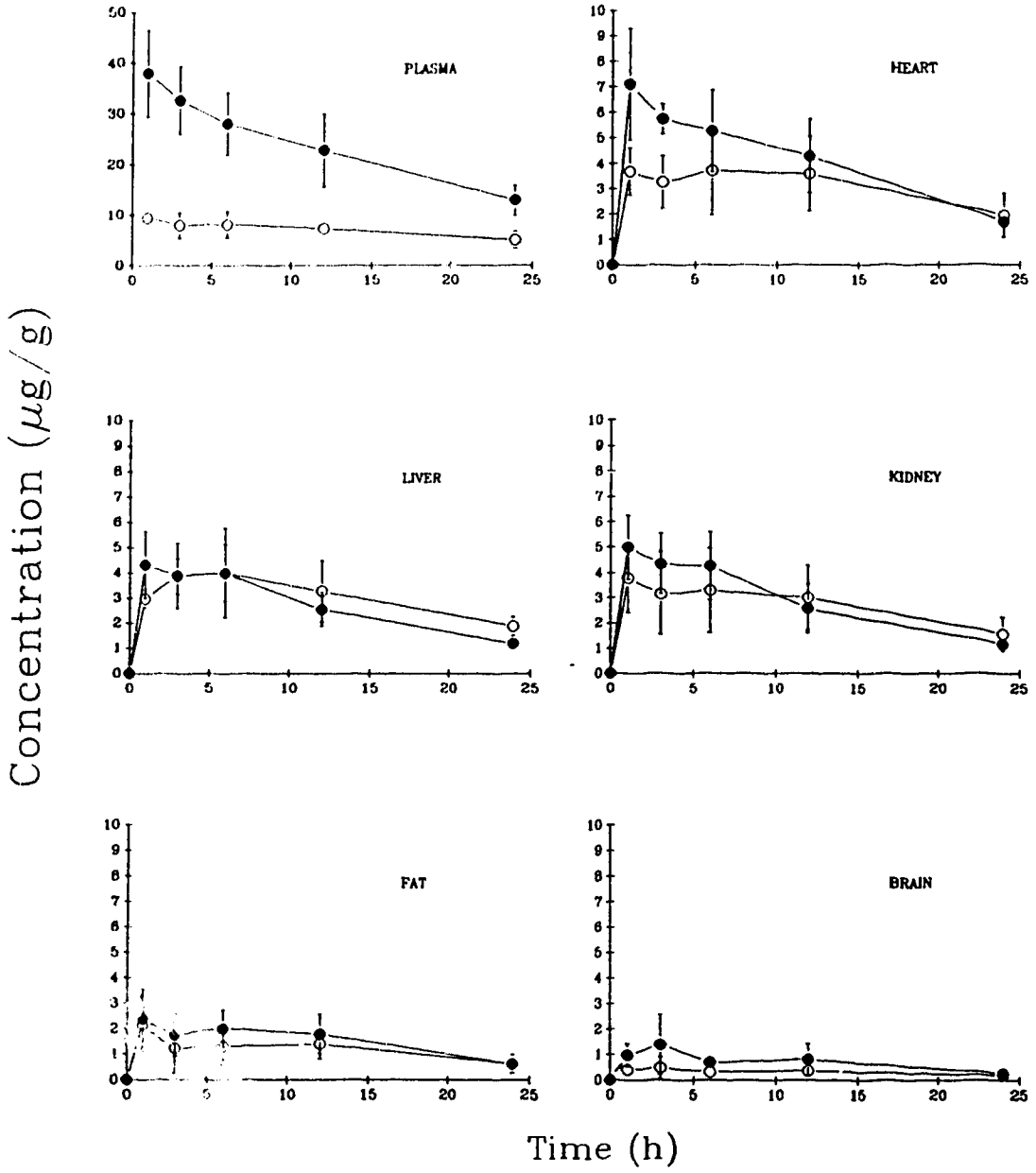


Figure 13 Total (bound + unbound) plasma and tissue concentration-time profiles of etodolac enantiomers given to rats as 10 mg racemate/kg *iv* bolus doses. Each point represents the mean±SD of 6-7 rats. Filled circles, *R*-etodolac; open circles, *S*-etodolac.

Table XI: Plasma and tissue-specific AUC_{0-24} of etodolac enantiomers following 10 mg/kg racemic bolus *iv* doses given to rats. Plasma AUC_{0-24} is expressed as μg·h/mL; tissue AUC_{0-24} as μg·h/g.

Specimen	AUC_{0-24}			Tissue:Plasma	
	S	*R*	*S*:*R*	*S*	*R*
Plasma	167	546	0.306	-	-
Liver	73.0	64.2	1.14	0.43	0.12
Kidney	65.0	67.7	1.01	0.39	0.12
Heart	74.8	97.8	0.765	0.45	0.18
Fat	28.6	36.9	0.775	0.17	0.068
Brain	7.70	17.0	0.453	0.046	0.031

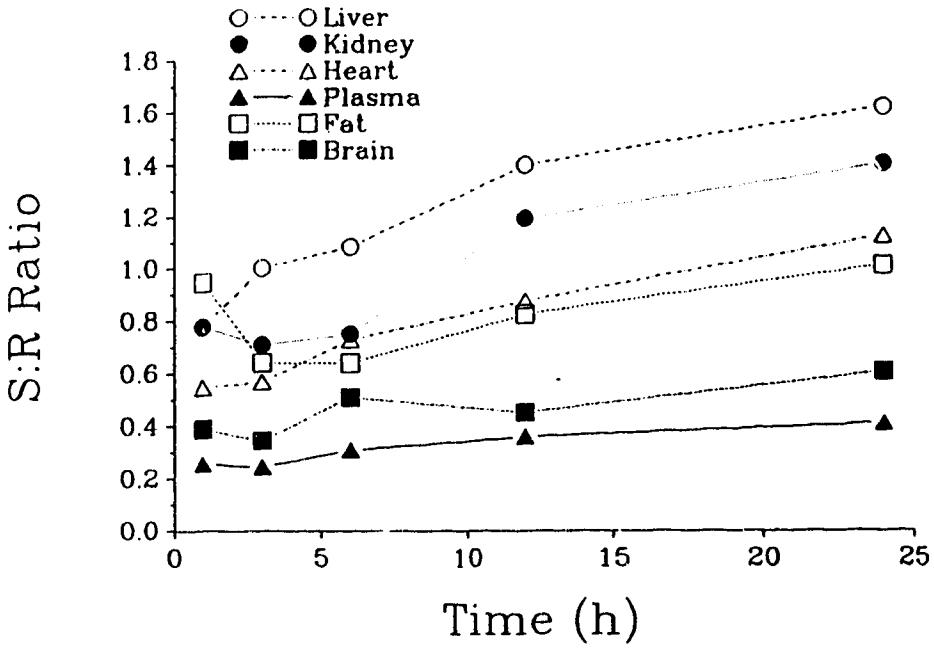


Figure 14 Relationship between the *S:R* concentration ratio of etodolac enantiomers *vs* time after a 10 mg/kg *iv* dose of (±)etodolac. Each point represents the mean of 6-7 rats.

differed, however, as only the 3 h *S*:*R* ratio was significantly different from the corresponding ratio in plasma (*Fig.* 13). For both enantiomers, concentrations were highest in heart, followed by kidney and liver, then fat and brain. The *S* enantiomer had a greater tissue distribution than *R*-etodolac relative to plasma (Table XI). With the progression of time, the *S*:*R* ratio increased in all tissues and in plasma (*Fig.* 14)

There was marked stereoselectivity reflected in the tissue binding results (*Fig.* 15), particularly in kidney and heart tissues. Because of phase separation upon dilution and homogenization, fat tissue could not be studied. The *S*:*R* ratios for unbound enantiomer were 1.15 ± 0.09, 2.14 ± 0.26, 3.69 ± 0.23, and 1.23 ± 0.03, in brain, heart, kidney, and liver tissues, respectively. In each tissue, binding of the *R*-enantiomer was significantly greater than *S*-etodolac (*Fig.* 15). With respect to order of binding, kidney and liver bound *S*-etodolac to a significantly greater extent than did brain and heart tissue. For *R*-etodolac, the order of binding was kidney > heart and liver > brain tissue.

3.2.3. *In vitro* metabolism

3.2.3.1. Acyl-glucuronidation

Preliminary studies indicated that, at the concentration used, UDPGA was not a rate limiting factor, and that the rate of net acyl-glucuronidation was linear with respect to time and protein concentration (*Fig.* 16). Microsomal protein recovered from hepatic tissue displayed pronounced glucuronidation activity, in favour of the *S*-enantiomer (*Fig.* 17). The Vmax values obtained for each enantiomer (1.15 ± 0.27

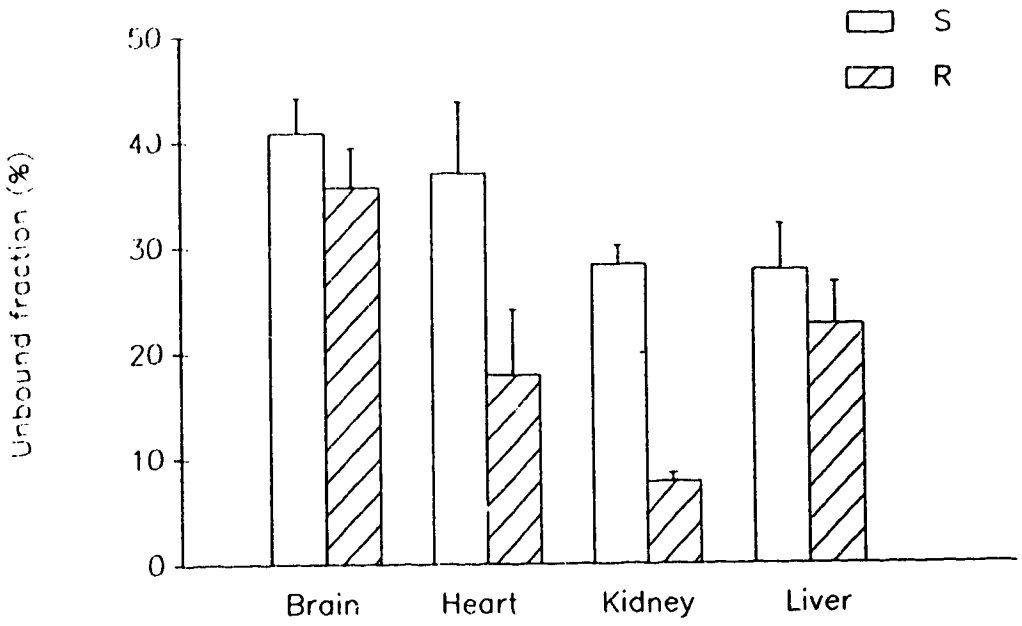


Figure 15 *In vitro* tissue binding of etodolac to drug-spiked blank rat tissues. Each bar represents the mean±SD of 5 determinations.

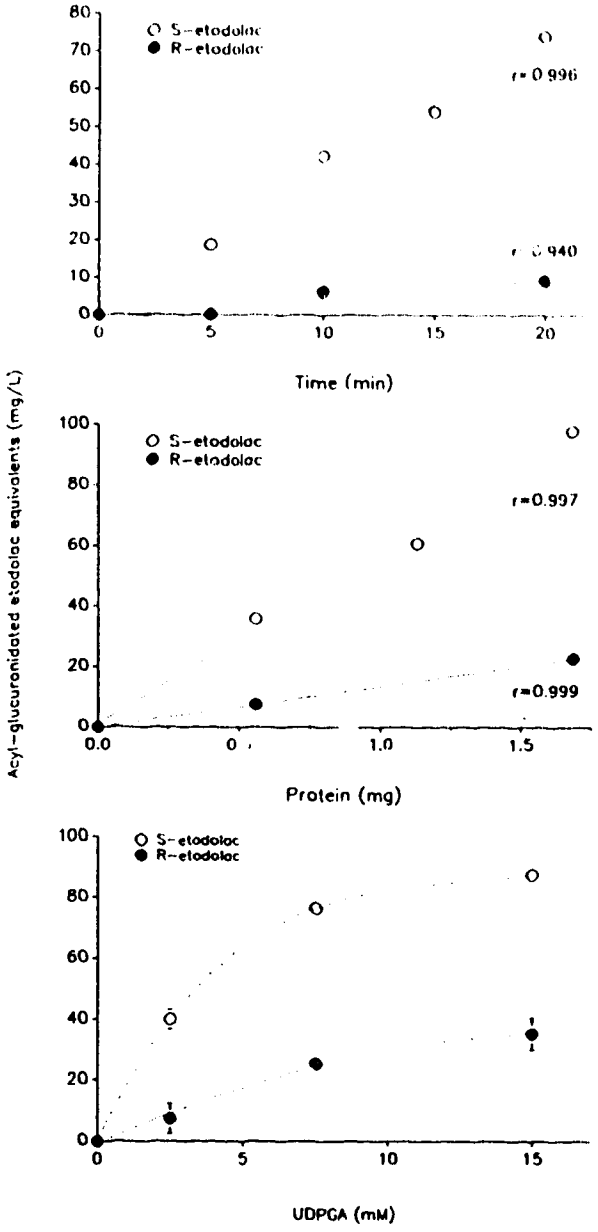


Figure 16 Relationship between *in vitro* formation of acyl-glucuronidated etodolac with time, protein and UDPGA concentration. Time and protein concentration determinations were done in duplicate in the presence of 187.5 mg/L of each enantiomer. The relationship between glucuronidation and UDPGA concentration was performed in quadruplicate using 125 mg/L of each enantiomer.

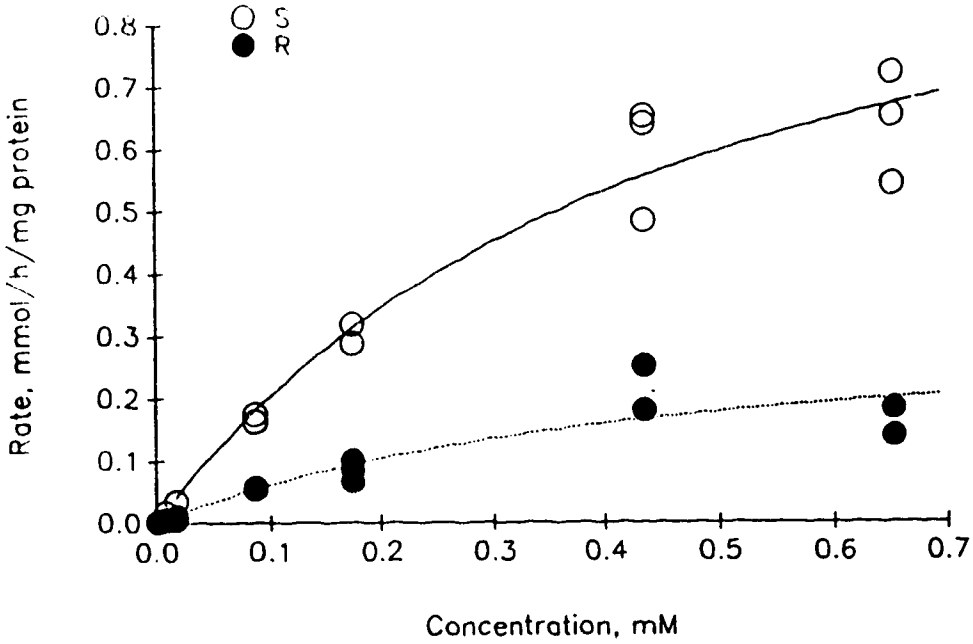


Figure 17 *In vitro* glucuronidation of etodolac enantiomers by rat liver microsomes. Lines represent the mean of the best fit lines (n=3).

and 0.337±0.11 mmol/h/mg protein, for *S*- and *R*-etodolac, respectively) differed significantly (*Fig.* 17), with *S*-etodolac having a 3.4 fold greater Vmax than *R*-etodolac. The Km values (0.47±0.11 and 0.45±0.15 mmol, for *S*- and *R*-etodolac, respectively), however, were not significantly different. No glucuronidation activity was seen in microsomes prepared from lung, jejunal, or kidney tissues, despite the positive result seen in concurrently incubated liver microsomes.

3.2.3.2. Microsomal oxidation

Microsomal oxidative activity was apparent in both liver and kidney microsomes (Table XII). Concentrations of both enantiomers were significantly reduced by liver microsomes after 4 h of incubation. Moreover, this decrease was stereoselective, based on the significantly greater *S*:*R* ratio of etodolac enantiomer concentrations in liver microsomal incubations. In kidney microsomes, the concentrations of *R*-etodolac did decline, although not significantly. A significant increase, however, was observed in the *S*:*R* ratio after 4 h. No *in vitro* oxidative or glucuronidation activity was observed in microsomes obtained from lung or gut microsomes.

