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THE CLINICAL PHARMACOKINETICS OF 2-ARYLPROPIONIC NONSTEROIDAL ANTIINFLAMMATORY DRUGS

By

KENNETH J. SKEITH

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

Doctor of Philosophy

In Pharmaceutical Sciences (Pharmacokinetics)

Faculty of Pharmacy & Pharmaceutical Sciences
Edmonton, Alberta

Fall 1999

UNIVERSITY OF ALBERTA

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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 CLINICAL PHARMACOKINETICS OF 2-ARYLPROPIONIC ACID NONSTEROIDAL ANTIINFLAMMATORY DRUGS** submitted by **Kenneth J. Skeith** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in **Pharmaceutical Sciences (Pharmacokinetics).**

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ACKNOWLEDGEMENTS

I wish to express my appreciation to my wife Mary and to my children for their interminable patience. This project that has barely escaped becoming a millenium celebration has tested the stamina and good spirits of my family and my supervisor Dr Jamali, all of whom deserve credit for seeing its completion.

I have gained the friendship of many fellow graduate students and instructors during the course of attaining this degree, and their assistance and insights have been greatly appreciated. This excursion into the realm of research, as initially prompted by Dr. G. King, has added a stimulating and satisfying dimension to my life and to my career.

I wish to acknowledge the financial support of the Medical Research Council, the Arthritis Society, the Alberta Heritage Foundation for Medical Research, the Canadian Society for Clinical Pharmacology, and Bayer SA (Germany) for these projects.

Abstract

Arthritis is one of the most prevalent clinical maladies affecting individuals world-wide. Nonsteroidal antiinflammatory drugs (NSAIDs) are used for symptom control for arthritis and related conditions and lead to significant adverse effects in some patients. Optimization of NSAID dosing regimens based on accurate characterization of their disposition in various clinical states can limit drug-induced toxicities.

The effects of dose, renal function, and arthritis on the stereoselective kinetics of ketoprofen in non-arthritic and arthritic subjects showed no significant differences in kinetic variables between groups or doses; values were similar to young adults. More conjugated ketoprofen enantiomers were present in arthritic subjects' plasma. Stereo-selective (R > S) renal clearance of conjugates was decreased in the arthritic group. Accumulation of unchanged ketoprofen did not occur.

Single 50 and 100 mg doses of racemic ketoprofen were given to 9 patients with renal function of CL_{CR} 6 ml/min to 110 ml/min. Reduced ketoprofen oral clearance (CL_O) and terminal elimination rate constant (ß) with decreased renal function was seen. Stronger correlations were observed between ketoprofen pharmacokinetic indices and renal clearance of KT_{conj} (CLr_{conj}). CLr_{conj} was reduced with diminished renal function.

CLr_{conj} was significantly reduced after the 100 mg dose suggestive of saturated clearance. Renal impairment reduces renal clearance of KT_{conj} and this appears to be rate-limiting for ketoprofen clearance.

Six subjects received 3 different ketoprofen formulations: enteric-coated, sustained-release, solution. Pharmacokinetic analysis from urine concentration data

showed maximal excretory rates for the solution, and for the S > R enantiomer. Mean urinary S:R ratios were significantly greater for EC formulation, perhaps related to intestinal site of absorption.

Twelve adult rheumatoid patients received 200, 400, 800, and 1200 mg ibuprofen doses every 8 hours, for two consecutive weeks with weekly clinical, laboratory and compliance measures. Trough enantiomeric ibuprofen levels weekly showed S-ibuprofen concentrations correlated better with activity variables than total ibuprofen concentration or R-ibuprofen or dose.

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NSAID, nonsteroidal anti-inflammatory drug

AUC, area under concentration-time curve

t1/2, half-life

CL/F, oral clearance

Vd/F, volume of distribution

A, arthritic

NA, non-arthritic

RA, rheumatoid arthritis

OA, osteoarthritis

HPLC, high performance liquid chromatography

$C_{last,}$, plasma concentration at last data point

$CL_{R(conj)}$, renal clearance of conjugated drug

$\Sigma X_{U(conj)}$, cumulative urinary excretion of conjugated drug

AUC_{conj}, area under concentration-time curve for conjugated drug

CL_{Cr}, creatinine clearance

Tmax, time taken after dosing to achieve maximal drug concentration

Cmax, maximal plasma concentration of drug

AUC_{0-24}, area under concentration-time curve to 24 hours

CHF, congestive heart failure

ß, terminal elimination rate constant

1 INTRODUCTION

1.1 RATIONALE FOR STUDYING NSAID KINETICS AND OPTIMIZING NSAID USE

1.1.1 PREVALENCE OF ARTHRITIS CONDITIONS

Arthritis and other rheumatic conditions are leading causes of physical disability, increased health-care use, and an impaired quality of life, especially in the population age 65 years or older. Data obtained from the 1980 Canada health survey indicate that approximately 16 percent of the Canadian population suffer from arthritis and rheumatism (Lee et al. 1985). In the 1990 Ontario health survey, musculoskeletal disorders ranked first in prevalence as a cause of chronic health problems, long-term disability, and consultations with a health professional (Ontario Ministry of Health 1992). With the aging of the Canadian population, the future impact of these conditions is likely to become greater.

There is a greater prevalence of these disorders among females than males, and the prevalence rates increase with age in a greater than linear fashion (Towheed 1998). The most frequent cause of disability in the Canadian health and disability survey (1983-84) was arthritis, affecting 7 percent of Canadians, and this also increased with age. Disability related to musculoskeletal disorders is projected to increase by 34 percent by 2020 (Badley & Crotty 1995).

The U.S. National Institute of Arthritis and Musculoskeletal and Skin Diseases, based on data from the U.S. 1989-1991 national health interview survey, has reported a

prevalence rate of arthritis and other rheumatic conditions of 15 percent in 1990, and this is projected to increase to 18.2 percent of the 2020 population (Helmick et al. 1995). Prevalence rates of arthritis were higher for older persons, women, residents of non-metropolitan areas, and those with less education or lower income.

Women with arthritis were significantly more likely to report fair or poor health, and a physician diagnosis of angina, myocardial infarction, hypertension, diabetes, stroke, lung disease, and hearing and vision problems (Hochberg et al. 1995). People with osteoarthritis (estimated prevalence of 12.1 percent of U.S. adults) may be more susceptible than non-arthritics to other common comorbid conditions that are prevalent in the elderly (Gabriel et al. 1995).

1.1.2 PREVALENCE OF USE OF NSAIDS

Nonsteroidal antiinflammatory drugs (NSAIDs) are among the most frequently used drugs in most countries. Use of NSAIDs increases with age, primarily for symptoms associated with osteoarthritis and other chronic degenerative and inflammatory musculoskeletal disorders. Population-based studies have shown that 10 - 20% of elderly people ($\geq$ 65 yrs) have a current or recent NSAID prescription (Griffin 1998). Over a 6 month period in Alberta, 27% of elderly people were prescribed NSAIDs. In Tennessee, USA, 40% of elderly people received at least one NSAID prescription annually, and 6% had NSAID prescriptions for > 75% of the year (Hogan et al. 1994).

Pharmacoepidemiologic studies have indicated that elderly females are more likely to be using NSAIDs than elderly males, and that elderly patients are less likely to change their NSAID prescription over time than younger patients. (Stuck et al. 1995)

1.1.3 PREVALENCE OF NSAID TOXICITY

NSAIDs cause a wide variety of side-effects. The most clinically important adverse effects are upper gastrointestinal (GI) tract dyspepsia, peptic ulceration, hemorrhage, and perforation, leading to death in some patients. The incidence of gastrointestinal adverse effects with NSAIDs is reported to be as high as 25%, and the most common NSAID-associated side-effect is epigastric pain/indigestion. (Griffin 1998)

Female users of NSAIDs have been reported to have a higher frequency of adverse effects than males. (Roth and Bennett, 1992). More complete analysis, however, has not substantiated gender as an important risk factor for serious gastrointestinal toxicity for these medications (Gabriel et al. 1991). Factors that increase the risk of serious peptic ulcer disease include older age, history of peptic ulcer disease, gastrointestinal hemorrhage, dyspepsia and/or previous NSAID intolerance, as well as the presence of co-morbidities. It must be noted that dyspepsia and similar symptoms alone have not been considered an accurate clinical correlate to the presence of significant NSAID-induced peptic ulcer disease or its complications. It has been estimated that approximately 10% of the excess mortality observed in rheumatoid arthritis may be attributable to medication use, in particular NSAIDs and corticosteroids (Hawker 1997).

There have been a number of studies that have confirmed that the risk of upper GI toxicity is dose-related. The low risk of gastrointestinal complications associated with ibuprofen appears to be attributable to the low doses that are prescribed routinely in clinical practice. Higher doses of ibuprofen were associated with relative risks similar to those of naproxen and indomethacin (Rodriguez 1998). A number of studies have provided comparative data on NSAIDs at 'high' and 'low' doses (as defined in the original reports), showing that the risk of toxicity was dose related (Rodriguez 1997).

One study identified higher plasma concentrations of piroxicam in patients presenting to hospital with an NSAID-related upper GI hemorrhage than in community controls matched for age, gender, dose, and time interval from last dose (Wynne & Rawlins 1993).

The point prevalence of ulcers in patients on long term NSAID treatment is about 20% and the annual incidence of complications in these patients is 1 - 4%; 1200 patients in the United Kingdom die each year as a result (Hawkey 1997). Many studies have shown that use of NSAIDs increases the risk of peptic ulcer complications by 3 to 5 times, and in several different populations it has been estimated that 15 - 35% of all peptic ulcer complications are due to NSAIDs (Griffin 1998). In the US alone, there are an estimated 41,000 hospitalizations and 3300 deaths each year among the elderly that are associated with NSAID use (Singh et al. 1996).

NSAIDs can produce a variety of other adverse effects including lower GI tract dysfunction (Davies 1995). Renal effects including acute or chronic renal failure, hyperkalemia, papillary necrosis, interstitial nephritis, impaired free water or sodium

excretion leading to edema, hyponatremia, or congestive heart failure, or aggravation of hypertension can produce significant morbidity or mortality (Bennett et al. 1996). Older individuals are more predisposed to the renal adverse effects of NSAIDs because of 1) age-associated changes in renal function 2) the prevalence of comorbid conditions (congestive heart failure, hypertension, hepatic cirrhosis, renal insufficiency) 3) the pervasive use of concomitant drugs that affect kidney function (diuretics, antihypertensives) (Ailabouni & Eknoyan 1996).

Much less frequently encountered drug-induced toxicities include hypersensitivity reactions, pulmonary airways reactivity and bronchospasm, hepatotoxicity, cutaneous reactions, and hematological disturbances (Skeith et al. 1994). Clinical guidelines have been published for general NSAID use, but the development of new COX-2 specific agents will alter these recommendations (Tannenbaum et al. 1996).

1.1.4 NONSTEROIDAL ANTIINFLAMMATORY DRUGS

These medications (NSAIDs) consist of a number of chemically different classes of compounds, but characteristic of all NSAIDs is their pharmacologic action of reducing inflammation and its resultant clinical symptoms. These drugs have numerous demonstrated cellular effects but it is believed that their major mechanisms of action include prostaglandin synthesis inhibition (Vane & Botting 1990) and neutrophil inactivation *via* interference with stimulus-receptor coupling (Abramson & Weissmann 1989).

The 2-arylpropionic acid class (2-APA) of NSAIDs is characterized by each member having a chiral centre: an asymmetric carbon α to the carboxylic acid moiety (other NSAIDs that possess a chiral center include sulindac, etodolac and ketorolac). The R-enantiomer of some of the 2-APAs may undergo *in vivo* irreversible inversion to the S-enantiomer, but this inversion is variable between drugs and between species. The members of this class that are in clinical use currently include ibuprofen, ketoprofen, naproxen, tiaprofenic acid, fenoprofen, oxaprozin, and flurbiprofen. The two compounds utilized in the following studies were ketoprofen and ibuprofen.

1.1.4.1 NSAID Disposition and Metabolism

Most of the NSAIDs available in this country have a number of shared properties. The absorption of these compounds is almost complete after oral administration. Most of these drugs are lipophilic, weakly acidic (pk_a of 3.5 to 6), and bind extensively to plasma proteins (> 90% bound to albumin for most). All of these compounds are ionized at physiologic pH and therefore are principally confined to the vascular compartment with little tissue binding (Vd of 0.1 - 0.3 L/kg).

Metabolic drug processes are usually divided into phase I steps, which are asynthetic reactions usually involving hydroxylation, reduction, or hydrolysis. In contrast, phase II processes concern conjugation reactions usually involving sulfate, glucuronic acid, glycine, or acetate. Most NSAIDs are involved primarily in phase I reactions followed by phase II reactions, but some such as ketoprofen and tiaprofenic acid, involve only phase II steps (acyl-glucuronidation). This elimination through hepatic metabolism

(oxidation and/or conjugation [glucuronidation or sulfation]) is associated with generation of active metabolites for some compounds (ASA, sulindac, nabumetone, diclofenac, oxaprozin, fenoprofen, phenylbutazone).

The principal routes of elimination of the 2-APAs are phase I oxidation and phase II conjugation with acyl glucuronide formation on the carboxylic acid moiety to give the primary urinary metabolite. The *in vivo* carboxylic acid glucuronidation is best described by reversible metabolism as the formed glucuronides are hydrolysable by esterase-catalyzed hydrolysis (Spahn-Langguth & Benet 1992). This process has been described as the 'futile-cycle' of conjugation-deconjugation (Rowe & Meffin 1984). This process for the 2-APA compounds appears to be stereoselective, preferentially favoring the R-enantiomer (Nakamura & Yamaguchi 1987). These glucuronide conjugates are diastereomers as glucuronic acid is chiral. It varies between drugs and between species whether 2-APA glucuronide formation is stereoselective.

It has been stated that the most important factor in determining observed distribution and elimination pharmacokinetics of NSAIDs is their plasma protein binding characteristics (Verbeek et al. 1983). These drugs bind preferentially to albumin rather than α_1 – acid glycoprotein or lipoproteins, specifically at site I (warfarin/azapropazone site) (Muller 1989). As a result of their extensive binding to plasma proteins (>99%), the 2-APAs are characterized by a relatively low volume of distribution (< 0.2 L/kg) and Cl_{TB} (< 100 ml/min) (Verbeek et al. 1983).

It has been demonstrated that a number of 2-APAs can be incorporated into lipid tissues as triacylglycerols, including fenoprofen, ibuprofen and ketoprofen. The prior

formation fo the acyl-CoA thioester was necessary and this reactive acyl-CoA could undergo chiral inversion or formation of glycerolipid. It has been found that only the R-enantiomer is capable of forming the hybrid triglyceride and subsequent incorporation into lipid tissue (Williams et al. 1986).

The synovial fluid (presumed site of action) kinetic profiles for these drugs almost always demonstrate slower elimination rates from that compartment than from measured plasma values, but this varies between individual NSAIDs. The elimination half-lives of these drugs vary considerably from 0.25 hrs to over 70 hrs, and this parameter affects dosing schedules (Verbeek 1988).

1.1.4.2 Stereochemistry of NSAIDs

Many of the NSAIDs currently in use exhibit molecular asymmetry or chirality, defined as the property of an object to display non-superimposability on its mirror image (Testa 1982). The chiral centre is the atom in the molecule to which are attached separate dissimilar chemical groups. These chiral compounds are administered as enantiomeric pairs (except for naproxen, a single enantiomer), with the individual isomers (R- or S-) having potentially distinct pharmacokinetics or pharmacodynamics. As the pharmacologic anti-inflammatory activity of the racemate resides almost entirely in the S-enantiomer (eutomer), analysis of individual enantiomeric disposition is crucial for optimal dosage regimens in clinical practice (Hutt & Caldwell 1984).

Absorption of individual enantiomers of the same compound would be expected to be identical, as physicochemical characteristics of these enantiomers (partition coefficient, pKa) are identical. The C_{max} and T_{max} of flurbiprofen (Jamali et al. 1988a) and ketoprofen

enantiomers (Foster et al. 1988) were shown to be identical, confirming this lack of stereoselectivity in absorption.

Several 2-APAs have shown similar T_{max} values but differences in C_{max}, including ibuprofen (Jamali et al. 1988b). The observation that the C_{max} (S/R) ratio increased proportional to the duration of residency in the GI tract led to speculation that this was a major site for enantiomeric inversion. Similar findings with ibuprofen were described by Avgerinos & Hutt (1990) with a C_{max} (S/R) ratio of 1.4. This process for ibuprofen has been characterized as stereoselective first-pass metabolism (chiral inversion) rather than stereoselective absorption.