3.2.4. Drug interaction studies

3.2.4.1. Effect of phenobarbital on the pharmacokinetics of etodolac enantiomers

A significant reduction of 23.9% was seen in the mean $AUC_{0\text{-}24}$ of *S*-etodolac after phenobarbital (Table XIII, *Fig.* 18); after phenobarbital the AUC were significantly lower at earlier time intervals as well. Specifically, the $AUC_{0\text{-}3}$ of

Table XII: **$NADP^+$-dependent microsomal oxidation of etodolac enantiomers. Each value represents the mean (±SD) concentration (μg/mL) of 4 determinations.**

	Liver microsomes			Kidney microsomes		
Time, h	*S*	*R*	*S*:*R*	*S*	*R*	*S*:*R*
0	1.00 ±0.023	1.00±0.027	1.00±0.039	0.98±0.031	1.00±0.13	1.00±0.15
4	0.74±0.078[a]	0.48±0.044[a,b]	1.55±0.066[a]	1.06±0.069	0.79±0.031	1.34±0.10[a]

a. Significantly different from 0 h. b. Significantly different from *S* enantiomer.

Table XIII: Pharmacokinetic parameters of etodolac enantiomers in control rats, after pretreatment with 75 mg/kg/day (n=3 days) phenobarbital, single doses of 50 mg cimetidine, and single or multiple (n=4 days) doses of 120 mg/kg/day of cimetidine.

Rat	AUC_{0-24} mg·h/L		*R*-etodolac[d]			
			$AUC_{0-\infty}$ mg·h/L	$t_{1/2}$ h	CL mL/h/kg	Vd_β L/kg
	S	*R*				
Controls						
1	56.5	290	426	15.4	5.87	0.131
2	73.8	277	375	12.8	6.66	0.123
3	64.8	258	318	10.5	7.86	0.119
4	62.9	295	523	21.9	4.78	0.151
5	61.1	305	525	22.2	4.76	0.152
6	86.5	261	311	9.52	8.04	0.111
7	55.9	244	366	18.0	6.84	0.178
Mean	**66.4[a]**	**276**	**406**	**15.8**	**6.42**	**0.138**
SD	**10.5**	**22.3**	**89.1**	**5.16**	**1.32**	**0.0236**
Phenobarbital						
8	42.9	181	213	9.17	11.7	0.155
9	53.2	143	157	7.23	15.9	0.166
10	42.6	198	246	11.1	10.2	0.163
11	55.4	273	379	13.5	6.60	0.129
12	50.3	249	280	7.98	8.94	0.103
13	58.7	178	189	6.00	13.3	0.114
Mean	**50.5[a,b]**	**204[b]**	**244[b]**	**9.16[b]**	**11.1[b]**	**0.138**
SD	**6.61**	**48.5**	**78.8**	**2.75**	**3.30**	**0.0268**
Cimetidine, 50 mg/kg						
14	81.8	404	522	11.8	4.79	0.0810
15	57.0	228	259	8.21	9.66	0.114
16	67.9	310	515	18.5	4.86	0.130
17	74.1	218	283	11.7	8.88	0.149
Mean	**70.2[a]**	**290**	**395**	**12.6**	**7.02**	**0.119**
SD	**10.5**	**86.5**	**143**	**4.3**	**2.58**	**0.0288**
Cimetidine, 120 mg/kg						
18	86.6	306	586	25.8	4.27	0.159
19	65.9	299	475	18.3	5.26	0.139
20	110	545	864	17.3	2.89	0.0722
21	98.6	393	472	9.60	5.30	0.0734
22	50.3	232	294	11.3	8.52	0.138
23	46.8	249	327	12.3	7.68	0.136
Mean	**76.3[a]**	**337**	**503**	**15.8**	**5.65**	**0.119**
SD	**25.9**	**116**	**207**	**6.0**	**2.09**	**0.0372**
Cimetidine, 120 mg/kg/day x 4 days[c]						
24	105	466	605	12.3	4.13	0.0733
25	74.0	295	339	8.70	7.38	0.0926
Mean	**89.5**	**381**	**472**	**10.5**	**5.76**	**0.0830**

a. Significantly different from *R*-etodolac. b. Significantly different from control rats.
c. Due to the small numbers, statistical analysis was not performed. d. Sampling was conducted for only 24 h; consequently, the $t_{1/2}$, $AUC_{0-\infty}$, CL and Vd_β of *S*-etodolac were not calculated due to enterohepatic recirculation.

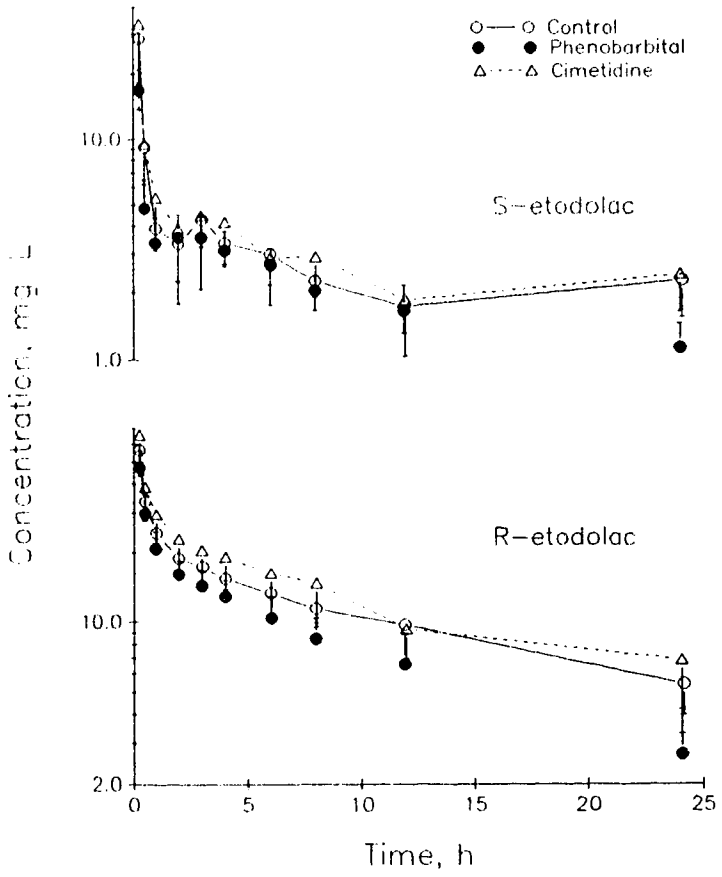


Figure 18 Plasma-concentration (mean±SD) *vs* time profiles of etodolac enantiomers in control rats, after phenobarbital (PB; 75 mg/kg/day for 3 days), and after a single dose of cimetidine (H-CIM; 120 mg/kg).

S-etodolac were 19.1 ± 3.25 and 13.9 ± 2.64 mg·L/h, and the $AUC_{0\text{-}12}$ were 42.3 ± 6.14 and 34.9 ± 5.80 mg·h/L, in the control and phenobarbital groups, respectively. The mean 24 h plasma concentration (*Fig.* 18) of *S*-etodolac in the intact phenobarbital rats (1.12 ± 0.31 mg/L) was noticeably and significantly lower than in the intact control rats (2.23 ± 0.70 mg/L).

Phenobarbital resulted in significant decreases in the $t_{½}$ (23.9%), $AUC_{0\text{-}24}$ (26.1%) and $AUC_{0\text{-}\infty}$ (40.0%), and an increase in CL (72.9%) of *R*-etodolac (Table XIII); the Vd_{β} was not influenced by phenobarbital. The $AUC_{0\text{-}3}$ of *R*-etodolac were 72.7 ± 4.5 and 62.0 ± 10.4 in the control and phenobarbital groups, respectively; this difference was significant.

The percentage of the dose of *S*-etodolac excreted in urine as glucuronide conjugates was 0.054 ± 0.048% and 2.5 ± 1.4%, in the control and the phenobarbital treated rats, respectively ($p < 0.05$). Little or no glucuronidated or unchanged *R*-etodolac was seen in the urine of either groups of rats.

After bile duct-cannulation there were no significant differences between phenobarbital and control rats (Table XIV) in the 3 h cumulative biliary recoveries of glucuronidated etodolac enantiomers. The $AUC_{0\text{-}3}$ of the enantiomers also did not significantly differ between the bile duct-cannulated phenobarbital and control rats, although there seemed to be a trend towards a lower $AUC_{0\text{-}3}$ of *S*-etodolac in the phenobarbital rats (Table XIV). Compared to the intact rats, the $AUC_{0\text{-}3}$ of *S*-etodolac were significantly reduced by 44.5 and 42.4% in the phenobarbital and control groups, respectively, after bile duct-cannulation. There were no significant differences

Table XIV: Pharmacokinetics of etodolac in bile duct-cannulated rats (n=4 each group) given 5 mg/kg racemate *iv*. Phenobarbital treated rats were given 3 daily doses of 75 mg/kg *ip* prior to study.

Group	ΣXb_{0-3}[a] % of dose		AUC_{0-3} mg·h/L		Bile flow μL/min/kg
	S	*R*	*S*	*R*	
Control	59.0±16.4[b]	7.72±2.80	11.0±5.12[b,c]	60.9±15.3	39.4±11.8
Phenobarbital	59.1±15.8[b]	11.1±3.01	6.03±3.23[b,c]	55.0±28.3	47.8±13.1

a. Cumulative biliary excretion from 0 to 3 h.
b. Significantly different from corresponding *R* enantiomer.
c. Significantly different from AUC_{0-3} of intact rats.

between the AUC_{0-3} of *R*-etodolac between intact and bile duct-cannulated rats.

3.2.4.2. Effect of phenobarbital on in vitro metabolism by liver microsomes

Between control and phenobarbital treated rats, there was no significant difference in the amount of conjugate formed at any of the concentrations studied. There were also no significant differences between the phenobarbital treated and control rats in either Vmax or Km of net glucuronidation (*Fig.* 19; Table XV). The Vmax for the net glucuronidation of *S*-etodolac was significantly higher than that of *R*-etodolac in both groups of rats.

After incubating 75 μL of human urine containing glucuronidated etodolac with the incubation mixture and microsomal protein for 20 min, 36.3 and 24.3% of the *S*- and *R*-etodolac glucuroconjugates were hydrolysed, respectively; in the presence of UDPGA, the net hydrolysis was 10.8 and 20.2% for *S*- and *R*-etodolac glucuronides, respectively.

Microsomal protein from the phenobarbital rats possessed significantly greater apparent oxidative activity than those from control rats (Table XV). The mean apparent oxidative activities of microsomes from phenobarbital treated rats were 82.9% and 44.1% greater for the *S*- and *R*-enantiomers, respectively. In both groups, microsomes had greater apparent oxidative activity in favour of the *R*-enantiomer.

3.2.4.3. Effect of cimetidine on the pharmacokinetics of etodolac enantiomers

Administration of cimetidine, either in low or higher doses, had no effect on

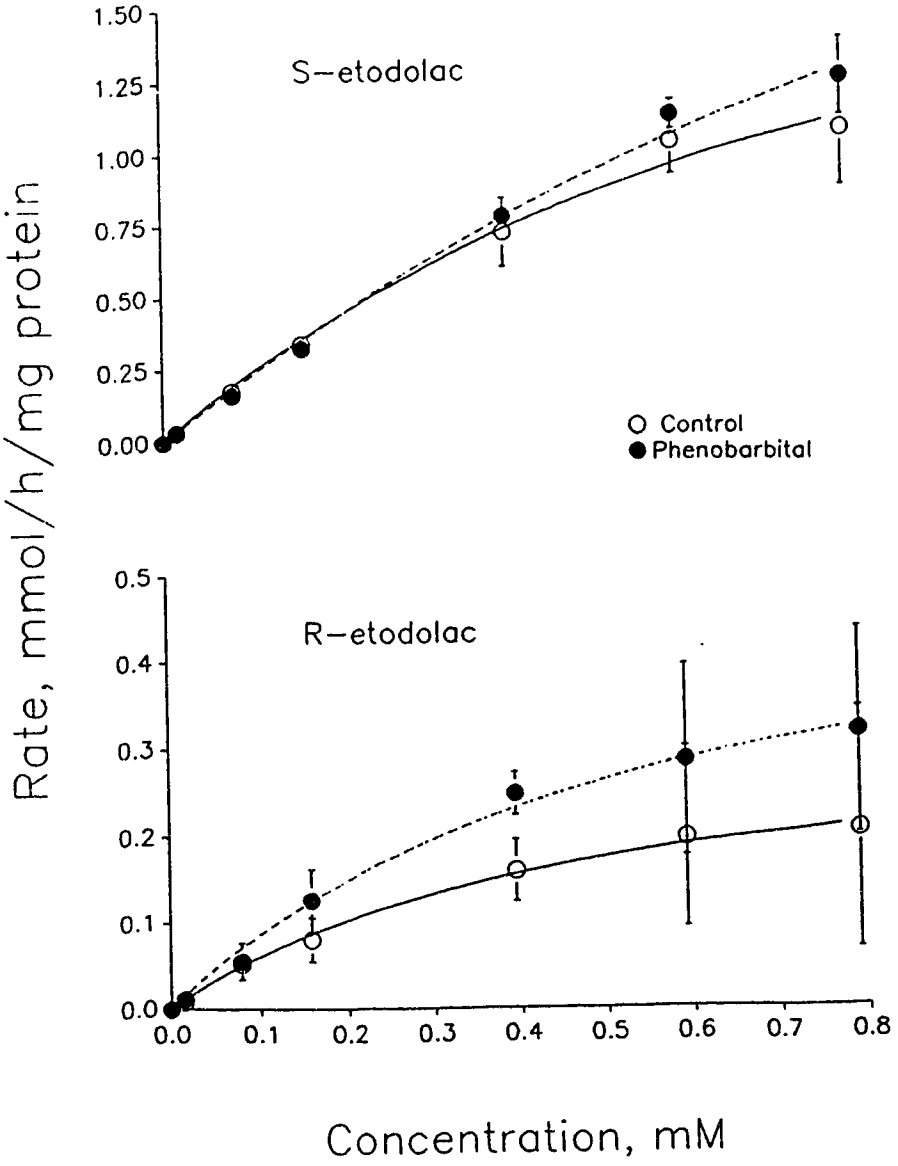


Figure 19 Rate of net glucuronidation *vs* concentration of etodolac enantiomers, *in vitro*, in control and phenobarbital treated rats.

Table XV: Apparent kinetic constants for *in vitro* net glucuronidation, and apparent *in vitro* oxidative activity of hepatic microsomes isolated from control (n=4) and phenobarbital treated (n=4) rats, for etodolac enantiomers.

Group	Net glucuronidation				Apparent oxidation	
	Vmax, mmol/h/mg protein		Km, mM		% decline over 4 h	
	S	*R*	*S*	*R*	*S*	*R*
Controls	2.5±0.68[a]	0.45±0.15	0.93±0.27	0.73±0.34	21.0±3.24[a,b]	46.0±10.2[b]
Phenobarbital	3.2±0.71[a]	0.68±0.16	1.2 ±0.35	0.87±0.55	38.4±7.52[a]	66.3±5.57

a. Significantly different from corresponding *R* enantiomer.
b. Significantly different from phenobarbital treated rats

the pharmacokinetics of the etodolac enantiomers (*Fig.* 18; Table XIII). In the two rats given multiple doses of cimetidine the pharmacokinetic parameters were within the ranges of the rats given single doses; consequently, no further rats were studied.

Because cimetidine did not influence the pharmacokinetics of the enantiomers of etodolac, the effects of cimetidine on the *in vitro* metabolism of etodolac enantiomers, and on the biliary excretion of conjugates, were not assessed.

3.2.5. Urinary excretion of etodolac in female rats

In the three female rats given racemic etodolac, there was virtually no enantiomer excreted as unchanged drug. However, after the addition of alkali (1 N NaOH) to the urine samples, 6.32, 9.22, and 13.1% of the administered dose of *S*-etodolac was seen, as acyl-glucuronidated enantiomer. There was no measurable excretion of the *R* enantiomer as unchanged or acyl-glucuronidated drug.

3.3. Human studies

3.3.1. Pharmacokinetic studies

3.3.1.1. Young subjects

In the young subjects (*Fig.* 20 & 21; Table XVI), plasma concentrations of *S*-etodolac were several-fold lower than those of *R*-etodolac (*S*:*R* AUC = 0.09 ± 0.03). There were significant differences between enantiomers in AUC ($S<R$), C_{max} ($S<R$), CL/F ($S>R$), and Vd_β/F ($S>R$) (Table XVI). No differences were seen between enantiomers in $t_{½}$, ß, or t_{max}. The AUC_{0-t} comprised $89 \pm 7\%$ and $91 \pm 6\%$ of $AUC_{0-\infty}$,

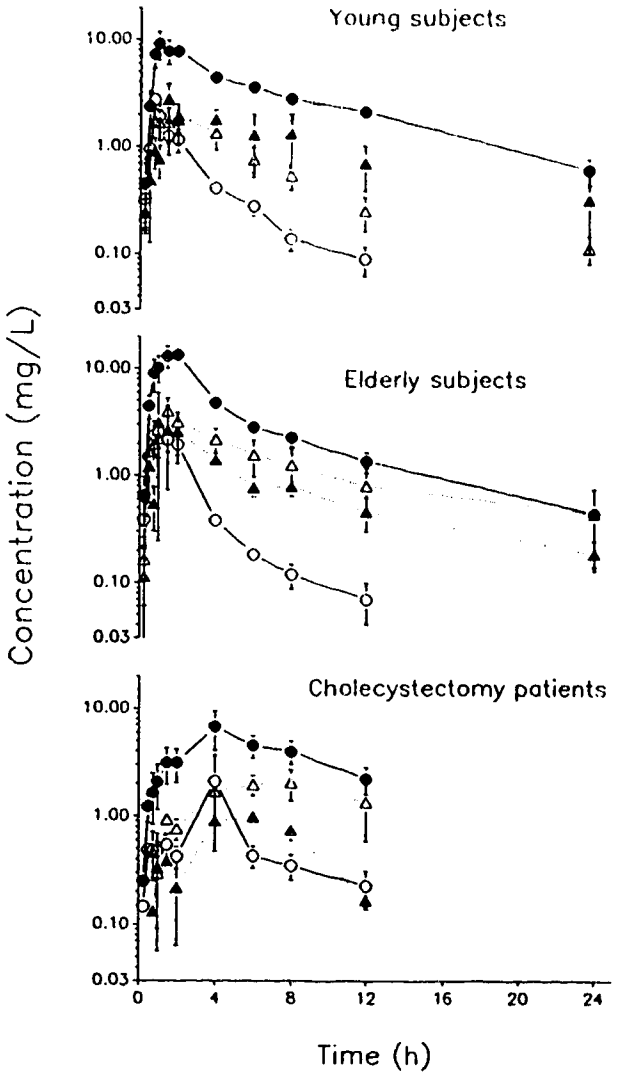


Figure 20 Mean (±SE) plasma concentrations *vs* time curves of unchanged and conjugated etodolac enantiomers after the oral administration of 200 mg of racemic etodolac to young subjects (n=6), elderly subjects (n=6), and cholecystectomy patients with T-tubes (n=3). Key: Circles, unchanged etodolac enantiomers; Triangles, acyl-glucuronidated etodolac enantiomers; Filled symbols, *R*-etodolac; Open symbols, *S*-etodolac.

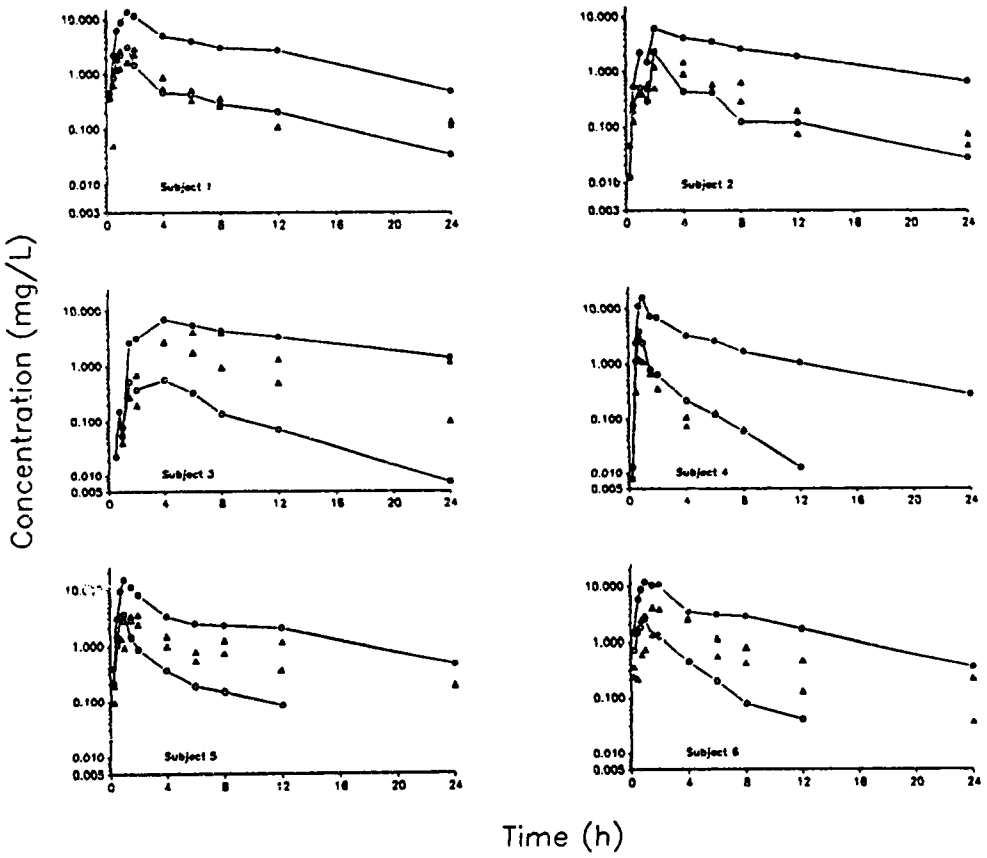


Figure 21 Plasma concentrations *vs* time curves of unchanged and conjugated etodolac enantiomers after the oral administration of 200 mg of (±)etodolac to 6 young healthy subjects. See *Fig.* 20 for key.

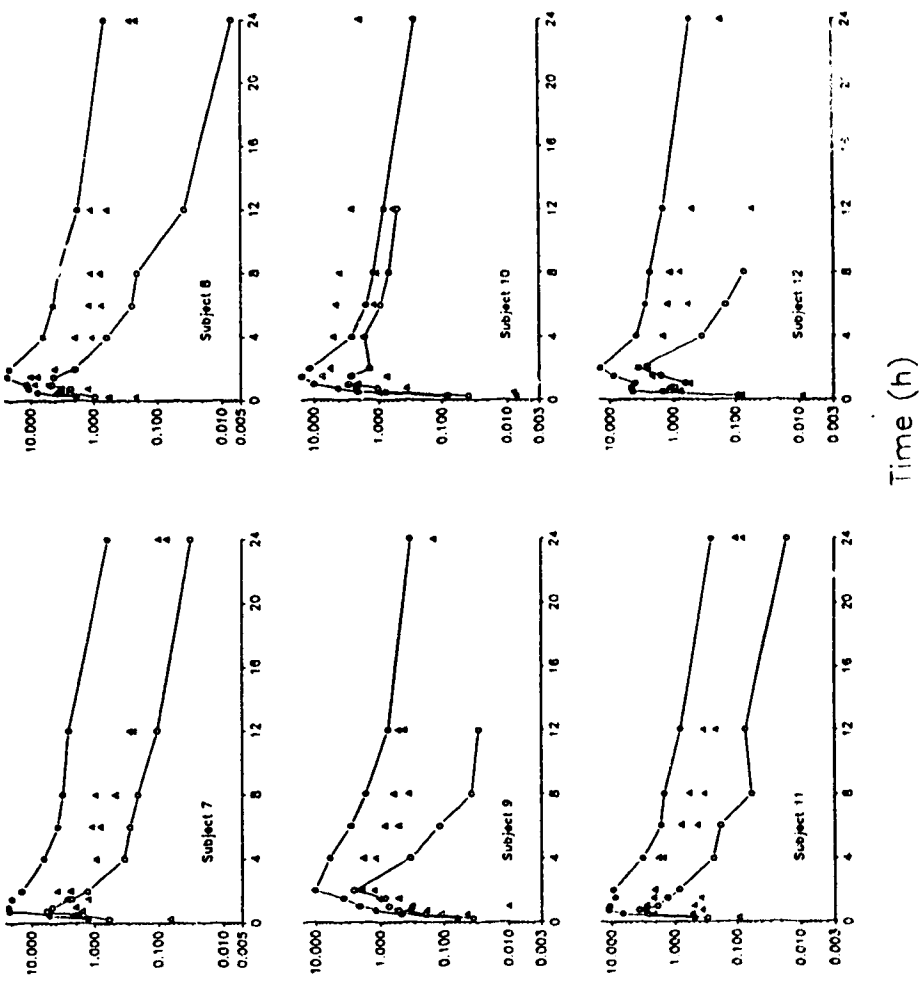


Figure 22 Plasma concentrations *vs* time curves of unchanged and conjugated etodolac enantiomers after the oral administration of 200 mg of racemic etodolac to 6 elderly healthy subjects. See *Fig.* 20 for key.

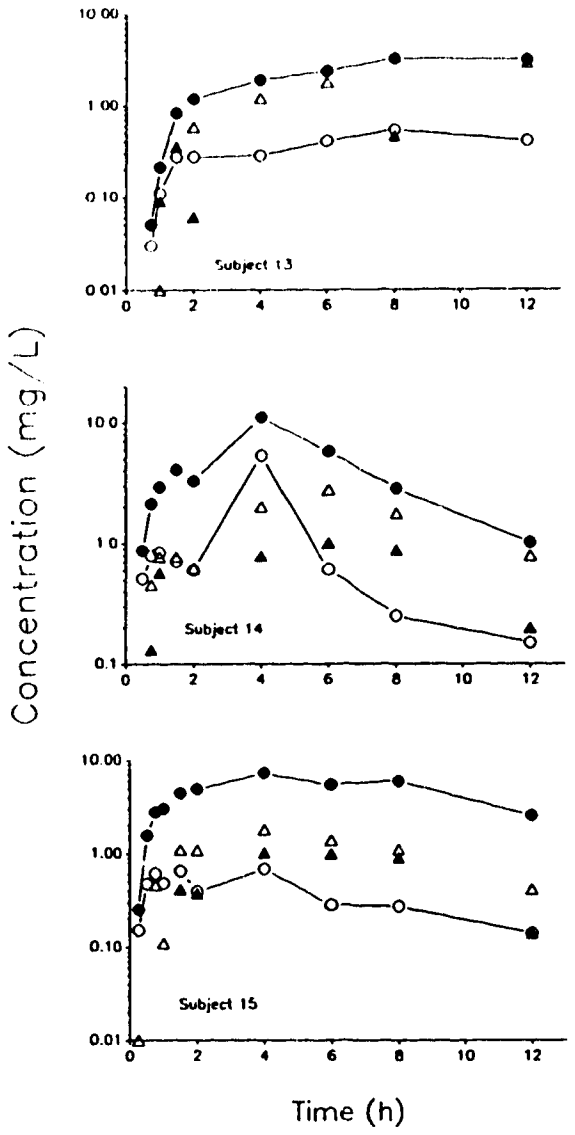


Figure 23 Plasma concentrations *vs* time curves of unchanged and conjugated etodolac enantiomers after the oral administration of 200 mg of (±)etodolac to 3 post-surgical cholecystectomy patients with biliary T-tubes. See *Fig.* 20 for key.

Table XVI: Pharmacokinetics of the enantiomers of etodolac in subjects[b] given 200 mg of the racemate orally

Subject	C_{max} mg/L		t_{max} h		AUC mg • h/L		$t_{1/2}$ h		CL/F mL/min/kg		Vd_β/F L/kg	
	S	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*
Young:												
1	3.1	13.5	1.5	1.5	9.7	83	4.7	4.8	2.2	0.25	0.89	0.11
2	2.3	6.20	2.0	2.0	6.7	60	5.6	7.9	3.1	0.35	1.5	0.23
3	0.55	7.01	4.0	4.0	3.6	99	3.8	10.0	9.4	0.34	3.1	0.29
4	3.9	16.3	0.75	1.0	4.3	51	1.9	6.2	5.1	0.42	0.84	0.23
5	3.6	15.0	0.75	1.0	6.6	65	5.5	5.7	3.8	0.39	1.8	0.19
6	2.6	11.7	1.0	1.0	6.0	67	4.1	5.3	5.0	0.46	1.8	0.21
Mean	**2.7[a]**	**11.6**	**1.7**	**1.8**	**6.2[a]**	**71**	**4.3**	**6.6**	**4.8[a]**	**0.37**	**1.6[a]**	**0.21**
SD	**1.2**	**4.18**	**1.2**	**1.2**	**2.1**	**17**	**1.4**	**1.9**	**2.5**	**0.073**	**0.83**	**0.059**
Elderly:												
7	5.7	22.2	0.8	1.0	9.7	102	6.9	5.8	3.3	0.32	2.0	0.16
8	4.6	22.4	1.0	1.5	10.5	105	4.9	8.4	2.9	0.28	1.2	0.21
9	2.5	10.3	2.0	2.0	5.5	52	9.8	10.9	5.2	0.55	4.4	0.52
10	2.8	14.7	1.0	1.5	21.0	50	7.5	7.2	0.91	0.38	0.59	0.24
11	3.6	11.3	0.8	0.8	5.8	49	5.6	6.5	3.1	0.37	1.5	0.21
12	3.6	14.4	2.0	2.0	7.3	67	1.8	7.5	2.3	0.26	0.36	0.17
Mean	**3.8[a]**	**15.9**	**1.3**	**1.5**	**10[a]**	**71**	**6.1**	**7.7**	**3.0[a]**	**0.36**	**1.7[a]**	**0.25**
SD	**1.2**	**5.26**	**0.6**	**0.5**	**5.8**	**26**	**2.7**	**1.8**	**1.4**	**0.10**	**1.5**	**0.13**
After cholecystectomy:												
13	0.53	3.2	8.0	8.0	9.7	ND	9.6	ND	2.3	ND	1.9	ND
14	5.3	11.0	4.0	4.0	15.7	55	5.4	2.3	2.0	0.55	0.9	0.11
15	0.70	7.2	4.0	4.0	5.2	59	4.3	3.3	5.0	0.36	1.9	0.10
Mean	**2.2**	**7.1**	**5.3**	**5.3**	**10**	**57**	**6.4**	**2.8**	**3.1**	**0.45**	**1.6**	**0.11**
SD	**2.7**	**3.9**	**2.3**	**2.3**	**5.3**	**2.8**	**2.8**	**0.7**	**1.6**	**0.13**	**0.6**	**0.011**

a. Significantly different from corresponding *R* enantiomer.

b. Subject characteristics are shown in Table V.

ND. Not determined due to fluctuation in terminal portion of concentration-time profile

Table XVII The pharmacokinetics of conjugated etodolac enantiomers in urine and plasma after 200 mg of racemic etodolac orally

Subject	$t_{1/2}$ h		AUC mg • h/L		CL_R mL/min/kg		ΣXu_{0-24} mg		ΣXb_{0-24}[a] mg	
	S	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*
Young:										
1	11.4	14.4	14.1	12.1	0.21	0.21	11.9	9.79		
2	18.2	10.0	7.75	10.3	0.54	0.49	17.0	22.2		
3	5.1	8.2	17.9	60.0	0.28	0.12	14.5	15.5		
4	5.0	ND	2.62	ND	1.1	ND	11.4	10.4		
5	12.3	8.1	18.4	26.9	0.17	0.09	10.4	9.49		
6	10.6	6.5	27.0	16.8	0.26	0.30	20.1	16.1		
Mean	**10**	**9.5**	**14.6**	**25.2**	**0.43**	**0.24**	**14.2**	**13.9**		
SD	**5.0**	**3.0**	**8.60**	**20.5**	**0.35**	**0.16**	**3.75**	**5.00**		
Elderly:										
7	6.0	5.4	14.4	20.6	0.36	0.19	15.6	11.4		
8	8.6	5.9	31.0	40.4	0.19	0.15	17.9	18.2		
9	7.4	4.0	16.5	9.62	0.69	1.2	35.6	33.5		
10	ND	12.3	62.0	23.4	0.058	0.042	18.8	3.9		
11	7.1	8.5	17.4	9.28	0.17	0.29	15.1	13.4		
12	7.4	ND	20.5	ND	0.17	ND	18.6	16.9		
Mean	**7.3**	**7.2**	**30.3**	**21.6**	**0.27**	**0.37**	**20.3**	**16.2**		
SD	**0.9**	**3.3**	**26.7**	**11.7**	**0.23**	**0.47**	**7.67**	**9.87**		
After cholecystectomy:										
13	18.1	5.0	40.7	5.1	0.36	1.48	48.5	17.0	0.08	0.05
14	4.4	5.0	23.0	9.4	0.20	0.23	13.1	7.00	0.28	0.34
15	4.5	6.5	15.4	8.9	0.17	0.30	9.90	12.5	1.0	6.5
Mean	**9.1**	**5.5**	**26.4**	**7.8**	**0.25**	**0.66**	**23.8**	**12.2**	**0.45**	**2.3**
SD	**7.8**	**0.9**	**13.0**	**2.4**	**0.10**	**0.68**	**21.4**	**5.01**	**0.48**	**3.6**

a. 24 h cumulative excretion in bile. ND. Not determined due to fluctuations in plasma concentrations

of the *S* and *R* enantiomers, respectively.