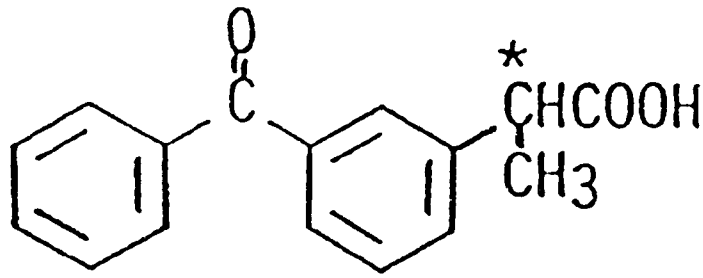
The 2-APA class of NSAIDs have the potential for enantiomeric inversion: a uni-directional *in vivo* enantiomeric inversion, whereby the R-enantiomer (distomer) may be inverted to the active antipode, the S-enantiomer (eutomer). The reverse reaction has been reported with some of these compounds in other species, but not in man. This inversion does not occur appreciably with tiaprofenic acid, indoprofen, carprofen, or flurbiprofen (Jamali 1988). This inversion process has been shown to be enzymatically mediated, with only the R-enantiomer of susceptible 2-APAs being capble of forming a CoA-thioester, with subsequent epimerization to its antipode (Nakamura et al. 1981, Knihinicki et al. 1989).

The majority of 2-APAs are bound stereoselectively. This can affect disposition as the clearance of each enantiomer is proportional to its respective unbound fraction in plasma, with the least bound having a higher clearance. Competitive enantiomer-

enantiomer protein binding has been suggested for a number of the 2-APAs, including ibuprofen (Lee et al. 1985).

1.1.4.3 Ketoprofen

This compound, 3-benzoyl-α-methyl-benzeneacetic acid, has the structure as



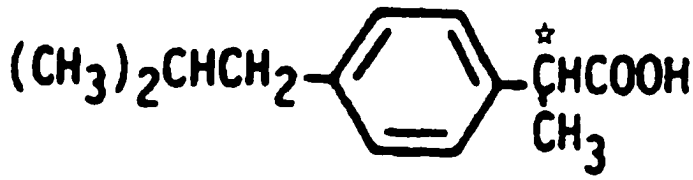
illustrated: Ketoprofen

(* denotes chiral centre)

Ketoprofen is practically insoluble in water. It is absorbed rapidly and completely after oral administration, with peak plasma levels in 0.5 to 2.0 hours. Its pharmacokinetics are linear over the usual prescribed dose range (up to 200 mg doses) with no accumulation on chronic dosing. It is > 99% protein bound and mean plasma elimination half-life ($t_{1/2}$) varies from 2 to 4 hours. Ketoprofen is predominantly metabolized by acyl-glucuronidation with subsequent renal elimination of the conjugates. It has been extensively used clinically for several decades at doses up to 300 mg/day and its enantioselective pharmacokinetics have been well documented (Jamali & Brocks 1990a).

1.1.4.4 Ibuprofen

This commonly used drug, (±)-α–methyl-4-(2-methylpropyl)-benzeneacetic acid,



Ibuprofen

has the following structure:

(* denotes chiral centre)

Ibuprofen is absorbed rapidly after oral administration and peak plasma levels are attained in 1 to 2 hours after dosing. It is only very slightly soluble in water with a pK_a of 5.2. Pharmacokinetics are linear in the usual dose range and the drug does not accumulate (maximum recommended daily dose of 3200 mg) (Evans et al. 1990). It is metabolized in the liver, mainly *via* oxidation, and metabolic products are eliminated in the urine. The plasma $t_{1/2}$ varies between 1.8 and 2.0 hours in most patients, and it is extensively protein bound (98 - 99%). In an evaluation of the protein binding and competitive inhibition parameters of R-ibuprofen and S-ibuprofen administered as single enantiomers or as the racemate, the intrinsic binding of Rr-ibuprofen was greater than S-ibuprofen, and the unbound fraction was significantly greater for S-ibuprofen *vs.* R-ibuprofen after a given dose of R-ibuprofen or racemate (Paliwal et al. 1993).

S-ibuprofen has been administered as a single enantiomer formulation to normal subjects and to arthritis patients. The bioavailability was independent of dose within the

usual range. The MRT of S-ibuprofen was significantly shorter for the S-enantiomer administered alone than after R-ibuprofen or racemate administration. Clearance values and $t_{1/2}$ were similar after single enantiomer or racemate dosing. S-ibuprofen was not inverted to R-ibuprofen when given as the single enantiomer. This formulation clinically appeared to exhibit efficacy as antirheumatic therapy (Brune 1990).

1.2 FACTORS AFFECTING NSAID KINETICS

1.2.1 NSAID FORMULATIONS

The specific formulation in which NSAIDs are administered can have a significant effect on observed disposition parameters and can be of major clinical relevance when these drugs are prescribed for specific clinical situations.

The stereospecific pharmacokinetics of ketoprofen enantiomers were evaluated for equivalent doses as an oral solution and as a regular release tablet. Values for Cmax were higher and for Tmax were reduced for the oral solution but AUC values were similar for both enantiomers (Stiegler et al. 1995).

1.2.2 DRUG INTERACTIONS

Many individuals that use NSAIDs have concurrent conditions that require pharmacological therapies. As well, because of the significant GI morbidity from these agents, many patients utilize GI tract medications to relieve symptoms and/or for prevention of serious bleeding consequences. These medications all have the potential for

interacting with NSAIDs to alter their pharmacokinetics and pharmacodynamics. These potential interactions have been evaluated with a number of currently used agents.

The concurrent use of antacids (magnesium hydroxide/citrate) was examined for ketoprofen enantiomer pharmacokinetic alterations and was found to result in higher Cmax values with reduced Tmax and MRT but no significant changes in AUC (Fuder et al. 1997). Overall, in a number of similar studies, the concurrent use of antacids has not had a significant effect on NSAID disposition.

Other medications that influence gastrointestinal physiology may indirectly alter NSAID pharmacokinetics by influencing the rate of gastric emptying and/or oro-cecal transit. Salicylic acid absorption was rate-limited by gastric emptying, influenced by metoclopramide, but not by codeine phosphate's reduction in oro-cecal transit time. The overall extent of absorption was not influenced by either drug (Riley et al. 1992).

1.2.3 CLINICAL AND PATHOPHYSIOLOGICAL FACTORS

1.2.3.1 GENDER

Although there are some examples of gender-related differences in the pharmacokinetics of several NSAIDs such as ketoprofen (Palylyk 1994) or etodolac (Brocks 1993) in animal species other than man, there is very limited data in human NSAID pharmacokinetic studies of significant differences between the sexes.

Acetylsalicylic acid (ASA) disposition does exhibit gender-related dimorphism. Acetyl-transferase, which metabolizes ASA, is less active in women than in men (Famaey

& Paulus 1992). Males also have a decreased oral but a more rapid intramuscular absorption rate for ASA with lower AUC values and greater elimination rates after oral dosing (Aarons et al. 1989).

1.2.3.2 AGE

As NSAIDs are used by patients of every age group, an accurate appraisal of age-related alterations in the pharmacokinetics and pharmacodynamics of these drugs is essential for the formulation of valid dosing guidelines. The demographics of developed and developing countries is changing, with an increasing proportion of people over the age of 65. This trend will continue and will become more prominent in the future. The physiologic consequences of aging affecting individuals to varying extents and at differing rates (Greenblatt et al. 1982), concurrent pathophysiologies and their prescribed therapies, genetic metabolic polymorphisms and the variable effect of each of these factors on individual drug disposition together produce a substrate of clinical complexity from which to draw accurate recommendations. However, a number of NSAID pharmacokinetic evaluations comparing different age groups have been carried out yielding clinically relevant data.

Human physiology changes with advancing age and there are a number of such changes that might be expected to affect the disposition of drugs. Absorptive processes are changed slowly with age. Achlorhydria is more common in old age and gastro-Intestinal motility is reduced, including delayed gastric emptying (Evans et al. 1981). Although the rate of absorption of a drug may be slowed somewhat, the total amount of drug absorbed is usually normal (Dawling & Crome 1989). This has been demonstrated

for a number of NSAIDs such as indomethacin, aspirin, and ketoprofen (Advenier et al. 1983).

Drug disposition may be affected by a number of aging changes in the body, including a decreased total body mass, decreased plasma albumin, an increased proportion of body fat, and reduced organ perfusion. Total lean body mass decreases between 20 and 30% between the ages of 30 and 80 (Novak 1972). There is a decrease in the cellular mass in the liver and other organs and this contributes to the general decline in the activity of hepatic drug metabolizing systems with age (Rossman 1979). Renal function diminishes with age, with the average glomerular filtration rate by age 80 only approximately 60% of that at age 30 (Rowe et al. 1976).

The elderly are at an increased risk of adverse drug reactions than are younger patients, with numerous contributing factors including the greater number of drugs used and aging physiologic changes (Levy et al. 1980).

The elimination of drugs that are excreted principally through the kidney would be expected to be reduced consequent to the reduced glomerular filtration in the elderly. Renal functional impairment in the elderly may affect the disposition of some NSAIDs, resulting in the accumulation of glucuronides of the two enantiomers, which may then be hydrolyzed in plasma, causing a significant elevation of the parent drug, the so-called 'futile-cycle' (Williams 1990). It has been suggested that reduced renal excretion of benoxaprofen glucuronides in the elderly, coupled with increased inversion of R-benoxaprofen to S-benoxaprofen, may have been partly responsible for the toxicity of this drug in elderly patients (Taggert & Alerdice 1982). Advenier et al. (1983) found a

significantly prolonged $t_{1/2}$ of ketoprofen in elderly patients, with increased AUC values. Other studies have shown that the S-enantiomer conjugates are increased in the plasma of elderly subjects (Foster et al. 1988). Tiaprofenic acid, another 2-APA NSAID, has been shown to have no alterations in disposition in elderly subjects with arthritis but normal renal function (Hosie & Hosie 1987).

Evaluations of NSAID pharmacokinetic alterations with age in animal models have yielded some potential insights. A study comparing ketoprofen disposition in young adult and senescent male Fischer 344 rats showed plasma clearance of free drug and steady-state Vd (free drug) were significantly lower in the aged rats, suggesting reduced metabolic activity and decreased ketoprofen binding to tissue components respectively (Satterwhite & Boudinot 1992).

Piroxicam steady state pharmacokinetics in children aged 7 to 16 with rheumatoid arthritis showed that clearance in this age group was higher than in young adults with a similar V/F (Makela et al. 1991).

In another pediatric study, ibuprofen pharmacokinetic parameters did not alter in an age range of 3 months to 10.4 years, but an antipyretic pharmacodynamic evaluation indicated that in younger children the antipyretic effect was of earlier onset and of greater magnitude (Kauffman & Nelson 1992).

Serum albumin levels tend to decrease with age and this has been shown to affect NSAID protein binding. Elderly patients with and without renal impairment had significantly decreased S-ibuprofen enantiomer binding compared to young adults. In this same study the S-ibuprofen pharmacokinetics were significantly different in the elderly

compared to young adults as well: the $t_{1/2}$ was increased and unbound clearance was decreased (Rudy et al. 1995). Unbound plasma piroxicam concentrations were found to increase with age in female osteoarthritis patients, with significantly higher levels in females than males (Hundal & Rugstad 1993).

A dosage reduction of 25% was recommended for indomethacin in elderly patients based on observed reductions in total clearance although oral bioavailability appeared to be reduced with aging also (Oberbauer et al. 1993).

An evaluation of flurbiprofen pharmacokinetics in younger and elderly patients with rheumatoid arthritis indicated that enantiomer pharmacokinetics are linear at all ages with no significant differences observed between the two age groups except for a significant correlation for free drug clearance with age (Kean et al. 1992). Another study of a sustained-release formulation of flurbiprofen in young and elderly subjects found no difference between the two age groups in any pharmacokinetic parameter (Hamdy et al. 1990).

Benorylate is an ester that is readily absorbed and rapidly hydrolysed in the blood to ASA and acetaminophen. The pharmacokinetics of this compound were studied in young adults and elderly arthritic subjects and higher total and free salicylic acid levels with a reduced renal clearance were identified in the elderly group (Taggart et al. 1991).

A frequently utilized formulation of various NSAIDs is topical percutaneous application and age-related physiologic cutaneous alterations affect absorption. The percutaneous permeation of ASA was significantly lower in aged subjects, mainly due to

diminished surface lipid content, and is typical of absorption patterns of compounds with limited lipid solubility (Roskos et al. 1989).

The pharmacokinetics of different NSAIDs are not predictably altered by aging, and studies to date have shown significant changes with salicylate, diflunisal, ibuprofen, naproxen, ketoprofen, indoprofen, azapropazone, dipyrone, sulindac, ketorolac, and nabumetone. Clinical alterations in use of these compounds must be based upon evaluation of available data. Although the prevalent clinical strategy of starting elderly patients with lower doses and gradually increasing their exposure to medication has been verified to be practical and appropriate, it is still imperative that such pharmacokinetic evaluations for each agent be conducted to prevent excessive morbidity or mortality, such as occurred with benoxaprofen.

1.2.3.3 PRESENCE OF ARTHRITIS OR INFLAMMATION

There are a variety of different types of arthritis that afflict patients with differing mechanisms and pathophysiologies. Osteoarthritis is a degenerative non-inflammatory condition with no systemic effects and is not expected to alter drug disposition in any manner. Rheumatoid arthritis and other autoimmune inflammatory disorders are characterized by a plethora of inflammatory mediator effects, including hepatic metabolic alterations, reduced plasma albumin, anemia and even pyrexia. These conditions may significantly alter the disposition of different compounds. Such alterations would be expected mostly for drugs with an extensive hepatic first-pass clearance (Kirch et al. 1983)

or highly protein bound drugs if plasma protein levels are significantly reduced. None of the available NSAIDs fall into the first category but most are highly protein bound.

To date a number of studies have attempted to determine if arthritis does affect the pharmacokinetics of a number of different NSAIDs with variable results (Crock et al. 1982, Eriksson et al. 1989). It has been shown that the volume of distribution of the total (bound and unbound) naproxen is increased in rheumatoid arthritis patients compared to normal subjects; however, the same parameter is reduced for the unbound drug (Van Den Ouweland et al. 1986). The clearance of naproxen has been shown to be 40% higher in patients with rheumatoid arthritis. This was attributed to a more extensive reduction in plasma protein binding than metabolic enzyme function, and was most marked during flares in disease activity with concurrent reductions in plasma albumin levels. (Van Den Ouweland et al. 1986, 1987).

Many of the attempted studies have been limited by the confounding factors of heterogenous arthritis populations, comorbidities such as variable renal function or heart failure, and the effects of aging on many participating subjects. NSAID metabolism and disposition varies between different drugs and each of the above factors will have varying influence, further limiting generalized conclusions regarding the presence of rheumatic disorders and NSAID dosing adjustments.

1.2.3.4 RENAL FUNCTION

There are a number of regulatory mechanisms that control glomerular and tubular function in the kidney. Prostaglandins produced within the kidney, both in cortical and medullary compartments, can be an important regulatory pathway due to their relaxant effects on vascular smooth muscle and glomerular mesangial cells (Garella & Matarese 1984). Some patients may have a prostaglandin-dependent renal blood flow and glomerular filtration rate. The use of NSAIDs, by their prostaglandin-inhibitory effects, can significantly affect this renal homeostasis.

The most common adverse renal effect of NSAIDs in patients is the reduction in renal blood flow and renal clearance as a consequence of renal prostaglandin inhibition (Downie 1991). This can result in fluid retention and edema and renal failure, which in turn can impede clearance of a number of medications, including some NSAIDs. This NSAID-induced renal insufficiency is more likely to occur in the presence of preexisting renal disease or reduced renal perfusion such as hypovolemia, diuretic use, congestive heart failure, cirrhosis or nephrosis. Some authors have reported in the past that renal disease is a frequent consequence of and cause of mortality in rheumatoid arthritis (Laakso et al. 1986), although with current treatment strategies this frequency has declined. Other medications used in the treatment of arthritis patients as well as comorbidities can produce declines in renal function.

Additional less frequent renal toxicities due to NSAID use include interstitial nephritis and the nephrotic syndrome. This is presumed to have an allergic basis and it is unlikely that pharmacokinetic factors play any role in this infrequent complication. It has been shown that prostaglandin I_2 represents the major cyclooxygenase product in human glomeruli and that it acts as a local modulator in the control of human juxtaglomerular function, enhancing the release of renin. The inhibition of prostaglandin I_2 production by NSAID use with reduced renin release will lead to reduced aldosterone secretion and as a consequence hyperkalemia.