The absorption of both enantiomers was rapid in most subjects, t_{max} being 2 h or less, with drug being detected in plasma within 15 min (*Fig.* 21; Table XVI). However, in subject 3 there was a delayed absorption.

Only 9-22% of the dose of etodolac enantiomer was recovered as conjugated etodolac enantiomer in the urine, and there was no significant stereoselectivity in the 24 h urinary recovery (Table XVII), virtually no unchanged drug being recovered. However, at 3, 6, and 12 h, but not at 24 h, the ΣXu_{0-t} of conjugated *S*-etodolac was significantly greater than that of conjugated *R*-etodolac (*Fig.* 24). In terms of ranking, the *S*:*R* ratio of ΣXu_{0-t} was significantly highest at 3 h, followed by 6 h, then 12 and 24 h. In most subjects, conjugates of both enantiomers had a longer $t_{½}$ than the unchanged etodolac. However, only for *S*-etodolac was the mean significantly different. There was a significant difference between the conjugated enantiomers in CL_c/F, which was 0.77 ± 0.43 and 0.058 ± 0.022 mL/min/kg, for *S*- and *R*-etodolac, respectively. There was no difference between enantiomers in mean CL_R (Table XVII). The AUC_{0-t} of conjugated etodolac enantiomers comprised $86\pm6\%$ and $87\pm11\%$ of $AUC_{0-\infty}$, of the *S* and *R* conjugated enantiomers, respectively.

3.3.1.2. Elderly subjects

The pharmacokinetics of etodolac in elderly volunteers (Table XVI; Figs. 20 & 22) paralleled those in the young subjects, with no significant differences being present between the young and the elderly subjects. As in the younger volunteers, the

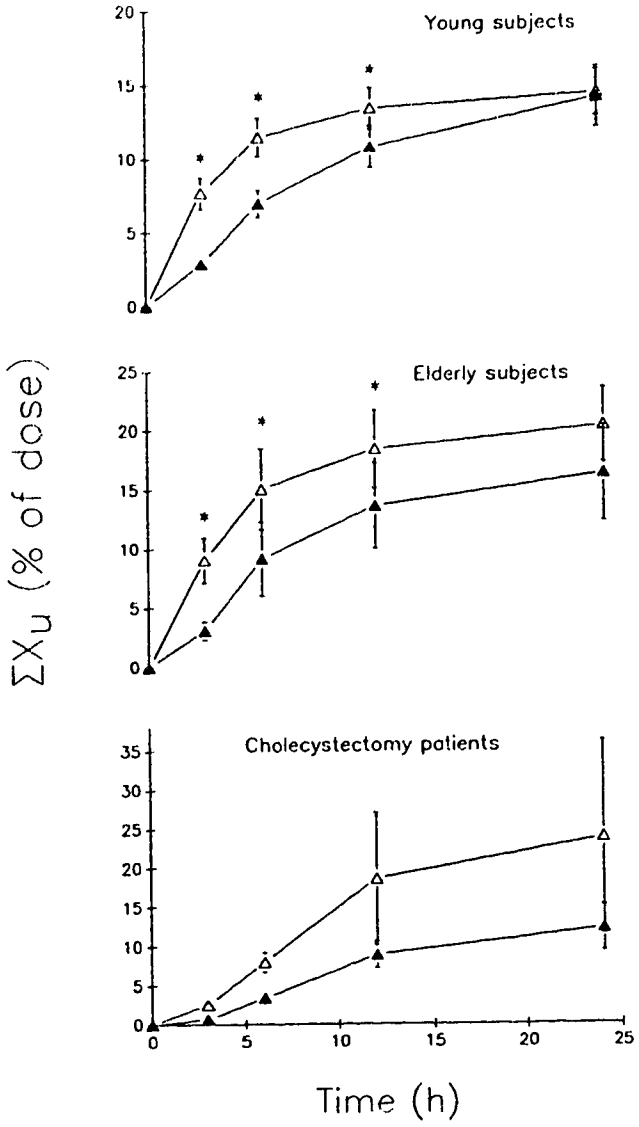


Figure 24 Mean (±SD) cumulative urinary excretion *vs* time curves of conjugated etodolac enantiomers in young subjects, elderly subjects, and cholecystectomy patients. No unchanged enantiomer was detected in the urine. The symbol (*) represents a significant difference between the concentrations of *S*-etodolac and *R*-etodolac, at that time. See *Fig.* 20 for key.

absorption of the enantiomers of etodolac was rapid, t_{max} being 2 h or less, and drug was detected in the plasma of all volunteers within 15 min. The AUC_{0-t} comprised 89±12% and 93±2% of $AUC_{0-\infty}$, of the *S* and *R* enantiomers, respectively.

The kinetics of conjugated etodolac in the elderly volunteers (Table XVII) were similar to those in the younger subjects. The CL_c/F was 0.84±0.71 and 0.092±0.076 mL/min/kg, for *S*- and *R*-etodolac, respectively ($p < 0.05$). There were no age related changes in CL_c/F.

Although renal function was good to excellent in both groups (Table V), there was a significant difference in CL_{CR} between the young and the elderly. No differences were seen between CL_R of conjugated enantiomers in the elderly volunteers (Table XVII). Also, similar to young subjects, ΣXu_{0-t} at 3, 6, and 12 h were significantly greater for *S*-etodolac than *R*-etodolac, with no difference at 24 h (*Fig.* 24). However, unlike the young subjects, the *S*:*R* ratios of ΣXu_{0-t} did not significantly differ between the collection times. This was due to the high ΣXu_{0-t} *S*:*R* ratios observed in subject 10. When this subject was excluded from the analysis, the mean *S*:*R* ratios became significantly greatest at 3 h followed by 6 h, then 12 and 24 h.

In the elderly, the AUC_{0-t} of conjugated enantiomers were 93±2% and 86±9% of the $AUC_{0-\infty}$, of the *S* and *R* conjugated enantiomers, respectively.

Although this study was not specifically designed to examine sex differences, 2 women were included in each of the elderly and young groups. In both groups, women had the lowest CL_{CR} (Table V). With respect to the AUC of the *R* enantiomer

in the women, subject 3 had the highest value in the young group, and both elderly women (subjects 7 & 8) had the highest values in their group (Table XVI). However, the women also had the lowest body weight (Table V); when normalized to body weight, no gender related differences seemed to be present in the CL/F or Vd_β/F of *R*-etodolac (Table XVI).

3.3.1.3. After cholecystectomy

Because only three subjects were studied, differences between *S*- and *R*-etodolac in cholecystectomy patients were not tested. However, there were similarities in the *S*:*R* ratios of pharmacokinetic parameters in these and other subjects, with the exception of absorption, which appeared to be delayed (*Figs.* 20 & 23; Table XVI).

Biliary excretion of the conjugated enantiomers of etodolac was a minor route of elimination in all three patients. Only in subject 15 was any notable amount of etodolac recovered in bile as conjugate (Table XVII).

Urinary excretion of conjugated drug in the cholecystectomy patients was variable. In patient 13 a high percentage of the dose of *S*-etodolac (48.5%) was recovered in the urine in 24 h; this coincided with a very high 24 h urine output (5.73 L; Table V) because of a high fluid intake (4 L). In the same subject the lowest biliary recoveries of conjugates were found (Table XVII).

3.3.2. Disposition into synovial fluid

The disposition of etodolac enantiomers in synovial fluid was stereoselective. As reflected by the *S*:*R* ratios in Table XVIII, in both synovial fluid and plasma, the concentrations of inactive *R*-etodolac greatly exceeded those of *S*-etodolac. Concentrations of *S*-etodolac were significantly greater in synovial fluid than in plasma ($p < 0.027$); no such difference was seen for *R*-etodolac. Although the synovial fluid:plasma concentration ratio for *R*-etodolac (0.91 ± 0.3) was not different from unity, it was significantly lower ($p < 0.026$) than that of *S*-etodolac (1.98 ± 0.8). There was no relationship between albumin concentration and etodolac enantiomer concentrations in either plasma or synovial fluid.

Following alkaline treatment of samples, concentrations of etodolac increased considerably, due to hydrolysis of glucuronide conjugates. Conjugated etodolac was found, not only in plasma, but also in synovial fluid (Table XVIII; *Fig.* 4). As reflected by the unchanged drug:conjugated drug ratios (Table XVIII), the relative proportion of metabolite to unchanged drug was greater for *S*- than for *R*-etodolac. No other metabolites could be detected in plasma or synovial fluid with the assay used.

Unchanged *R*- and *S*-etodolac, and conjugated *S*-etodolac, all showed excellent and significant correlations between their respective concentrations in plasma and synovial fluid (fig. 25). Conjugated *R*-etodolac, however, displayed a poor, insignificant, correlation between plasma and synovial fluid concentrations. The slopes determined between the synovial fluid and plasma concentrations of both unchanged *R*-enantiomer (0.99) and conjugated *S*-enantiomer (1.25) were close to unity.

Table XVIII: Concentrations of etodolac and conjugated etodolac in plasma and synovial fluid.

Patient	Etodolac			Conjugated etodolac-equivalents			Unchanged:Conjugated	
	S	*R*	*S*:*R* Ratio	*S*	*R*	*S*:*R* Ratio	*S*	*R*
	mg/L			mg/L				
Plasma								
1	0.19	2.9	0.066	6.7	0.20	33	0.028	14
2	0.070	1.7	0.041	3.5	2.8	1.2	0.020	0.61
3	0.70	15	0.047	5.0	4.4	1.1	0.14	3.4
4	0.19	1.7	0.11	1.8	0.0		0.11	
5	0.16	1.4	0.11	1.6	0.10	16	0.10	14
6	0.22	5.1	0.043	2.1	1.4	1.5	0.10	3.6
Mean[a]	**0.17[b,c,d]**	**2.6**	**0.075**	**3.1**	**0.90**	**13**	**0.072[b]**	**8.1**
SD	**0.058**	**1.5**	**0.034**	**2.1**	**1.2**	**14**	**0.044**	**7.0**
Synovial fluid								
1	0.36	3.0	0.12	8.3	1.9	4.5	0.043	1.6
2	0.22	2.3	0.096	2.6	0.57	4.6	0.085	4.0
3	1.7	15.0	0.11	5.3	2.8	1.9	0.31	5.4
4	0.17	1.5	0.12	1.5	0.21	7.3	0.11	6.9
5	0.34	0.81	0.43	0.94	0.36	2.6	0.37	2.3
6	0.41	3.6	0.11	1.6	0.91	1.7	0.26	3.9
Mean[a]	**0.30[b]**	**2.2[d]**	**0.17[e]**	**3.0**	**0.79**	**4.1**	**0.17[b]**	**3.7**
SD	**0.10**	**1.1**	**0.14**	**3.0**	**0.67**	**2.2**	**0.14**	**2.0**

a. Patient 3 excluded from calculation of mean concentrations, ratios, and associated statistical analysis as samples were obtained 2 h post-dose. All other patient samples were obtained at 12 h post-dose.

b. Significantly different from corresponding *R* enantiomer. c. Significantly different from synovial fluid.

d. Significantly different from corresponding conjugated enantiomer. e. Significantly different from conjugated *S*:*R* ratio in synovial fluid.

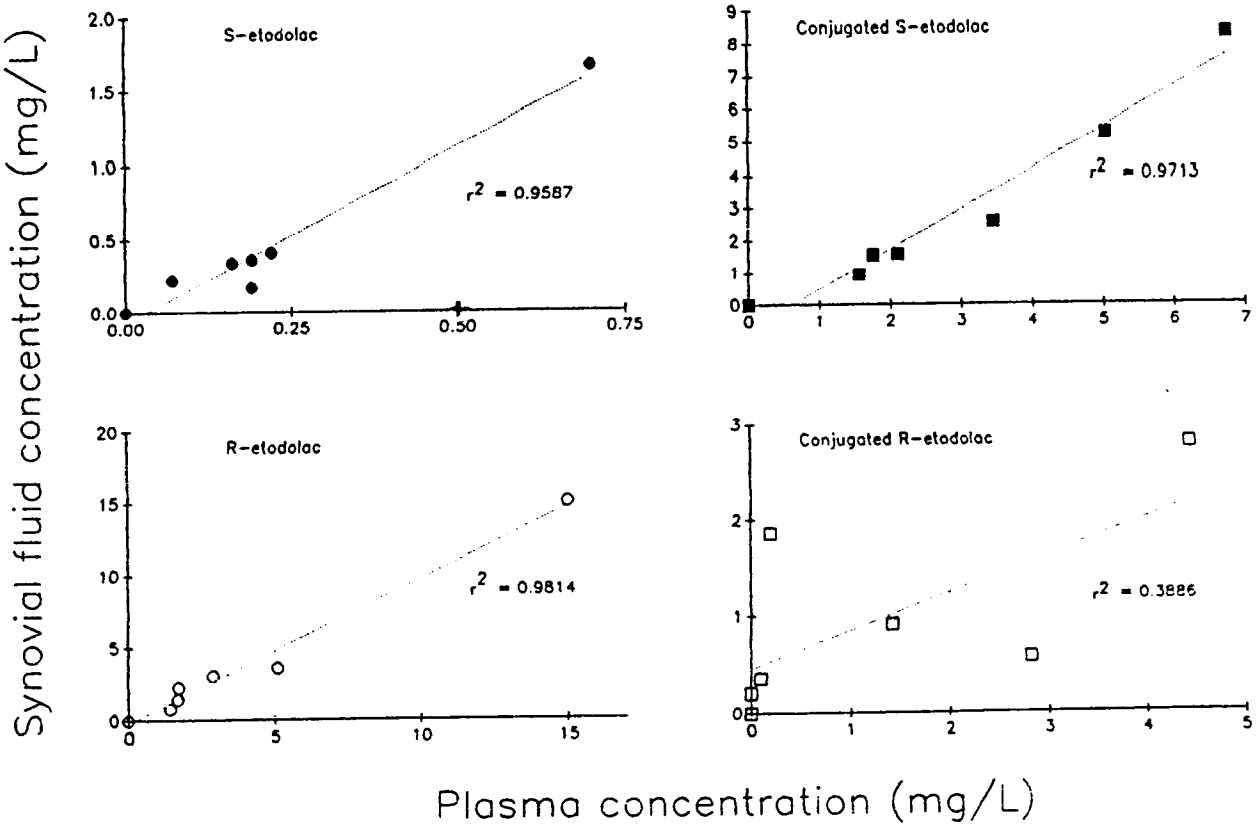


Figure 25 Relationship between concentrations of etodolac enantiomers in synovial fluid and plasma of 6 arthritic patients. Best-fit line is shown.

However, for unchanged *S*-etodolac, a slope of 2.4 was seen. When the 2 h sample from patient 3 was omitted, r^2 values became 0.67, 0.84, and 0.95, and slopes became 1.5, 0.72, and 1.26, for the *S*, *R*, and conjugated *S* enantiomers, respectively. Each of these slopes were significantly different from zero.

3.3.3. Protein binding studies

The synovial fluid:plasma ratios of mean albumin and total protein concentrations were 0.67 and 0.60, respectively. The albumin concentrations in pooled plasma and synovial fluid used for the *in vitro* protein binding experiments were 45.4 and 21.3 g/L, respectively. The total protein concentrations of the pooled blank plasma from healthy volunteers used in binding experiments was 65 g/L respectively.

In the *in vitro* binding experiments, no pertinent volume shift was noted (<5%), and no binding of etodolac to the membrane was seen. In both plasma and synovial fluid, the unbound fractions of *S*-etodolac was significantly greater than that of *R*-etodolac (Tables XIX & XX). Also, both enantiomers were bound to a greater extent by plasma than synovial fluid. Over a ten-fold range of arthritic plasma concentrations of etodolac, the unbound fraction increased 2.0 fold for *S*-etodolac, and 2.7 fold for *R*-etodolac (Table XIX).

There was a small but significantly higher unbound fraction of *S*-etodolac in pH adjusted synovial fluid (Table XX). There was no difference in the binding of the enantiomers between unadjusted plasma from arthritic (5 mg enantiomer/L; Table XIX) and healthy (10 mg enantiomer/L; Table XX) subjects. When synovial fluid was

competitively dialysed vs plasma, 49.3±2.8% of *S*-etodolac and 48.8±6.0% of *R*-etodolac were found in synovial fluid, respectively. This difference was not statistically significant.