Whether due to arthritis or to concomitant diseases, NSAIDs or concurrent medications, impaired renal function occurs in a portion of arthritis patients prescribed NSAIDs. Attempts to evaluate the pharmacokinetic alterations of various NSAIDs in the presence of variable levels of renal failure have been carried out with the aim of rationalizing dosing regimens in these patients. Given the varied metabolic and excretory pathways of different compounds, these disposition studies and derived dosing recommendations have to be specific for each individual NSAID. For these evaluations of chiral NSAIDs administered as active and inactive enantiomers, stereospecific kinetic analysis is necessary to characterize enantiospecific alterations in drug disposition induced by altered renal function.

Relative to normal healthy subjects, it has been shown that uremic subjects ingesting flurbiprofen exhibited a significantly greater oral clearance and V(z) and percent unbound for both enantiomers. Unbound clearance and V(z) did not differ from normal

subjects, indicating that adjustment of dosing schedules for flurbiprofen is not needed on the basis of pharmacokinetics (Knadler et al. 1992).

In an evaluation of ibuprofen in elderly patients with and without renal impairment it was found that unbound clearances of glucuronidation and hydroxylation were reduced in renal impairment (Rudy et al. 1995). Patients with renal impairment given ibuprofen showed elevated S-ibuprofen levels and AUC values. It appeared that the metabolic activation of R-ibuprofen to S-ibuprofen was increased in renally compromised patients. This reduced renal clearance coupled with increased metabolic inversion resulted in elevated s-ibuprofen levels that potentially could exacerbate renal hemodynamic abnormalities in these subjects by inhibiting prostaglandin synthesis (Chen & Chen 1994). Renal failure was shown to produce elevated s-ibuprofen AUC values and higher S/R AUC ratios, with advanced age and hypertension being two independent clinical variables that correlated with these pharmacokinetic findings (Chen & Chen 1995).

Diflunisal dosage reductions in renal failure were also recommended due to identified reductions in overall clearance. An accumulation of glucuronide and sulphate conjugates in plasma with hydrolysis to the parent diflunisal itself were reported (Dickinson et al. 1991).

1.2.3.5 OTHER FACTORS

The effect of food on the plasma concentration-time profile of a number of NSAIDs has been studied. Results indicate that while qualitative changes in the plasma concentration-time curves are primarily influenced by the nature of the formulation and the

type of meal, bioavailability is influenced by the absorption characteristics of the drug as well. This was studied with sustained release formulations of ibuprofen and flurbiprofen and indicated that food produced a significantly increased Cmax and AUC with a delayed Tmax (Pargal et al. 1996). The single dose pharmacokinetics of oral meloxicam were not significantly affected by food including high-fat meals (Turck et al. 1995).

Circadian variations in the effectiveness, toxicity and pharmacokinetics of different NSAIDs have been demonstrated, as well as similar fluctuations in disease states, adding another factor to consider when dosage schedules are recommended (Labreque et al. 1995).

There have been hypotheses advanced that the elimination half-life of individual NSAIDs is of primary importance in determining the risk of serious adverse effects. Should an adverse event occur, the prolonged presence of the drug in the body might aggravate this reaction. Initial correlations between serious GI events and deaths with NSAID half-life did support this relationship (Adams 1987). Many other studies since then utilizing various methodologies have failed to demonstrate in a consistent fashion such a correlation, however (Hochberg 1992). Recent meta-analysis of the variability in risk in gastrointestinal complications with individual NSAIDs showed inconsistent results with both short $t_{1/2}$ (ketoprofen) and long $t_{1/2}$ (piroxicam) agents ranked as most toxic (Henry et al. 1996).

1.3 FURTHER OPTIMIZATION OF NSAID DOSING REGIMENS

1.3.1 DOSE-DEPENDENT PHARMACOKINETICS

Alterations in the pharmacokinetics of a number of different NSAIDs with increasing dose have been demonstrated, although this may occur only at the upper end of the recommended dose range or in excess of pharmacologic doses. These pharmacokinetic alterations might be on the basis of dose-dependent protein binding (salicylates, diflunisal, phenylbutazone, nabumetone, sulindac, naproxen, ibuprofen and oxaprozin) or due to saturable metabolic pathways (salicylates and oxaprozin).

Oxaprozin clearance for the total drug was increased while that of the unbound drug was decreased after repetitive dosing. This inverse pharmacokinetic behavior has been attributed to the two noncompensatory kinetic effects: concentration dependent protein binding and saturable metabolism of oxaprozin (Karim 1996).

1.3.2 NSAID PHARMACODYNAMICS

To date there has been limited success in demonstrating a valid dose or concentration-response for different NSAIDs in relation to anti-rheumatic efficacy. A number of different reasons for this have been advanced including: intersubject variability in response to these agents, the imprecise nature of some measured clinical response variables, variability in pharmacokinetic profiles for different individuals, limited relationship between plasma and synovial fluid pharmacokinetic profiles for many

NSAIDs, intrinsic disease variability over time, and measurement of total rather than active enantiomeric drug concentrations in many of these studies.

A significant dose-response relationship has been demonstrated for naproxen in rheumatoid arthritis patients (Day et al. 1982) (administered as the single active enantiomer), and for fenclofenac (Dunagan er al. 1986) and carprofen (Furst et al. 1988). However, many other evaluations of different NSAIDs have failed to demonstrate a significant correlation between efficacy measures and either dose or concentration in plasma or synovial fluid.

1.4 Hypotheses

1. It is possible to improve optimal NSAID dosing recommendations if the pharmacokinetic variability induced by clinical, pathophysiologic and pharmaceutical cofactors is characterized.

2. Pharmacokinetic variation due to alterations in age, the presence or absence of rheumatic disorders or variable drug dose can be determined for individual NSAIDs.

3. Renal impairment can interfere with clearance of some NSAIDs and their metabolites and can result in increased exposure to the drug and can possibly alter enantiomeric inversion.

4. Different formulations of an NSAID can exhibit pharmacokinetic variability in the same individual, and for the 2-APA compounds, may affect presystemic enantiomeric inversion and enantiomeric bioavailability due to absorption site variability.

5. A significant correlation (pharmacodynamic relationship) between active 2-APA NSAID enantiomer plasma levels and clinical effects can be determined in patients with active inflammation.

1.5 Objectives

1. To assess the pharmacokinetic profiles of different doses of ketoprofen in elderly subjects, with comparison between healthy arthritic and non-arthritic study populations, as well as evaluating the effects of variable levels of renal function in elderly subjects.

2. To accurately assess the differences in pharmacokinetics of ketoprofen and ketoprofen acyl-glucuronide conjugates observed in individuals with varying levels of renal function.

3. To determine the variability in pharmacokinetics, including enantiomeric inversion, as assessed using urinary excretion data, in healthy subjects receiving sequentially different formulations of ketoprofen including oral solution, enteric-coated, and sustained release.

4. To characterize the pharmacodynamics (clinical and laboratory assessments) of ibuprofen enantiomers in moderately active rheumatoid arthritis patients receiving in a randomized fashion four different ibuprofen doses, measuring trough (pre-dose) enantiomer levels.

2 KETOPROFEN PHARMACOKINETICS IN THE ELDERLY: INFLUENCE OF RHEUMATIC DISEASE, RENAL FUNCTION, AND DOSE

2.1 ABSTRACT

An age-related accumulation of ketoprofen due to a reduced clearance has been reported in the elderly. Other studies have not observed these changes in the kinetics of unchanged ketoprofen, but have reported increased plasma levels and reduced urinary excretion of conjugated ketoprofen. We examined the effects of dose, renal function, and the presence of arthritis on the stereoselective kinetics of ketoprofen in 5 non-arthritic and 6 arthritic subjects. There was a significant difference in renal function (CL_{Cr}, ml/min; arthritic 71.8±12.3, non-arthritic 91.4±11.1) but not in age or weight between the 2 groups. Subjects received 50 mg and then 150 mg of enteric-coated racemic ketoprofen, and plasma and urine samples were collected for 24 hours. No significant differences in CL/F, AUC, $t_{1/2}$, Tmax or Cmax were found between groups or between doses, and values were similar to those previously reported in young adults. Urinary ketoprofen conjugate S:R ratio was 1.6±0.25 and 1.65±0.27 for arthritic and non-arthritic subjects. Greater amounts of conjugated ketoprofen enantiomers were present in the plasma of the arthritic compared to non-arthritic subjects. Renal clearance of ketoprofen conjugates exhibited stereoselectivity (R > S), and was decreased in the arthritic group. Significant

(This chapter published in part: J Clin Pharmacol 33: 1052 – 1059, 1993)

accumulation of unchanged ketoprofen was not found to occur in elderly subjects in the presence or absence of rheumatic disease or moderate renal impairment. No dosage adjustment is necessary for ketoprofen based on age *per se*.