No binding of etodolac to hyaluronic acid was seen. The ratio of etodolac in the buffer side vs that in the hyaluronic acid side was approximately one, at both 1 and 20 mg/L.

3.3.4. In vitro glururonidation activity

No glucuronidation of etodolac enantiomers was present in microsomes from synovial membrane, or in synovial fluid.

Table XIX: Percent unbound etodolac enantiomer (mean±SD) following *in vitro* equilibrium dialysis of spiked isotonic phosphate buffer vs fluids from patients with arthritis.

	Plasma		Synovial Fluid
	5 mg/L	50 mg/L	5 mg/L
S	0.723±0.074[a,b,c]	1.48±0.087[a,c]	4.83±1.7[a]
R	0.285±0.093[b,c]	0.750±0.19[c]	3.26±1.5

a. Significantly different from corresponding *R* enantiomer.
b. Significantly different from 50 mg/L in plasma.
c. Significantly different from synovial fluid.

Table XX: Percent unbound etodolac enantiomer (mean±SD) following *in vitro* equilibrium dialysis of spiked (20 mg/L racemic etodolac) isotonic phosphate buffer vs samples.

	pH adjusted		pH unadjusted	
Enantiomer	Plasma (n = 11)	Synovial fluid (n = 11)	Plasma (n = 6)	Synovial fluid (n = 5)
S	1.28±0.51[a,b]	3.85±1.7[a,c]	0.848±0.20[a,b]	1.96±0.29[a]
R	0.851±0.48[b]	2.36±1.4	0.472±0.34[b]	1.10±0.28

a. Significantly different from *R* enantiomer in same fluid
b. Significantly different from synovial fluid
c. Significantly different from unadjusted synovial fluid

4. DISCUSSION

4.1. Disposition studies in the rat

4.1.1. Pharmacokinetics of etodolac in the rat

The disposition of etodolac in the rat differs from that reported for humans in several ways. It is known that in humans etodolac is metabolized to a significant extent by phase I metabolism to oxidized metabolites (Ferdinandi *et al.* 1986). In the rat, however, >75% of the racemic dose is recovered in bile as conjugated drug. Human urinary excretion of conjugates accounts for 10-20% of elimination of etodolac (Table XVII; *Fig.* 24), while in the rat, urine is a negligible route of elimination. The elimination of conjugates in rat bile is apparently so rapid that none could be detected in plasma. Humans differ in this respect, as conjugates are found in plasma in considerable concentrations (Jamali *et al.* 1988; *Figs.* 20-23; Table XVII). Nevertheless, in both species plasma concentrations of *S*-etodolac are greatly exceeded by *R*-etodolac. This makes the rat a suitable model for study of certain aspects, such as tissue distribution and plasma protein binding, of etodolac enantiomers in humans.

The considerably greater free fraction of *S*-etodolac in plasma (Table X) probably contributes substantially to the differences between enantiomers. Furthermore, the higher unbound fraction of *S*-etodolac at 100 mg/L suggests that the binding of *S*-etodolac is saturable at higher plasma concentrations. These findings may help to explain the greater initial rapid decline in *S*-etodolac plasma concentrations (*Fig.* 10), as a greater free fraction is available for distribution to tissues and sites of

metabolism at higher plasma concentrations. The initial phase of rapid decline in the plasma time courses of *S*-etodolac is also due to its rapid rate of metabolism, as reflected in the biliary excretion of etodolac as acyl-glucuronides (*Fig.* 12) and the values for biliary clearance of etodolac as conjugates (Table VIII).

The lack of significant differences in the pharmacokinetics of enantiomers administered individually (Table IX), and as racemate (Table VII), indicates that an enantiomeric interaction, such as that reported for flurbiprofen (Berry & Jamali 1989), is absent.

The bile (*Fig.* 12) and urine analyses suggested stereoselective phase I metabolism of *R*-etodolac to the oxidized and ureide metabolites previously described in the rat (Ferdinandi *et al.* 1987), because $>65\%$ of the administered *R*-etodolac was not recovered, either as intact drug or conjugate. With the assay used here, we were unable to detect these oxidized and ureide metabolites.

Enterohepatic recirculation exerts considerable influence on the pharmacokinetic pattern of *S*-etodolac. This is exemplified in comparing $AUC_{0\text{-}24}$ between bile duct-cannulated and non-bile duct-cannulated rats (Tables VII & VIII), as $>70\%$ of the $AUC_{0\text{-}24}$ of non-bile duct-cannulated rats is attributable to recirculated drug. In contrast, only 26% of the *R*-etodolac $AUC_{0\text{-}24}$ can be attributed to recirculated drug. As the enantiomers shared similar values for F_{ba}, no stereoselectivity in deconjugation and subsequent reabsorption from the intestine seems to exist. The longer $t_{½}$ of the *S* enantiomer caused by enterohepatic recirculation is reflected in the increase in the *S:R* concentration ratio in tissues and plasma over time (*Fig.* 14).

The extensive enterohepatic recirculation of *S*-etodolac complicates data analysis to an appreciable degree. The elimination rate constant β normally describes first order elimination processes, such as those seen in bile duct-cannulated rats. However, under conditions of recycling, β, as determined for *S*-etodolac, is an apparent β in the sense that it reflects the average decline of the fluctuating plasma concentrations over time. Furthermore, the increases in plasma concentration that result from recycled drug cause values such as CL and Vd_{ss} to lose their defined meaning. Therefore the values for $CL_{(c)}$ and $Vd_{ss(c)}$ are more representative of the true values. This is reflected in their closer agreement with the values obtained from bile duct-cannulated rats.

Pharmacokinetic models are available which predict and describe the disposition of drugs which recycle in bile. Several of these models attribute fluctuations in plasma concentrations to periodic emptying of the gall bladder (Colburn 1984). However, because the rat does not possess a gall bladder, other intermittent processes must be responsible for the fluctuations seen in the plasma concentration-time courses of *S*-etodolac. One alternate model developed for phenolphthalein (Colburn *et al.* 1979) may be useful for describing the plasma-time course of *S*-etodolac in the rat, as it incorporates an intestinal-transit lag time rather than a gall bladder. This could help explain the characteristic peak at 3-6 h in the plasma-concentration profiles of rats given *S*-etodolac, as within 2-4 h almost the entire dose was recovered in the bile.

In accordance with our results, a previous non-stereoselective study by Cayen

et al. (1981) also indicated extensive excretion (80% of the dose) of etodolac in rat bile, and a significant reduction in AUC following bile duct-cannulation. However, they reported that almost all etodolac was excreted as unchanged drug. This discrepancy may be explained on the basis of differences in methodology. They incorporated a digestion step in their bile-sample preparation, which might have caused inadvertent hydrolysis of conjugates. Moreover, β-glucuronidase was used to hydrolyse the conjugates. As has been previously demonstrated, the rearrangement products of acyl-glucuronide conjugated NSAIDs are not readily susceptible to cleavage by β-glucuronidase (Verbeeck *et al.* 1988; Loewen *et al.* 1988). In our study alkaline conditions were employed, which allowed for hydrolysis of the conjugated etodolac metabolites when desired (*Fig.* 6).

Etodolac is unique amongst the NSAIDs administered as racemates, because the concentrations of the active enantiomer following a dose are much lower than those of the inactive enantiomer. The differences between the enantiomers cannot be attributed to chiral inversion, as no antipode was detected in plasma or urine following administration of individual enantiomers. This is expected, as unlike the 2-arylpropionic acids, the chiral centre in etodolac is located within an alicyclic ring (*Fig.* 1). The observed differences in the pharmacokinetics of etodolac enantiomers, therefore, can be attributed to stereoselectivity in plasma protein binding, conjugation and elimination in bile, and perhaps in oxidative phase I metabolism of enantiomers of etodolac.

In the preliminary study involving female Sprague-Dawley rats, we observed

9.55±3.41% of the given dose of *S*-etodolac in the urine, as the acyl-glucuronidated species. This was in contrast to that seen in the urine of male rats, in which typically, less than 2% of the dose of the *S* enantiomer is excreted as unchanged and acyl-glucuronidated drug, even after phenobarbital administration. A sex difference in urinary excretion of ketoprofen enantiomers has also been observed in the Sprague-Dawley rat (Palylyk & Jamali 1992). This observation, with respect to etodolac, would seem to warrant more study.

4.1.2. Tissue distribution

The greater *S:R* ratios of etodolac concentrations in tissues as compared to plasma indicate that in the rat, the enantiomeric ratio in plasma is not indicative of that present in the tissues, where pharmacological and toxic effects primarily occur. Plasma concentrations, especially of *R*-etodolac, were considerably higher than those in the tissues (*Fig.* 13; Table XI). To some extent this may be explained by the binding of etodolac enantiomers to rat plasma, which is extensive for both enantiomers, but stronger for *R*- than *S*-etodolac (Table X). This is thought to be a contributing factor for the difference in Vd_{ss} between etodolac enantiomers, which was 5.4 fold greater for *S*- than *R*-etodolac (Table VII). The lower tissue concentrations of enantiomers relative to plasma are in line with those of Cayen *et al.* (1981), who studied the nonstereospecific tissue distribution of racemic etodolac in rats given 10 mg/kg orally. From their results, we calculated $AUC_{0\text{-}24}$ in serum, liver, heart, kidneys, fat, and brain to be 494, 208, 162, 160, 68, and 9.2 μg·h/g, respectively.

Both those and the present results may be explained, in part, by a relatively low degree of tissue binding, as seen in the present study. However, tissue binding does not appear to fully explain why the *S:R* distribution ratio in tissues was generally higher than that seen in plasma. For example, although the *S:R* ratio for $AUC_{0\text{-}24}$ in kidney was close to unity (Table XI), marked stereoselectivity was seen in kidney binding (*S:R* unbound fraction = 3.3)(*Fig.* 15). This apparent paradoxical result can be explained, at least partially, by the greater binding of *R*- than *S*-etodolac in plasma, which balances the greater binding of *R*-etodolac in tissues.

If it is assumed that the amount of drug that can enter tissues is dependent on the concentration of free drug in plasma (C_u), and that the unbound fraction in plasma is fairly constant, then the enantiomeric ratio of tissue concentrations normalized for plasma concentrations (i.e., tissue AUC:plasma AUC) should be similar to the corresponding ratio of C_u. The *S:R* ratios (Table XI) of the tissue $AUC_{0\text{-}24}$, when normalized to plasma $AUC_{0\text{-}24}$, were calculated to be 3.6, 3.3, 2.5, 2.4, and 1.7 in liver, kidney, heart, fat, and brain tissues, respectively. Interestingly, with the exception of brain, these values appear to be in line with the *S:R* ratio of C_u of etodolac in rat plasma, which was 3.12 ± 1.13 at 20 mg/L (Table X). This observation seems to support the notion that free drug concentrations in plasma are the major controlling factor in the distribution of etodolac to the tissues.

Some of these findings parallel those seen in the disposition of etodolac into the synovial fluid of 5 arthritic subjects (Table XVIII). Twelve h after receiving 200 mg of etodolac, concentrations of *S*-, but not *R*-etodolac, were significantly greater

in synovial fluid than in plasma. In both etodolac spiked human synovial fluid and in rat tissues there were greater *S:R* concentration ratios than in plasma, and a greater binding of *R*- than *S*-etodolac. There was, however, a greater binding of enantiomers to drug-spiked human synovial fluid (96 and 97% for *S*- and *R*-etodolac, respectively at 10 mg/L enantiomer) than to the diluted rat tissue homogenates. This may be due to the presence of considerable concentrations of albumin, the major plasma protein implicated in binding of NSAIDs, in synovial fluid.

The tissue binding results should be viewed with some degree of caution, because the dilution step might have altered the binding characteristics of the tissues. Although dilution might compromise the ability of the binding experiments to determine the absolute degree of binding of etodolac to tissues, the experiments do reflect the relative binding affinity of enantiomers to tissues. Another confounding variable in the tissue studies is the presence of residual blood in the tissues. Although this undoubtedly influenced the results by some degree, it did not appear to have a profound influence; the *S:R* concentration ratio of drug in plasma was quite dissimilar to that present in the rat tissues (Table XI).

4.1.3. In vitro metabolism by the rat microsomal fractions

With respect to *in vitro* glucuronidation, a good parallel was seen between the maximal rate of glucuronidation by hepatic-microsomal glucuronyl transferases, and the data on the biliary excretion of conjugates in the rat (*Figs.* 12 & 17). With the Km values seen, glucuronidation of neither enantiomer is saturated at the concentrations

seen in plasma during the post-distributive phase following a 10 mg/kg *iv* dose. The mean *S:R* ratio for cumulative excretion of conjugates in bile was 3.2. A similar *S:R* ratio in Vmax for *in vitro* glucuronidation (3.46±0.45) was seen. These similarities appear to suggest that the differences in biliary excretion of enantiomers as conjugates are due to enzymatic velocity, and not to secretion of conjugates into bile.

Virtually the complete dose of *S*-etodolac is recovered as conjugated enantiomer in rat bile following *iv* administration (*Fig.* 12). However, only 33% of the *R*-enantiomer is likewise recovered. Etodolac had previously been reported to undergo microsomal oxidative metabolism in the rat (Ferdinandi *et al.* 1987; *Fig.* 3); hence it seemed likely that the *R*-enantiomer is predominately biotransformed to those metabolites. Although we were unable to assay these metabolites, and pure oxidized metabolites were not available for assay development, we nevertheless felt that by determining the disappearance of etodolac enantiomers in the presence of an $NADP^+$ microsomal oxidizing system, insight could be gained into the oxidative metabolism of etodolac. As hypothesized, the concentrations of *R*-etodolac fell to a significantly greater extent than the corresponding concentrations of *S*-etodolac after 4 h incubation in the presence of hepatic microsomes (Table XII). Concentrations of *S*-etodolac were also significantly reduced after 4 h, suggesting that it too was capable of being metabolized by hepatic microsomes. Given the high rate of formation of glucuroconjugated *S*-etodolac, however, which is further enhanced by a lower extent of plasma protein binding, glucuronidation is the major metabolic fate of the *S*-enantiomer. This is confirmed by the virtual complete recovery of *S*-etodolac in bile

as conjugated drug (*Fig.* 12).

An unclear situation appeared in the kidney oxidative microsomal incubations (Table XII), in which a significant increase in the *S:R* concentration ratio, and a non-statistically significant reduction in the mean concentrations of *R*-etodolac, were seen. It is likely that there was some oxidation of *R*-etodolac by kidney microsomal protein, because use of *S:R* ratios involves a statistical pairing of the *R*- with *S*-etodolac concentrations, which in turn normalizes the variation present amongst both sets of data. The *S* enantiomer does not appear to be metabolized by the kidney microsomal fraction. Confirmation of the findings from both liver and kidney microsomes, using an assay which can measure the formation of the oxidized metabolites, would be most desirable.

4.1.4. Drug interaction studies

Phenobarbital caused increases in the CL of both etodolac enantiomers (*Fig.* 18; Table XIII) in the rat. This barbiturate is known to induce the cytochrome P-450 catalyzed oxidation of many substrates (Hansten & Horn 1989), and oxidation seems to be primarily responsible for the elimination of *R*-etodolac. Indeed, there was an increase in the apparent oxidative activity *in vitro* for both enantiomers (Table XV). Acyl-glucuronidation, which accounts for over 90% of the dose of *S*-etodolac which is recovered in the bile of bile duct-cannulated rats (*Fig.* 12), was not influenced by phenobarbital (Table XIII, XIV & XV; *Fig.* 19). In rats with an intact biliary system, oxidation may be a more important pathway of metabolism than expected based on

bile recovery studies. This is due to hydrolysis of conjugates and enterohepatic recycling of the liberated etodolac, which allows repeated exposures of recycled drug to the cytochrome P-450 oxidative enzymes. When the latter process is interrupted by biliary cannulation, the amount of drug exposed to the cytochrome P-450 enzymes is expectedly less than in the intact animals. There is some evidence in the literature supporting this proposition. The elimination of pirprofen, a non-steroidal anti-inflammatory drug which has recently been withdrawn from the pharmaceutical markets due to hepatotoxicity, has been studied in Wistar rats with intact and cannulated bile ducts (Thompson *et al.* 1979). In intact animals, 77.4% of the dose of ^{14}C-pirprofen was recovered in the urine within 48 h after a 5 mg/kg *iv* dose of racemate. However, within only 4 h after giving the same dose to bile duct-cannulated rats, over 40% of the dose is recovered in the bile, mostly as acyl-glucuronidated drug.

The upward incline in the 24 h plasma concentration of *S*-etodolac in control rats (*Fig.* 18), which was absent in the phenobarbital group, may be attributed to enterohepatic recycling of *S*-etodolac. The noticeably lower mean concentration at 24 h in the phenobarbital group might be explained by an increase in the proportion of *S*-etodolac metabolized *via* the oxidative pathways, and to the increase in urinary excretion of *S*-etodolac acyl-glucuronides, each of which results in less drug being available for enterohepatic recycling.

Although urinary excretion was a minor route of elimination of conjugated *S*-etodolac, it was nevertheless almost 5-fold greater in the intact phenobarbital treated

rats than in the control rats. Therefore, a small part of the reduced AUC in the phenobarbital rats can be attributed to this change. An increase in renal conjugative activity could also explain the increased renal excretion of acyl-glucuronides in rat urine; renal glucuronidation has been reported for other NSAIDs, including ketorolac and ibuprofen, in rabbit (Mroszczak *et al.* 1987) and rat (Ahn *et al.* 1991) kidneys, respectively. Another possibility is that phenobarbital causes a shift in the route of excretion of etodolac acyl-glucuronides from bile to urine, as is seen for glucuronidated acetaminophen (Gregus *et al.* 1990).

The microsomal preparations used in this study appeared to possess β-glucuronidase activity. Therefore, the Vmax values for net glucuronidation (Table XV) may be underestimates of the true Vmax for glucuronidation. The hydrolytic enzymes present in the microsomal fraction are derived from the smooth endoplasmic reticulum, and/or they represent impurities derived from other cellular organelles such as lysosomes (Dutton 1980). Regardless, the net microsomal glucuronidation of the etodolac enantiomers may still provide a valid reflection of the *in vivo* glucuronidation activities, because *in vivo* the conjugates can also be expected to be exposed to the same hepatic hydrolytic enzymes as those present in the microsomal preparations.

There is ample evidence that cimetidine can interfere with drug metabolism mediated by the cytochrome P-450 mixed function oxidase system (Hansten & Horn 1989), although it does not seem to interfere with UDP-glucuronyltransferase (Somogyi & Muirhead 1987). We had anticipated a possible effect of cimetidine on the pharmacokinetics of *R*-etodolac, because it seems to undergo oxidation as its

primary route of elimination in the rat. Nevertheless, the lack of an interaction between cimetidine and etodolac is consistent with results of other studies in which cimetidine failed to interfere with the metabolism of most other NSAIDs (Verbeeck 1990; Tonkin & Wing 1988). It would therefore appear that cimetidine does not bind to the isoenzymes of cytochrome P-450 which are responsible for the metabolism of etodolac enantiomers, or that the binding of etodolac enantiomers to cytochrome P-450 is much stronger than binding of cimetidine.

A recent report (Gumbleton & Benet 1991) showed that urethane causes a reduction in hepatic blood flow, which in turn lowers the intrinsic hepatic CL of antipyrine in the rat, presumably by reducing oxygen transport to the hepatocytes. In our bile recovery study, we might have expected a smaller recovery of acyl-glucuronides of the etodolac enantiomers after phenobarbital, since a higher proportion of the dose would have been biotransformed to oxidized metabolites. However, in the event that urethane caused a reduction in oxidative metabolism of etodolac, a reduced recovery of etodolac as acyl-glucuronides after phenobarbital may not have occurred in the bile duct-cannulated rats.

In recent reports, the hepatic *in vitro* glucuronidations of (±)-pirprofen (Magdalou *et al.* 1990), (±)-flurbiprofen (Magdalou *et al.* 1990), (±)-ibuprofen (Magdalou *et al.* 1990), and (±)-naproxen (El Mouelhi & Bock 1991) were all increased after the administration of phenobarbital to rats. Although the induction of NSAID metabolism in humans has received little attention, it has been said to be of minor clinical significance because of a lack of an established concentration-effect

relationship (Tonkin & Wing 1988). There is, however, some evidence for a relationship between anti-inflammatory activity and plasma concentrations of *S*-ketoprofen in the rat (Jamali *et al.* 1989; Jamali & Brocks 1990). Positive serum concentration-response relationships have also been described for carprofen and for naproxen in patients with rheumatoid arthritis (Furst *et al.* 1988; Day *et al.* 1982). There are also reports that the analgesic effects of ketoprofen (Kohler *et al.* 1985; D'Arienzo *et al.* 1984), and ibuprofen (Laska *et al.* 1986) may be related to plasma concentrations in humans. These observations may therefore give the phenobarbital-induced metabolism of NSAIDs some measure of clinical importance.

4.2. Disposition studies of etodolac enantiomers in humans

4.2.1. Pharmacokinetics

The stereoselectivity in the kinetics of the enantiomers of etodolac have been confirmed. The results also agreed well with those of non-stereoselective studies of etodolac in humans. The data for mean CL/F, $AUC_{0-\infty}$, and C_{max} of (R+S)-etodolac in young healthy volunteers given oral doses of 200 mg of the racemate were 0.68 mL/min/kg (tablets; Cayen *et al.* 1981), 72 mg·h/L (capsules; Kraml *et al.* 1984), and 16 mg/L (capsules; Kraml *et al.* 1984), respectively; in the present study the corresponding values for (R+S)-etodolac were 0.67 mL/min/kg, 70 mg·h/L, and 14 mg/L.

The stereoselectivity of the pharmacokinetics of etodolac may be attributed to differences in absorption, protein binding, or metabolism of enantiomers. Absorption

for most drugs is presumed to be a passive process (Jamali *et al.* 1989), which renders it as an unlikely contributor to the stereoselectivity observed in the kinetics of etodolac. In humans the unbound fraction (f_u) of *S*-etodolac in pH unadjusted plasma is 2 to 2.5-fold higher than that of *R*-etodolac (Table XIX & XX). This may contribute to the lower C_{max} of *S*-etodolac. However, the difference in f_u cannot by itself explain the ten-fold greater AUC of *S*-etodolac (Table XVI). Alternatively, higher intrinsic metabolic clearance of *S*-etodolac may explain the difference. Our calculations of CL_c/F support this suggestion, where the CL_c/F of *S*-etodolac was 11.9 fold greater than *R*-etodolac in the young subjects, and 9.3 fold greater in the elderly subjects. The observed differences may also be due to stereoselectivity in the oxidative clearance of the enantiomers of etodolac. Etodolac is known to be extensively metabolized through oxidation to 6- and 7-hydroxyetodolac, 8-1'-hydroxyetodolac, and 4-ureidoetodolac (*Fig.* 3), each of which is pharmacologically inactive (Ferdinandi *et al.* 1986; Humber *et al.* 1988; Ferdinandi *et al.* 1987). These metabolites account for most (67%) of the radioactively labelled material recovered in urine in 24 h (Ferdinandi *et al.* 1986). Unfortunately, stereoselective oxidative clearance could not be studied, because we did not have any purified oxidized metabolite.

The absolute systemic availability (F) of racemic etodolac in humans has not been established. However, in other species (*e.g.*, dogs and rats), the nonstereospecific F of etodolac has been estimated to be over 0.94 (Cayen *et al.* 1981). Assuming passive gastrointestinal absorption, coupled with a relatively low CL and CL/F in all three species, it is likely that the F of (R+S)-etodolac in humans is

also near 1. However, whether both enantiomers share the same F is unclear. Estimates of F in young subjects can be made by using values of 331 mL/min and 26 mL/min (from Table XVI) as mean intrinsic hepatic CL (CL_i) of *S*- and *R*-etodolac, respectively, and by assuming mean hepatic blood flow (Q_H) of 1500 mL/min, and haematocrit (HCT) to be 0.4. Using the relationship $F=Q_H\cdot(1\text{-HCT})/\{[Q_H\cdot(1\text{-HCT})]+CL_i\}$ (Gibaldi & Perrier 1982), F is estimated to be 0.73 and 0.97 for *S*-etodolac and *R*-etodolac, respectively. These calculations, which are valid assuming complete absorption of each enantiomer, negligible extra-hepatic metabolism, negligible extra-renal excretion of conjugates, and linear pharmacokinetics (Gibaldi & Perrier 1982), show that *S*-etodolac is less bioavailable than *R*-etodolac in humans. This 24% difference in F may also contribute to the stereoselectivity in AUC of the enantiomers of etodolac.

The Vd_β/F of the *S* enantiomer of etodolac in humans is higher than that of the *R* enantiomer, and is greater than that of most other NSAIDs (Lin *et al.* 1987). A similar finding was also seen in the rat with respect to the Vd_{ss} of the enantiomers. A lower degree of plasma protein binding of the *S* enantiomer as compared to its antipode, in both humans (Tables XIX and XX) and the rat (Table X), may be responsible for the observed stereoselectivity. The larger distribution of *S*-etodolac than *R*-etodolac is reflected by its greater concentrations in the synovial fluid, relative to plasma (Table XVIII). Furthermore, in the rat, despite a low ratio of *S:R* concentrations in plasma, ratios of close to unity were observed in most of the examined tissues (Table XI; *Fig.* 13). Another contributing factor in humans is the

lower apparent F of *S*-etodolac as compared to the *R* enantiomer, which may cause the Vd_β/F of *S*-etodolac to be overestimated in humans.

The lack of change in unchanged and conjugated etodolac enantiomers in plasma or urine of the elderly subjects corroborates the finding that the pharmacokinetics of (*S*+*R*)-etodolac are not affected by aging, in the presence of normal renal and hepatic function (Scatina *et al.* 1986).

The $\Sigma Xu_{0\text{-}t}$ of conjugates of *S*- and *R*-etodolac differed significantly over the first 12 h, but not at 24 h, in both young and elderly subjects (*Fig.* 24). Because there were no differences between conjugated enantiomers in CL_R (Table XVII), it appears that once formed, the conjugates of both enantiomers are cleared from the plasma at the same rate. However, differences in CL_R of enantiomers may have gone undetected due to the large interindividual variation in the AUC of conjugates in the plasma (Table XVII). The mean CL_c/F of *S*-etodolac was substantially different from *R*-etodolac in both the elderly and in the young groups, suggesting that the differences between $\Sigma Xu_{0\text{-}t}$ of conjugated enantiomers at different times is primarily due to a difference in CL_c/F.

The validity of the calculation of CL_c/F requires that the renal pathway is the predominant route of elimination of conjugates. This condition appeared to be met, as little conjugated etodolac was excreted in bile over 24 h in our cholecystectomy patients. However, CL_c/F may be underestimated, as 1) biliary excretion of conjugates in cholecystectomy patients may not reflect that of normal subjects, and 2) hydrolysis of conjugates back to parent drug is possible; this has been speculated to occur with

ketoprofen in the elderly (Verbeeck *et al.* 1984). The extent of deconjugation of the conjugated etodolac enantiomers, however, appeared to be small, as the $t_{½}$ of conjugates were longer than those of the corresponding unchanged enantiomers in the majority of our subjects (Tables XVI & XVII). In the presence of considerable *in vivo* hydrolysis of conjugates (i.e. reversible metabolism), the $t_{½}$ for conjugated and unchanged drug should have been similar (Jamali *et al.* 1988).

Bile was a minor route of excretion of conjugates (Table XVII). This is in contrast to rats, in which biliary excretion and enterohepatic recycling (Cayen *et al.* 1981), particularly of *S*-etodolac (*Figs.* 8 & 9), is extensive. Although the bile output seen in our subjects (Table V) was within the range reported in humans (1.5-15.4 μL/min/kg; Erlinger 1982), the biliary excretion of the enantiomers of etodolac may still be underestimated, because of incomplete collection of bile from T-tubes and surgically-induced alterations in bile flow (Rollins & Klassen 1979). Nevertheless, biliary elimination is unlikely to contribute to the gastrointestinal toxicity of etodolac in humans. The low C_{max} and long t_{max} seen in the cholecystectomy patients may have been due to decreased gastrointestinal motility secondary to surgery and post-surgical medications.

Non-arthritic subjects were studied, which eliminated many of the variables which might affect drug handling in arthritic patients. The arthritic diseases may by themselves cause changes in renal and hepatic function (McCarty 1989), and years of administration of other anti-arthritic medications, most notably NSAIDs, may have deleterious effects on renal, gastrointestinal, and perhaps hepatic function. Although

a previous report showed that hepatic cirrhosis had no effect on the pharmacokinetics of (*S*+*R*)-etodolac, the data did not necessarily reflect the time course of the active enantiomer (Lasseter *et al.* 1988). Hence, studies of the time courses of etodolac enantiomers in patients with arthritis, and in those with hepatic and/or renal dysfunction, are warranted.

4.2.2. Uptake into synovial fluid

Stereoselectivity in the disposition of etodolac is evident by the preponderance of the pharmacologically inactive *R*-enantiomer in both synovial fluid and plasma. The observed 16.1±1.7.50±3.5 *R:S* concentration ratios in plasma and synovial fluid, respectively (Table XVIII), are consistent with previous works reporting relative plasma concentrations of etodolac enantiomers (Singh *et al.* 1986; Jamali *et al.* 1988). Differences were also present in the relative concentrations of each enantiomer in the two body fluids studied. Specifically, concentrations of *S*-etodolac were found to be almost 2-fold greater in synovial fluid than in plasma. In contrast, the synovial fluid levels of the *R* enantiomer were approximately equal to those in plasma. These observations have clinical relevance as the therapeutic benefits of etodolac are mainly ascribed to the *S*-enantiomer (Humber *et al.* 1986) which, despite its relatively low plasma concentration, tends to concentrate in synovial fluid, the proposed site of antiinflammatory action (Wallis & Simkin 1983).

Most other NSAIDs studied for their disposition into synovial fluid have yielded synovial fluid:plasma or serum ratios of less than 1 (Netter *et al.* 1989). This

might be expected, because NSAIDs are highly bound to albumin, which attains higher concentrations in plasma than synovial fluid. The influence of protein concentration on extravascular fluid uptake is illustrated by comparing concentrations of ketoprofen in plasma and cerebrospinal fluid (Netter *et al.* 1985). Total ketoprofen concentrations in the cerebrospinal fluid, which is a protein-poor medium, approximate those of unbound-drug plasma concentrations. However, no relationship was seen between albumin concentrations and the *in vivo* concentrations of the (bound + unbound)-etodolac enantiomers in synovial fluid. Similar to *S*-etodolac, some other NSAIDs such as meclofenamic acid (Koup *et al.* 1988) and diclofenac (Fowler *et al.* 1986) also have synovial fluid:plasma ratios (3.3 to 5.5 at 12 h post-dose) greater than unity. Ketoprofen also shows a progressive increase in synovial fluid:serum level with time (Netter *et al.* 1987; Foster *et al.* 1989). When synovial fluid was dialysed against etodolac-spiked plasma, the concentration of each etodolac enantiomer did not differ between plasma and synovial fluid, despite the 2-fold higher concentration of albumin in plasma than in synovial fluid. This finding can be explained by the high degree of binding of etodolac enantiomers to albumin in both fluids (range 95.2-99.7%, Table XIX). Interestingly, it has been shown that the binding of racemic ketoprofen, at 1 and 10 mg/L, is independent of albumin concentrations >10 g/L (Netter *et al.* 1987). Protein concentration, therefore, cannot wholly explain the synovial fluid dispositional characteristics of all NSAIDs.

Other drugs also provide examples of the independence between synovial fluid drug concentration and protein binding. For example, methotrexate has a 24 h

post-dose synovial fluid:plasma concentration ratio of 3.3 (Tischler *et al.* 1989), despite its limited extent of binding (50%) to proteins (Benet & Sheiner 1985). Furthermore, because gastrointestinal absorption of L-methotrexate is active (Hendel & Brodthagen 1984), it is not inconceivable that the uptake of methotrexate into synovial fluid might also be mediated by an active process. It is of interest that synovial cells appear to possess specialized systems for intrasynovial transport of small molecules such as glucose (Ropes *et al.* 1953; Simkin & Pizzorno, 1974).

Stereoselective binding to macromolecules other than albumin might also explain the disposition of etodolac enantiomers in synovial fluid. Consequently, we probed the possibility of binding to hyaluronic acid. With the exception of this glycoprotein, which is present in normal synovial fluid in concentrations of 0.3 g/100 mL (McCarty 1989), the composition of synovial fluid is qualitatively similar to plasma. Nevertheless, binding of etodolac to hyaluronic acid was not seen, and therefore does not appear to contribute to the relatively slow exit of *S*-etodolac from synovial fluid. Another suggestion that has been made to explain the low rate of exit of NSAIDs from synovial fluid is related to the large volume:surface area ratio of synovial fluid (Koup *et al.* 1988). This had previously been used to explain the high concentrations of β-lactam antibiotics attained in blister fluid (Fleishaker & McNamara 1985). However, because enantiomers share the same physico-chemical properties, and synovial transport of NSAIDs is thought to largely involve diffusion, this cannot by itself explain the relatively larger concentrations of *S*-etodolac, than *R*-etodolac, in synovial fluid relative to plasma.