2.2 RATIONALE

Ketoprofen is a nonsteroidal anti-inflammatory drug (NSAID) that is widely utilized and well-tolerated in the elderly population of patients with rheumatic conditions (Le Loet, 1989). Significant alterations in ketoprofen pharmacokinetics in the elderly compared to young adults have been reported by some investigators (Advenier *et al.* 1983, Dennis *et al.* 1985) but not by others (Foster *et al.* 1988). Advenier *et al.* (1983) observed a substantial accumulation of ketoprofen with increased AUC and $t_{1/2}$ and reduced CL/F and Vd/F in elderly subjects. They proposed that these findings were due to an age-related reduction in glucuroconjugation. A reduction in Vd/F in these patients from aging-related decreases in total body water was also thought to be contributory. Another postulated mechanism was *in vivo* hydrolysis of conjugated ketoprofen back to the parent drug resulting in the observed larger AUC compared to young adults (Verbeeck *et al.* 1984). More recently Dennis *et al.* (1985) in a study of controlled release ketoprofen in elderly subjects found AUC values 65% greater than in previously reported young volunteers. Neither of these studies utilized stereospecific methods: ketoprofen is a racemic drug with antiinflammatory effects ascribed mainly to the S enantiomer (Jamali 1988).

Foster *et al.* (1988) using a stereospecific approach, did not observe any significant differences in ketoprofen pharmacokinetic indices between young or elderly patients with arthritis. They postulated that the greater age of the patients in Advenier's study, or the greater dose of ketoprofen used, may

have been responsible for the contrasting results. In an attempt to clarify these different observations, and to determine whether other variables such as renal function or the presence of rheumatic disease may be more influential than age, we studied two groups of elderly subjects that differed in these characteristics. In addition, the effect of dose was also studied by giving single 50 and 150 mg doses of racemic ketoprofen.

2.3 METHODS

2.3.1 Patient Study

The project was conducted according to the principles of the Declaration of Helsinki with institutional ethics committee approval. Six arthritic (A) and 5 non-arthritic (NA) elderly volunteers were recruited for the study. All of the arthritic subjects had either rheumatoid arthritis (RA) or osteoarthritis (OA) and several had additional rheumatic diagnoses. Some of the subjects in both groups had other non-rheumatic medical conditions that were controlled or in remission at the time of the study. (see **Table 2-1: Patient Characteristics**) All of the arthritic subjects were using NSAIDs on a chronic basis but none received any NSAID for 2 to 5 days prior to study onset. All concurrent medications were continued during the study.

In a cross-over fashion subjects received initially 50 mg and then 150 mg of racemic ketoprofen in the form of 50 mg enteric-coated tablets (Orudis E-50; Rhone-Poulenc Pharmaceutical, Montreal, Canada). There was a 24 hour wash-out period between doses. After an overnight fast, subjects received their ketoprofen tablet(s), along with 200 ml of water. No food was allowed for 2 hours after dosing. Venous blood was collected *via* an indwelling catheter inserted into a forearm vein into

heparinized tubes at 0, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 15, 18, 21, and 24 hours post-dose. After collection of blood samples, plasma was separated by centrifugation. Total urine output was collected for 24 hours. All samples were stored in acid-rinsed tubes at -70° C prior to analysis.

2.3.2 Assay

Plasma and urinary concentrations of ketoprofen enantiomers present as intact and ester conjugates were measured by a reverse-phase stereospecific HPLC method (Foster & Jamali 1987). Sample volume used for analysis was 0.5 ml for plasma and 0.1 ml for urine. Calibration curves prepared using plasma and urine showed consistent linearity ($r^2 > 0.995$) between peak area ratios (drug:internal standard) and concentrations. The % error was calculated as the difference between estimated and added concentrations of drug and was less than 15%. The reproducibility (precision) was reported as intra- and inter-day assay coefficient of variation of nine samples (three replicates of each concentration/day for three days) and again was determined to be less than 15%. Samples were assayed before and after alkaline hydrolysis, and the difference between these 2 values constituted the concentration of conjugated ketoprofen enantiomers.

2.3.3 Data Analysis

Ketoprofen enantiomer concentrations were plotted versus time. The area under the plasma log-concentration time curve (**AUC**) was calculated by the linear trapezoidal rule. The area from the last data point (C_{last}) to infinity was calculated by $C_{last}/ß$. Due to excessive fluctuation in plasma

concentrations, AUC calculations for conjugated ketoprofen were carried out for 24 hours only. The apparent elimination rate constant ß was calculated from the log-linear terminal phase of the curve. Oral clearance **(CL/F = Dose/AUC)** and apparent volume of distribution **(Vd/F = CL/ß)** were also corrected for body weight in each subject and dose. Renal clearance of conjugated ketoprofen ($CL_{R(conj)}$), was assessed as $\sum X_{U(conj)}/AUC_{conj}$, where $\sum X_{U(conj)}$ is the cumulative urinary excretion and AUC_{conj} is the AUC of conjugated ketoprofen.

Statistical significance for comparison between groups was evaluated using Student's paired or unpaired t-test. The level of significance was set at $\alpha = 0.05$. Values are expressed as mean $\pm$ standard deviation.

2.4 RESULTS

The arthritic group had a significantly lower creatinine clearance (CL_{Cr}) than the non-arthritic group ($p = 0.023$) (see **Table 2-1: Patient Characteristics**), but there was no difference in age or body weight between groups. Pharmacokinetic parameters obtained from non-arthritic and arthritic subjects are summarized (see **Table 2-2: Pharmacokinetic indices of ketoprofen enantiomers in elderly non-arthritic subjects.**and see **Table 2-3: Pharmacokinetic indices of ketoprofen enantiomers in elderly arthritic subjects.**) Representative plasma concentration-time profiles for ketoprofen and ketoprofen conjugates for illustrated for each group (see Figure 2-1: Ketoprofen and conjugated ketoprofen plasma concentration-time profile in a representative non-arthritic patient (no. 3) and Figure 2-2: Ketoprofen and conjugated ketoprofen plasma concentration-time profile in a representative arthritic patient (no. 2)).

Although the differences between groups was not statistically significant, there was a trend for T_{max} to be longer for the arthritic group compared to the non-arthritic group for both doses and both enantiomers; with values as late as 4 hours for 2 arthritic patients for the 50 mg dose. Consistent with these results were the prolonged lag times of up to 4 hours seen in the arthritic group for the 50 mg dose. Within groups, the mean C_{max} values were proportional to the administered dose.

The only significant difference observed in the elimination $t_{1/2}$ between groups was an increase in the $t_{1/2}$ of S-ketoprofen in the arthritic group at the 50 mg dose, compared to the non-arthritic.