In the other study involving the synovial fluid uptake of etodolac, it was found that despite comparable total (bound+unbound) AUC, the AUC of unbound drug was significantly greater in synovial fluid than in serum (Kraml *et al.* 1988). On this basis they speculated that unbound etodolac is actively transported into synovial fluid. This should be viewed cautiously, however, because unbound concentrations in their patient samples were determined by equilibrium dialysis of synovial fluid vs buffer: The free concentration of drug in an extravascular fluid separated from plasma by a membrane should be equal (Gibaldi & Perrier 1982; Wise 1986). Likewise, *in vivo*, equilibrium is achieved only when the unbound concentration between the two fluids becomes equal. When synovial fluid is dialysed vs buffer, the influence of the plasma components on free-drug concentration in synovial fluid is absent (*Fig.* 26). Hence, free drug concentrations derived from plasma or serum vs buffer binding experiments may more closely reflect the true, *in vivo*, free-concentration in synovial fluid, and as such, synovial fluid equilibrium dialysis binding experiments should be limited to the determination of relative binding capacity of drug to the fluid.

Considerable concentrations of conjugated *S*-etodolac, and smaller concentrations of conjugated *R*-etodolac, were seen in both plasma and synovial fluid. Due to their inherent instability, these metabolites may represent a potential reservoir for both enantiomers, particularly as arthritic synovial fluid is known to contain considerable β-glucuronidase activity (Bartholemew 1972). Consequently, hydrolysis of only a small fraction of the conjugated *S*-etodolac may yield considerably greater concentrations of pharmacologically active enantiomer at the site of action. The

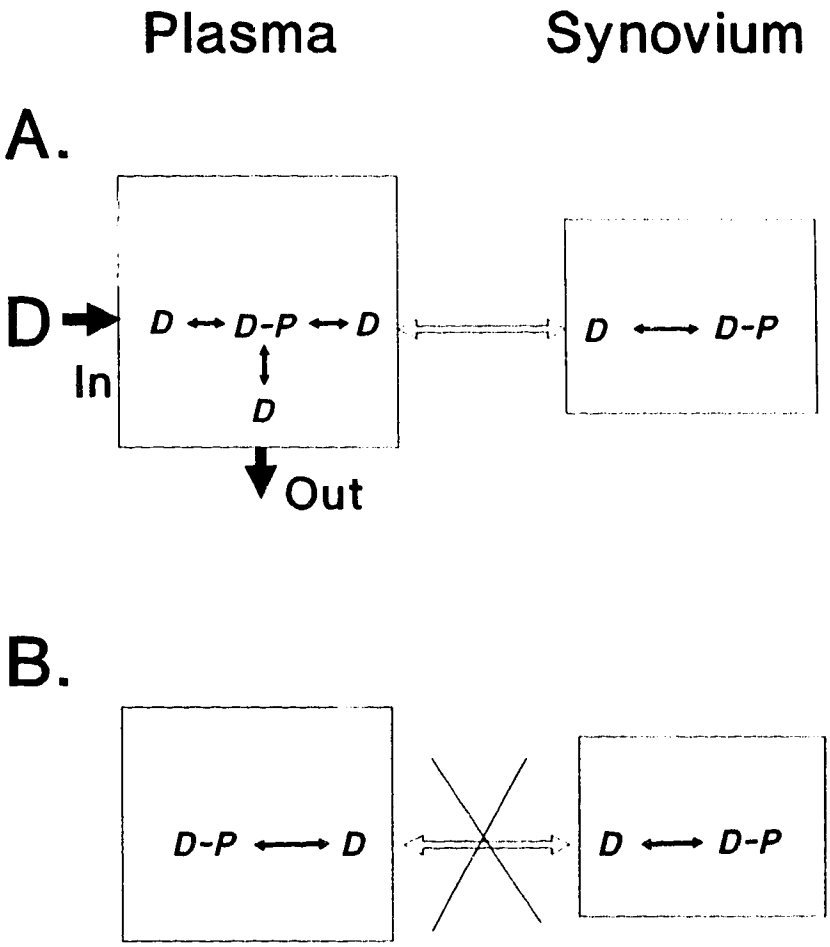


Figure 26: Relationship between concentrations of unbound drug (D), protein-bound drug (D-P), and plasma synovial transport. Panel A depicts the *in vivo* situation, in which the unbound drug concentration is determined by the protein binding of the drug on both sides of the membrane. During the post-absorptive and post-distributive phase, the unbound drug concentration should be equal in the plasma and synovial fluid. When plasma and synovial fluid are removed (Panel B), the influence of trans-synovial distribution is removed. During subsequent dialysis, a redistribution of the unbound species may occur, which may result in unbound concentrations different from those actually present *in vivo*.

presence of substantial concentrations of alkaline hydrolysable conjugated NSAID in synovial fluid, as seen here, has not been previously reported. It is of note, however, that considerable concentrations of hydroxylated diclofenac metabolites have been detected in synovial fluid (Fowler *et al.* 1986).

The concentrations of acyl-glucuronidated etodolac enantiomer seen in the arthritic patients (*Fig.* 25; Table XVIII) were somewhat higher than seen in the young and elderly subjects at 12 h post-dose (*Fig.* 20). Unfortunately, we did not have the data for the renal function of the arthritic patients. Due to the severity of their disease, which likely included years of therapy with other nonsteroidal antiinflammatory drugs, it is possible that some degree of diminished renal function was present. This could explain the higher concentrations of acyl-glucuronides in the arthritic plasma.

A question arises as to the source of conjugates in synovial fluid. On the basis of their relatively high polarity, it might be hypothesized that conjugates do not cross the synovial membrane, but are rather formed by the synovium itself. This seems unlikely, as no glucuronidation activity was seen in isolated microsomes from synovial membrane. Alternatively, the presence of acyl-glucuronides may be secondary to the disease process, in which inflammatory changes in the synovial membrane lead to altered synovial permeability. The possibility of trans-synovial diffusion of conjugates is supported by the strong correlation between plasma and synovial fluid concentrations of *S*-etodolac conjugates (*Fig.* 25).

The strength of our results may be weakened by our inability to administer it as multiple doses to patients. This was due to the present investigational status of

etodolac in Canada, which in turn limited our access to sufficient quantities of etodolac. Nevertheless, our results of concentrations of (R+S)-etodolac at 12 h post-dose agreed well with those of Kraml *et al.* (1986), who administered etodolac as multiple doses to 5 patients. It was also beyond our means to collect serial samples of synovial fluid and plasma, such that a concentration-time relationship for synovial fluid disposition of etodolac enantiomers could have been derived.

Due to assay sensitivity constraints, it was not possible to determine free drug concentration in our patient samples. Also, because considerable concentrations of conjugated etodolac were found in plasma and synovial fluid, the possibility of inadvertent hydrolysis of conjugates existed with equilibrium dialysis. Despite these obstacles, it was felt that by using etodolac-spiked blank samples, some estimate of relative enantiomer binding could be achieved. Because the etodolac concentrations used in binding studies were higher than those observed in our patient samples, the unbound fractions may be an overestimate of the values actually observed with the lower concentrations present *in vivo*. The extent of this may be minimal, however, because the unbound fraction of both enantiomers increased only 3 fold with a 10 fold increase in enantiomeric concentrations (Table XIX).

It should also be noted that the enantiomeric concentration ratios *in vivo* were substantially different from unity. Therefore, because the binding of *R*-etodolac was greater than *S*-etodolac in the presence of equal concentrations of the 2 enantiomers (Table XIX & XX), the difference between the binding of the 2 enantiomers *in vivo* might be even greater, assuming displacement of *S*-etodolac by the greater

concentrations of *R*-etodolac.

5. Conclusions

The stereoselectivity observed for etodolac in humans and rats can be attributed to differences between the enantiomers in their binding to plasma proteins, and to enantioselective metabolic clearance. There are several similarities between the species in their dispositional handling of the enantiomers of etodolac (Table XXI). In the plasma of both species, the binding of the *R* enantiomer is higher than that of the *S* enantiomer; this coincides with a higher Vd for *S*- than *R*-etodolac. The difference between the enantiomers in plasma protein binding is accompanied by a greater metabolic clearance of the *S* than the *R* enantiomer. Due to the almost complete metabolism of both enantiomers, the total body clearance, which is much greater for *S*- than *R*-etodolac in humans and rats, is mostly reflective of metabolic clearance. In the rat this is supported by a faster rate of *in vitro* hepatic microsomal acyl-glucuronidation of *S*- than *R*-etodolac, and by the rapid and nearly complete biliary excretion of *S*-etodolac as the acyl-glucuronidated species.

The distribution of etodolac enantiomers to the tissues and extravascular fluids is a reflection of the enantioselective plasma protein binding. In rat tissues, the *S* enantiomer achieves similar concentrations to the *R* enantiomer, despite a ≈4-fold lower total (bound+unbound) *S*-etodolac concentration in the corresponding plasma.

Table XXI: Summary of the pharmacokinetic and metabolic differences between humans and rats.

Inter-species differences	
Humans	**Rats**
Unbound fraction in plasma:	
S > R	S > R
Volume of distribution:	
S >>> R	S >> R
AUC:	
S<<<R	S << R
CL:	
S >>> R	S << R
Major metabolic fate:	
1. S-etodolac	
Oxidized	Acyl-glucuronidated
2. R-etodolac	
Oxidized	Oxidized
Major route of excretion of metabolites:	
Urine	Bile and feces

In contrast, the unbound fraction of *S*-etodolac is ≈4-fold higher in plasma, which explains the similar concentrations of the enantiomers in tissues. In human synovial fluid, the concentrations of the *S* enantiomer, which were higher than in plasma, also seems to be explainable to some degree by its higher unbound fraction in plasma.

The enantiomers of etodolac are differentially metabolised in the rat. Most of the *S* enantiomer is eliminated from plasma *via* acyl-glucuronidation, whereas oxidative metabolism is largely responsible for most of the elimination of *R*-etodolac. We studied the elimination of the enantiomers as acyl-glucuronides, and found almost 100% of the *S* enantiomer recovered in bile. After phenobarbital administration the AUC of the *S* enantiomer decreased to the same degree as that of its antipode, although there was no increase in the acyl-glucuronidation activity of *S*-etodolac. This leads us to believe that bile duct-cannulated rats eliminate more of the *S* enantiomer as acyl-glucuronides than occurs in intact rats, in which enterohepatic recycling permits a larger percent of the dose of *S*-etodolac to be eliminated as oxidized metabolite. This should be viewed as a caution in the interpretation of drug excretory results obtained from bile duct-cannulated animals.

The metabolism of etodolac was not influenced in the rat by cimetidine, which might alleviate to some degree concerns about higher etodolac concentrations with cimetidine coadministration. However, in humans a larger percentage of the dose is eliminated as oxidized etodolac; this might be cause for a drug interaction study in humans.

Between the species there were only a few qualitative differences in the drug

metabolism and pharmacokinetics of the enantiomers of etodolac. These differences mostly involved the pathways of metabolism, and the route of elimination of the metabolites. In humans, the clearance of both enantiomers is heavily dependent on the formation of oxidized-etodolac metabolites; acyl-glucuronidation only accounts for 10-20% of the elimination of each enantiomer. In contrast, in the rat the *S* enantiomer undergoes a high degree of acyl-glucuronidation as opposed to the *R* enantiomer, which, like humans, undergoes primarily oxidative metabolism. The acyl-glucuronides of the enantiomers are primarily excreted in the urine in humans, in contrast to the rat, where almost all of the enantiomer is excreted in the bile. Female rats appear to excrete more of the *S*-etodolac acyl-glucuronides in the urine than male rats. This may have some importance in considering the use of the rat as an animal model. The female rat may be somewhat more comparable to humans than the male rat, when one considers that humans seem to preferentially excrete the acyl-glucuronides of both etodolac enantiomers in the urine.

In the studies described in this thesis, there were some findings which were somewhat unexpected based on previous works. For example, it had not been previously reported that acyl-glucuronides can transverse into synovial fluid. Acyl-glucuronides were also found in bile from rats, which was in disagreement the data reported in a previous study.

Our studies have illustrated the importance of considering stereoselectivity with respect to the pharmacokinetics of etodolac, because concentrations of inactive *R*-etodolac account for the majority of the drug found in plasma following a racemic

dose. Studies which have used non-stereospecific techniques to measure etodolac concentrations yield plasma-time courses that essentially reflect those of inactive *R*-etodolac; unfortunately, information derived from such studies may be of little use and/or potentially misleading. Indeed, because stereoselectivity was not considered, the predisposition of pharmacologically active *S*-etodolac to the proposed site of action (i.e., synovial fluid) was not previously elucidated.

We have established that in healthy elderly subjects with normal hepatic and renal function, there does not seem to be a need for dosage adjustment. It might be anticipated that concentrations of conjugated etodolac enantiomers accumulate in patients with renal disease. Also, in patients with hepatic diseases, an elevated concentration of *S*-etodolac could easily be obscured by the already high concentrations of the *R* enantiomer. Consequently, there is a need to study the pharmacokinetics of etodolac enantiomers in patients with compromised renal or hepatic function. Our human pharmacokinetic study involved only single doses; it would be informative to see the effects of multiple dosing on the pharmacokinetics of etodolac enantiomers and its acyl-glucuronide metabolites. In light of these possibilities and unanswered questions, it is clear that additional studies are warranted for etodolac. Moreover, it is essential that a stereospecific approach be incorporated into the experimental designs of any future studies which investigate the pharmacokinetics and pharmacodynamics of etodolac.

6. References

Ahn HY, Jamali F, Cox SR, Kittayanond D, Smith DE. Stereoselective disposition of ibuprofen enantiomers in the isolated perfused rat kidney. *Pharm Res* 8: 1520-1524, 1991

Anonymous. Etodolac. *Med Letter Drugs Ther* 33: 79-80, 1991

Ariens EJ. Stereochemistry, a basis for sophisticated nonsense in pharmacokinetics and clinical pharmacology. *Eur J Clin Pharmacol* 26: 663-668, 1984

Ariens EJ, Wuis EW. Bias in pharmacokinetics and clinical pharmacology. *Clin Pharmacol Ther* 42: 361-363, 1987

Ariens EJ, Wuis EW, Veringa EJ. Stereoselectivity of bioactive xenobiotics. *Biochem Pharmacol* 37: 9-18, 1988

Bartholemew BA. Synovial fluid glycosidase activity. *Scand J Rheumat* 1: 69-74, 1972

Benedetti MS, Frigerio E, Tamassia V, Noseda G, Caldwell J. The dispositional enantioselectivity of indobufen in man. *Biochem Pharmacol* 43: 2032-2034, 1992

Benet LZ, Sheiner LB. Design and optimization of dosage regimens; pharmacokinetic data. In: Goodman Gilman A, Goodman LS, Rall TW, Murad F, eds. *The Pharmacological Basis of Therapeutics 7th Ed.* New York: MacMillan, *pp* 1663-1733, 1985

Benhamou CL. Large-scale open trials with etodolac (Lodine®) in France: An assessment of safety. *Rheumatol Int* 10(Suppl): 29-34, 1990

Berry BW, Jamali F. Enantiomeric interaction of flurbiprofen in the rat. *J Pharm Sci* 78: 634-636, 1989

Bopp RJ, Nash JF, Ridolfo AS, Shepard ER. Stereoselective inversion of (R)-(-)-benoxaprofen to the (S)-(+)enantiomer in humans. *Drug Metabol. Dispos.* 356-359, 1979

Brocks DR, Jamali F. Clinical pharmacokinetics of ketorolac tromethamine. *Clin Pharmacokinet* 23: 415-427, 1992

Brune K, Beck WS, Geisslinger G, Menzelsoglowek S, Peskar BM, Peskar BA. Aspirin-like drugs may block pain independently of prostaglandin synthesis inhibition. *Experimentia* 47: 257-261, 1991

Cayen MN, Kraml M, Ferdinandi ES, Greselin E, Dvornik D: The metabolic disposition of etodolac in rats, dogs, and man. *Drug Metab Rev* 12: 339-362, 1981

Chapron DJ: Influence of advanced age on drug disposition and response. In: Delafuente JC, Stewart RB, eds. *Therapeutics in the elderly.* London: Williams & Wilkins, *pp* 107-120, 1988

Colburn WA, Hirom PC, Parker RJ, Milburn P. A pharmacokinetic model for enterohepatic recirculation in the rat: Phenolphthalein, a model drug. *Drug Metabol Dispos* 7: 100-102, 1979

Colburn WA. Pharmacokinetic analysis of concentration-time data obtained following administration of drugs that are recycled in bile. *J Pharm Sci* 73: 313-317, 1984

D'Arienzo M, Pennisi M, Zanolo G, Borsa M. Ketoprofen lysine: Ketoprofen serum levels and analgesic activity. *Drugs in Exp Clin Res* 10: 863-866, 1984

Day RO, Furst DE, Dromgoole SH, Kamm B, Roe R, Paulus HE. Relationship of serum naproxen concentration to efficacy in rheumatoid arthritis. *Clin Pharmacol Ther* 31: 733-740, 1982

Demerson CA, Humber LG, Philipp AH, Martel RR. Etodolic acid and related

compounds. Chemistry and antiinflammatory actions of some potent di- and trisubstituted 1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acids *J Med Chem* 19: 391-395, 1976

Demerson CA, Humber LG, Abraham NA, Schilling G, Martel RR, et al. Resolution of etodolac and anti-inflammatory activity and prostaglandin synthetase inhibiting properties of the enantiomers. *J Med Chem* 26: 1778-1780, 1983

Dey M, Enever R, Marino M, Michelucci J, Smith D, *et al.* Sustained-release etodolac bioavailability and dose proportionality: Corrleation between *in vivo* and *in vitro* performance. *Int J Pharm* 49: 121-128, 1989

Drayer DE. Pharmacodynamic and pharmacokinetic differences between drug enantiomers in humans: An overview. *Clin Pharmacol Ther* 40: 125-133, 1986

Durnas C, Loi CM, Cusack BJ: Hepatic drug metabolism and ageing. *Clin Pharmacokinet* 19: 359-389, 1990

Dutton GJ. Metabolic pathways immediately preceding and succeeding glucuronidation -- anabolic pathways. In: *Glucuronidation of drugs and other compounds.* Boca Raton: CRC, *pp* 83-96, 1980.

El Mouelhi M, Bock KW. Stereoselective (S)- and (R)-naproxen glucuronosyltransferases of rat liver. *Drug Metabol Dispos* 19: 304-307, 1991

Erlinger S: Bile flow. In: Arias I, Shafritz DA (ed.) *The liver: Biology and pathobiology.* New York: Raven, *pp* 407-427, 1982

Evans AM, Nation RL, Sansom LN, Bochner F, Somogyi AA: Stereoselective drug disposition: Potential for misinterpretation of drug disposition data. *Br J Clin Pharmacol* 26: 771-780, 1988

Evans AM. Enantioselective pharmacodynamics and pharmacokinetics of chiral non-steroidal anti-inflammatory drugs. *Eur J Clin Pharmacol* 42: 237-256, 1992

Ferdinandi ES, Sehgal SN, Demerson CA, Dubuc J, Zilber J, Dvornik D, Cayen MN: Disposition and biotransformation of ^{14}C-etodolac in man. *Xenobiotica* 16: 153-166, 1986

Ferdinandi ES, Cochrane D, Gedamke R: Identification of the etodolac metabolite, 4-ureidoetodolac, in mouse, rat, dog, and man. *Drug Metab Dispos* 15: 921-924, 1987

Fleishaker JC, McNamara PJ: Performance of a diffusional clearance model for

β-lactam antimicrobial agents as influenced by extravascular protein binding and interstitial fluid kinetics. *Antimicrob Agents Chemother* 28: 369-374, 1985

Fowler PD, Dawes PT, John VA, Shotton PA. Plasma and synovial fluid concentrations of diclofenac sodium and its hydroxylated metabolites during once-daily administration of a 100 mg formulation. *Eur J Clin Pharmacol* 31: 469-472, 1986

Furst DE, Cladwell JR, Klugman MP, Enthoven D, Rittweger K, Scheer R, Sarkissian E, Dromgoole S. Serum concentration and dose-response relationships for carprofen in rheumatoid arthritis. *Clin Pharmacol Ther* 44: 186-194, 1988

Gibaldi M, Perrier D. *Pharmacokinetics 2nd ed.* New York: Marcel Dekker, 1982.

Gregus Z, Madhu C, Klaassen CD. (1990): Effect of microsomal enzyme inducers on biliary and urinary excretion of acetaminophen metabolites in rats. *Drug Metabol Dispos* 18: 10-19, 1990

Gumbleton M, Benet LZ. Drug metabolism and laboratory anesthetic protocols in the rat: Examination of antipyrine pharmacokinetics. *Pharm Res* 8: 544-546, 1991

Guzman A, Yuste F, Toscano RA, Young JM, Van Horn AR. Absolute

configuration of (-)-5-benzoyl-1,2-dihydro-3H-pyrrolo[1,2-a]pyrrole-1-carboxylic acid, the active enantiomer of ketorolac. *J Med Chem* 29: 589-591, 1986

Hansten PD, Horn JR: *Drug interactions: clinical significance of drug-drug interactions, 6th ed.* Philadelphia: Lea & Febiger, 1989

Hendel J, Brodthagen H. Entero-hepatic cycling of methotrexate estimated by use of the D-isomer as a reference marker. *Eur J Clin Pharmacol* 26: 103-107, 1984

Humber LG, Demerson CA, Swaminathan P, Bird PH: Etodolac (1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acid): A potent antiinflammatory drug. Conformation and absolute configuration of its active enantiomer. *J Med Chem* 29: 871-874, 1986

Humber LG, Ferdinandi ES, Demerson CA, Ahmed S, Shah U, Mobilio D, Sabatucci J, De Lange B, Labbadia F, Hughes P, DeVirgilio J, Neuman G, Chau TT, Weichman BM: Etodolac, a novel antiinflammatory agent. The synthesis and biological evaluation of its metabolites. *J Med Chem* 31: 1712-1719, 1988

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

Hutt AJ, Caldwell J. The importance of stereochemistry in the clinical pharmacokinetics of the 2-arylpropionic acid non-steroidal anti-inflammatory drugs. *Clin Pharmacokinet* 9: 371-373, 1984

Inoue K, Fujisawa H, Sasaki Y, Nishimura T, Nishimura I, et al. Pharmacological properties of the new non-steroidal anti-inflammatory agent etodolac. *Arzneimittel Forsch* 41: 228-235, 1991a

Inoue K, Motonaga A, Nishimura T, Yokota N, Miki N, et al. Mechanism of anti-inflammatory action of etodolac. *Arzneimittel Forsch* 41: 235-239, 1991b

Jacob G, Messina M, Kennedy J, Epstein C, Sanda M, et al: Minimum effective dose of etodolac for the treatment of rheumatoid arthritis. *J Clin Pharmacol* 26: 195-202, 1986

Jamali F. Pharmacokinetics of enantiomers of chiral non-steroidal anti-inflammatory drugs. *Eur J Drug Metabol Pharmacokinet* 13: 1-9, 1988

Jamali F, Alballa RS, Mehvar R, Lemko CH: Longer plasma half-life for procainamide utilizing a very sensitive high performance liquid chromatography assay. *Ther Drug Monitor* 10: 91-96, 1988

Jamali F, Mehvar R, Lemko C, Eradiri O. Application of a stereospecific high-performance liquid chromatography assay to a pharmacokinetic study of etodolac enantiomers in humans. *J Pharm Sci* 77: 963-966, 1988

Jamali F, Mathers D, Russell AS. Concentration-effect relationship of S-ketoprofen in adjuvant arthritic rats. *Pharm Res* 6(Suppl.): S211, 1989 (Abstract)

Jamali F, Mehvar R, Pasutto FM: Enantioselective aspects of drug action and disposition: Therapeutic pitfalls. *J Pharm Sci* 78: 695-715, 1989

Jamali F, Brocks DR. Clinical pharmacokinetics of ketoprofen and its enantiomers. *Clin Pharmacokinet* 19: 197-217, 1990

Jamali F, Russell AS, Foster RT, Lemco C. Inversion of R-ketoprofen and lack of pharmacokinetic interaction in humans. *J Pharm Sci* 79: 460-461, 1990

Kochak GM, Mason WD: The pharmacokinetics of α-methyldopa in dogs. *J Pharmacokinet Biopharm* 13: 405-423, 1985

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

Koup JR, Thomas D, Tucker E, Black A, Ruderman M, Dixon JA, Kinkel A. Plasma and synovial fluid meclofenamic acid concentrations in patients with rheumatoid arthritis of the knee. *Eur J Clin Pharmacol* 35: 199-202, 1988

Kraml M, Cosyns L, Hicks DR, Simon J, Mullane JF, Dvornik D: Bioavailability studies with etodolac in dogs and man. *Biopharm Drug Dispos* 5: 63-74, 1984

Kraml M, Hicks DR, McKean M, Panagides J, Furst J. The pharmacokinetics of etodolac in serum and synovial fluid of patients with arthritis. *Clin Pharmacol Ther* 43: 571-576, 1988

Laska EM, Sunshine A, Marrero I, Olson N, Siegel C, McCormick N. The correlation between blood levels of ibuprofen and clinical analgesic response. *Clin Pharmacol Ther* 40: 1-7, 1986

Lasseter K, Shamblen E, Murdoch A, Marino M, Minor M, Kraml M: Pharmacokinetics of etodolac in patients with hepatic cirrhosis. *J Clin Pharmacol* 28: 933, 1988 (Abstract)

Lin JH, Cocchetto DM, Duggan DE: Protein binding as a primary determinant of the clinical pharmacokinetic properties of non-steroidal anti-inflammatory drugs. *Clin Pharmacokinet* 12: 402-432, 1987

Loewen GR, MacDonald JI, Verbeck RK. High-performance liquid chromatographic analysis for the simultaneous quantitation of diflunisal and its glucuronide and sulfate conjugates in human urine. *J Pharm Sci* 78: 250-255, 1988

Lowry OH, Rosebrough NJ, Farr AJ, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265-275, 1951

Lynch S, Brogden RN. Etodolac: A preliminary review of its pharmacodynamic activity and therapeutic use. *Drugs* 31: 288-300, 1986

Magdalou J, Chajes V, Lafaurie C, Siest G. Glucuronidation of 2-arylpropionic acids pirprofen, flurbiprofen, and ibuprofen by liver microsomes. *Drug Metabol Dispos* 18: 692-697, 1990

Martel RR, Klicius J. Anti-inflammatory and analgesic properties of etodolic acid in rats. *Can J Physiol Pharmacol* 54: 245-248, 1976

Martel RR, Klicius J. Comparison in rats of the anti-inflammatory and gastric irritant effects of etodolac with several clinically effective anti-inflammatory drugs. *Agents Actions* 12: 295, 1982

McCarty DJ. Synovial fluid. In: McCarty DJ ed. *Arthritis and Allied Conditions:*

A Textbook of Rheumatology 11th Ed. Philadelphia: Lea and Febiger, *pp* 69-90, 1989

Mroszczak EJ, Lee FW, Combs D, Sarnquist FH, Huang BL, Wu AT, Tokes LG, Maddox ML, Cho DK. Ketorolac tromethamine absorption, distribution, metabolism, excretion, and pharmacokinetics in animals and humans. *Drug Metabol Dispos* 15: 618-626, 1987

Netter P, Lapicque F, Bannwarth B, Tamisier JN, Thomas P, et al. Diffusion of intramuscular ketoprofen into the cerebrospinal fluid. *Eur J Clin Pharmacol* 29: 319-321, 1985

Netter P, Bannwarth B, Lapicque F, Harrewyn J, Frydman A, et al. Total and free ketoprofen in serum and synovial fluid after intramuscular injection. *Clin Pharmacol Ther* 42: 555-561, 1987

Netter P, Bannwarth B, Royer-Marrot. Recent findings on the pharmacokinetics of non-steroidal anti-inflammatory drugs in synovial fluid. *Clin Pharmacokinet* 17: 145-162, 1989

Palylyk EL, Jamali F. Gender related differences in the pharmacokinetics of ketoprofen enantiomers in the rat. *Pharm Res* 1992, Submitted for publication

Rainsford, KD: Toxicity of currently used anti-inflammatory and antirheumatic drugs. In: Lewis AJ, Furst DE (ed) *Nonsteroidal anti-inflammatory drugs: mechanisms and clinical use.* New York: Marcel Dekker, *pp* 215-244, 1987

Rollins DE, Klaassen CD: Biliary excretion of drugs in man. *Clin Pharmacokinet* 4: 368-379, 1979

Ropes MW, Muller AF, Bauer W. The entrance of glucose and other sugars into joints. *Arth Rheumat* 3: 496-513, 1960

Rubin A, Knadler MP, Ho PPK, Bechtol LD, Wolen RL. Stereoselective inversion of (R)-fenoprofen to (S)-fenoprofen in humans. *J Pharm Sci* 74: 82-84, 1985

Schattenkirchner M. An updated safety profile of etodolac in several thousand patients. *Eur J Rheumatol Inflamm* 10: 56-65, 1990

Scatina J, Hicks D, Kraml M, Weidler D, Garg D, Sanda M. Etodolac kinetics in the elderly. *Clin Pharmacol Ther* 39: 550-553, 1986

Serni U. Global safety of etodolac: Reports from worldwide postmarketing surveillance studies. *Rheumatol Int* 10(Suppl): 23-27, 1990

Simkin PA, Pizzorno JE. Transynovial exchange of small molecules in normal human subjects. *J Applied Physiol* 36: 581-587, 1974

Simkin PA. Synovial physiology. In *Arthritis and Allied Conditions: A Textbook of Rheumatology 11th Ed.* McCarty DJ, ed. Philadelphia: Lea and Febiger, *pp* 207-221, 1989

Singh NN, Jamali F, Pasutto FM, Coutts RT. Stereoselective gas chromatographic analysis of etodolac enantiomers in human plasma and urine. *J Chromatogr* 382: 331-337, 1986

Sioufi A, Colussi D, Marfil F, Dubois JP. Determination of the (+)- and (-)-enantiomers of pirprofen in human plasma by high-performance liquid chromatography. *J Chromatogr* 414: 131-137, 1987

Somogyi A, Muirhead M. Pharmacokinetic interactions of cimetidine 1987. *Clin Pharmacokinet* 12: 321-366, 1987

Taha AS, McLaughlin S, Sturrock RD, Russell RI. Evaluation of the efficacy and comparative effects on gastric and duodenal mucosa of etodolac and naproxen in patients with rheumatoid arthritis using endoscopy. *Br J Clin Rheum* 28: 329-332, 1989

Thompson TA, Borman CH, Goodblatt RS, Roth WJ. Effects of aspirin on ^{14}C-pirprofen disposition in rats. *J Pharm Sci* 68: 996-998, 1979

Tishler M, Capsi D, Graff E, Segal R, Peretz H, et al. Synovial and serum levels of methotrexate during methotrexate therapy of rheumatoid arthritis. *Br J Rheumatol* 28: 422-423, 1989

Tonkin AL, Wing LMH. Interactions of non-steroidal anti-inflammatory drugs. *Bailleres Clin Rheumatol* 2: 455-483, 1988

Tse FLS, Ballard F, Skinn J. Estimating the fraction reabsorbed in drugs undergoing enterohepatic reabsorption. *J Pharmacokinet Biopharm* 10: 455-461, 1982

Verbeeck RK, Wallace SM, Loewen GR: Reduced elimination of ketoprofen in the elderly is not necessarily due to impaired glucuronidation. *Br J Clin Pharmacol* 17: 783-784, 1984

Verbeeck RK, Dickinson RG, Pond SM. Biliary excretion of diflunisal conjugates in patients with T-tube drainage. *Eur J Clin Pharmacol* 34: 423-426, 1988

Verbeeck RK. Pharmacokinetic drug interactions with nonsteroidal

anti-inflammatory drugs. *Clin Pharmacokinet* 19: 44-66, 1990

Wainer IW, Marcotte AL. Stereochemical terms and concepts: an overview. In Wainer IW, Drayer DE, eds. *Drug Stereochemistry: Analytical methods and pharmacology*. New York: Marcel Dekker, *pp* 31-41, 1988

Wallis WJ, Simkin PA. Antirheumatic drug concentrations in human synovial fluid and synovial tissue: Observations on extravascular pharmacokinetics. *Clin Pharmacokinet* 8: 496-522, 1983

Wise R. The clinical relevance of protein binding and tissue concentrations in antimicrobial therapy. *Clin Pharmacokinet* 11: 470-482, 1986

Wright MR, Jamali F. Methods for the analysis of racemic drugs: Application to pharmacological and pharmacokinetic studies. *J Pharmacol Toxicol Meth* 1992, Accepted for publication

Yamaoka K, Tanigawara Y, Nakagawa T, Uno T. A pharmacokinetic analysis program (Multi) for microcomputer. *J Pharm Dyn* 4: 879-885, 1981