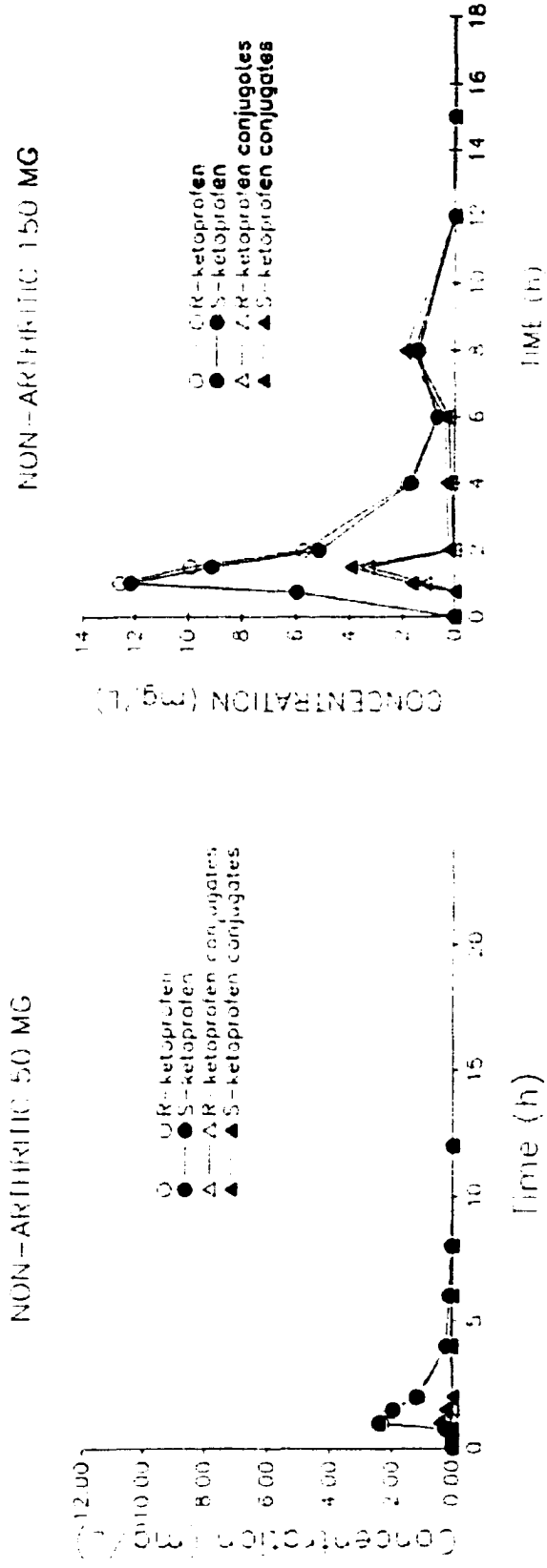


Figure 2-1: Ketoprofen and conjugated ketoprofen plasma concentration-time profile in a representative non-arthritic patient (no. 3)

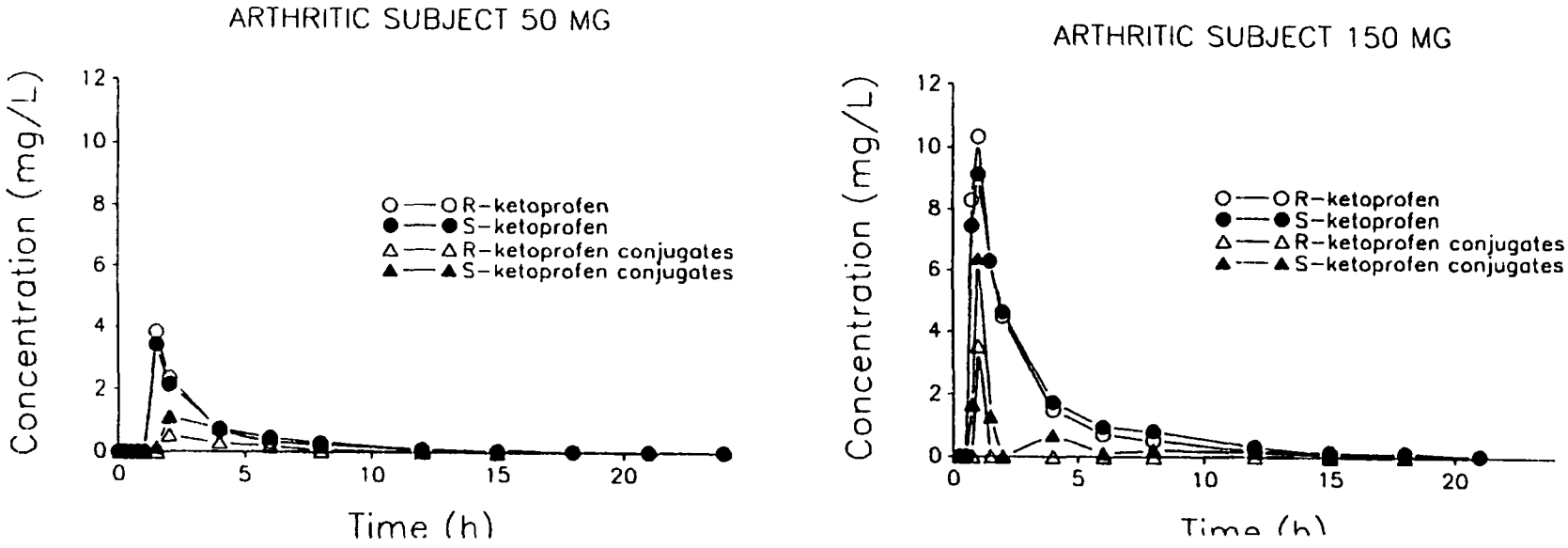


Figure 2-2: Ketoprofen and conjugated ketoprofen plasma concentration-time profile in a representative arthritic patient (no. 2)

Table 2-1: Patient Characteristics

Subject	Sex	Age Year	Weight kg	Cr_{Cl} ml/min	Rheumatic Diagnosis	Other Diagnoses	Medications
Arthritic:							
1	F	73	95	73	OA, PMR	Nil	Nil
2	M	78	82	70	RA	COPD, CHF	Prednisone, D-Penicillamine, Digoxin
3	F	85	63	84	RA	Nil	Aurothiomalate
4	F	75	67	80	OA	Depression, Hypothyroid	Amitriptyline, Lorazepam, Levothyroxine
5	F	77	57	49	OA, Pseudogout	Hypertension	Enalapril, Chloral hydrate
6	F	71	72	75	RA	Osteoporosis	Prednisone, NaF
Mean		**76.5**	**72.7**	**71.8***			
SD		4.9	13.8	12.3			
Non-arthritic:							
1	M	77	93	90	Nil	Prostate CA	Oxybutynin
2	F	72	46	89	Nil	Nil	Nil
3	F	70	74	80	Nil	Nil	Nil
4	M	77	63	88	Nil	Nil	Nil
5	M	73	84	110	Nil	Nil	Nil
Mean		**73.8**	**72.0**	**91.4***			
SD		3.1	18.3	11.1			

*Significant difference between groups. **Abbreviations**: OA, Osteoarthritis; RA, Rheumatoid Arthritis; PMR, Polymyalgia Rheumatica; COPD, Chronic Obstructive Pulmonary Disease; CHF, Congestive Heart Failure.

Table 2-2: Pharmacokinetic indices of ketoprofen enantiomers in elderly non-arthritic subjects.

Subject	Dose mg	Lag Time h R & S	T_{max}, h S	T_{max}, h R	C_{max} mg/L S	C_{max} mg/L R	$t_{½}$, h S	$t_{½}$, h R	$AUC_{0-\infty}$ (mg/L)h S	$AUC_{0-\infty}$ (mg/L)h R	Cl/F L/h/kg S	CL/F L/h/kg R	V_d/F L/kg S	V_d/F L/kg R
1	50	0.75	0.75	0.75	4.00	3.75	2.25	2.23	6.84	6.27	0.08	0.09	0.26	0.28
2	50	0.75	0.75	0.75	5.66	5.81	2.46	3.60	9.29	10.8	0.12	0.10	0.42	0.52
3	50	0.75	1	1	1.76	1.73	2.23	2.38	3.83	4.23	0.18	0.16	0.57	0.55
4	50	0.5	1.5	1.5	3.37	3.56	2.30	2.97	5.95	7.32	0.13	0.11	0.44	0.47
5	50	0.5	1	1	3.48	3.79	0.86	0.74	5.77	6.03	0.10	0.10	0.13	0.10
Mean		0.65	1	1	3.65	3.73	2.02*	2.38	6.34	6.93	0.12	0.11	0.36	0.38
SD		0.14	0.27	0.27	1.26	1.29	0.59	0.95	1.98	2.44	0.04	0.03	0.17	0.19
1	150	0.75	1	1	6.40	6.02	2.65	2.73	21.76	19.55	0.07	0.08	0.28	0.33
2	150	0.25	0.75	0.75	13.6	14.1	5.90	11.4	30.74	31.56	0.11	0.10	0.90	1.70
3	150	0.75	1.5	1.5	8.25	8.77	3.30	3.65	18.89	20.01	0.11	0.10	0.51	0.53
4	150	1.5	1.5	1.5	7.45	7.86	2.05	1.88	23.73	25.56	0.10	0.09	0.30	0.25
5	150	1	1.5	1.5	8.35	8.71	4.56	4.00	18.27	18.68	0.10	0.10	0.64	0.55
Mean		0.85	1.25	1.25	8.80	9.10	3.69	4.73	22.69	23.27	0.10	0.09	0.53	0.67
SD		0.45	0.32	0.32	2.48	2.71	1.38	3.42	5.02	5.34	0.02	0.01	0.26	0.59

* significantly different from arthritic

Table 2-3: Pharmacokinetic indices of ketoprofen enantiomers in elderly arthritic subjects.

Subject	Dose mg	Lag Time h S & R	T_{max} ,h S	T_{max}, h R	C_{max} mg/L S	C_{max} mg/L R	$t_{½}$, h S	$t_{½}$, h R	$AUC_{0-\infty}$ (mg/L)h S	$AUC_{0-\infty}$ (mg/L)h R	CL/F L/h/kg S	CL/F L/h/kg R	V_d/F L/kg S	V_d/F L/kg R
1	50	0.75	1.5	1.5	2.12	1.95	3.80	2.78	7.06	4.87	0.07	0.11	0.41	0.43
2	50	1.5	1.5	1.5	3.45	3.86	2.81	2.53	8.63	8.31	0.07	0.07	0.29	0.27
3	50	0.5	1	1.5	2.2	2.28	2.49	2.40	5.95	5.95	0.13	0.13	0.49	0.46
4	50	4	4	4	1.15	1.18	2.47	2.04	4.75	4.31	0.16	0.17	0.56	0.51
5	50	4	4	4	0.65	0.77	4.95	1.91	3.00	2.63	0.29	0.33	2.09	0.92
6	50	0.75	1.5	1.5	2.9	3.19	4.44	2.77	8.04	7.53	0.09	0.09	0.55	0.37
Mean		1.92	2.25	2.33	2.08	2.21	3.49*	2.41	6.24	5.60	0.14	0.15	0.73	0.49
SD		1.64	1.37	1.29	1.05	1.17	1.06	0.37	2.12	2.11	0.09	0.09	0.67	0.22
1	150	0.75	1.5	1.5	4.53	4.55	3.03	2.82	18.80	16.44	0.08	0.10	0.37	0.39
2	150	0.75	1	1	9.16	10.3	3.42	3.28	24.84	22.34	0.07	0.08	0.36	0.39
3	150	0.5	1	1	8.95	8.97	2.56	2.13	19.82	19.71	0.12	0.12	0.44	0.37
4	150	0.75	2	2	4.8	5.32	2.68	2.76	15.66	16.19	0.14	0.14	0.55	0.55
5	150	1.5	1.5	1.5	4.01	4.07	3.72	2.21	13.31	11.76	0.20	0.22	1.06	0.71
6	150	0.5	1.5	1.5	6.5	7.4	2.31	2.11	22.88	24.48	0.09	0.09	0.30	0.26
Mean		0.79	1.42	1.42	6.33	6.78	2.95	2.55	19.22	18.49	0.12	0.13	0.51	0.45
SD		0.37	0.38	0.38	2.27	2.54	0.54	0.48	4.32	4.63	0.05	0.05	0.28	0.16

* significantly different from non-arthritic.

subjects ($p = 0.025$). There appeared to be some degree of stereoselectivity in AUC values for both doses and groups, with a significantly greater plasma enantiomer S:R ratio present in the arthritic patients (1.13 ± 0.16) than the non-arthritic subjects (0.93 ± 0.11) for the 50 mg dose ($p = 0.038$).

Clearance values corrected for body weight (CL/F) were consistently greater for the arthritic subjects compared to the non-arthritic group, but again were not significant. No significant differences were found between groups for volume of distribution (Vd/F). As well, the values obtained for these parameters were similar to previous reports in young adult subjects (Jamali & Brocks 1990). No significant differences in kinetic parameters were seen between the 50 mg and 150 mg doses in either group.

Quantitation of the cumulative urinary excretion of ketoprofen enantiomers (see Figure 2-3: Mean percent excretion of ketoprofen enantiomer dose for non-arthritic (NA) and arthritic (A) subjects.) showed significant differences between enantiomers and between the arthritic and non-arthritic groups. S-ketoprofen excretion was 60% greater than R-ketoprofen in both groups at each dose (urine S:R ratio: 1.60 ± 0.25 and 1.55 ± 0.16 for arthritic subjects, 50 mg and 150 mg doses; 1.65 ± 0.27 and 1.59 ± 0.12 for non-arthriticsubjects, 50 mg and 150 mg doses). This difference reached significance in each group for both doses except for the non-arthritic group for the 50 mg dose, likely due to small sample size ($n = 4$). Urinary excretion results were not available for non-arthritic subject number 5 for the 50 mg dose. The percentage of enantiomer dose excreted in urine was significantly smaller in the arthritic group compared to the non-arthritic subjects in each study period for each enantiomer. (See Figure 2-3: Mean percent excretion of ketoprofen enantiomer dose for non-arthritic (NA) and arthritic (A) subjects.)

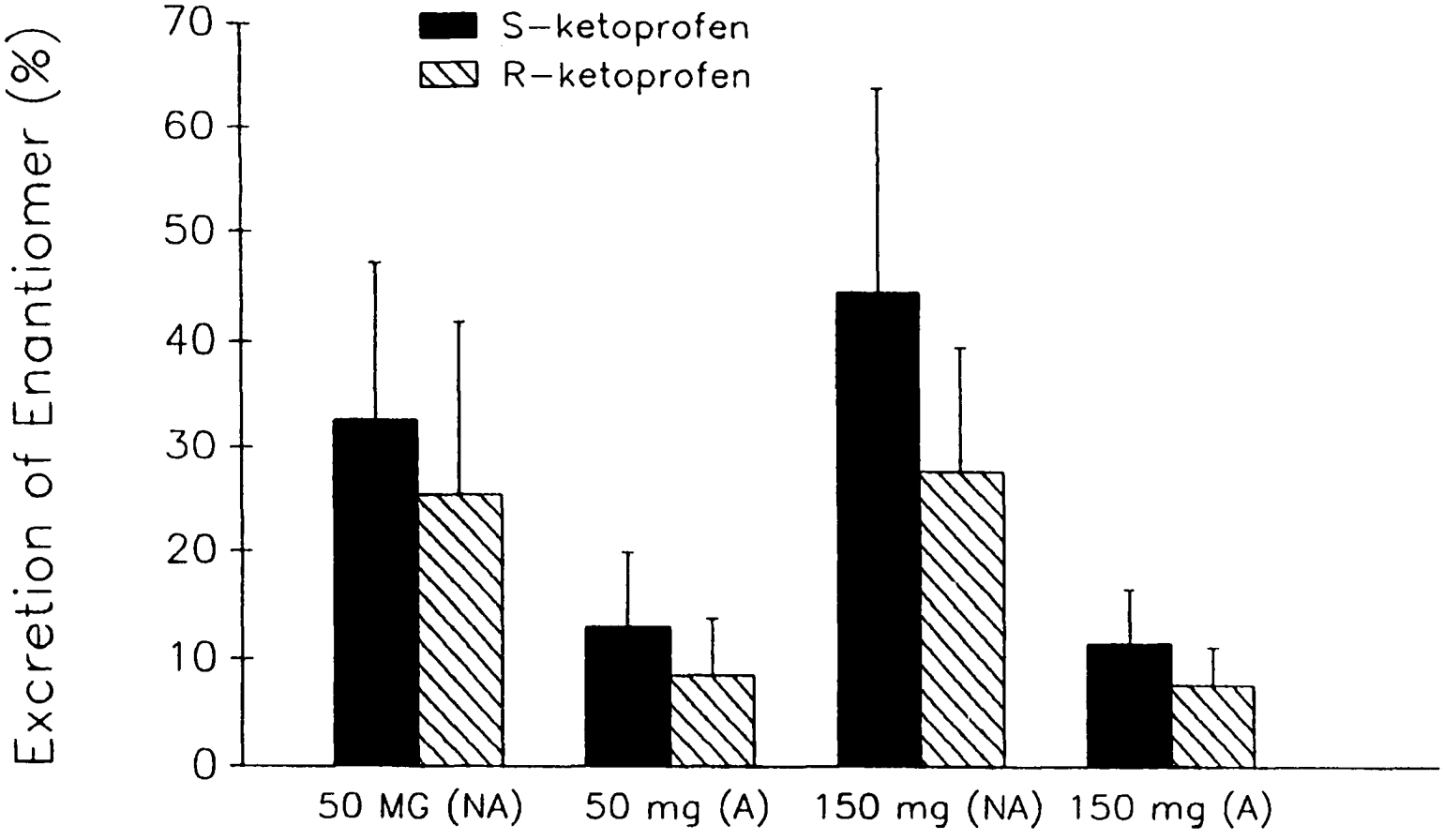


Figure 2-3: Mean percent excretion of ketoprofen enantiomer dose for non-arthritic (NA) and arthritic (A) subjects.

A greater amount of conjugated S-ketoprofen and R-ketoprofen was present in the plasma of the arthritic subjects compared to non-arthritic subjects for each dose, as measured by AUC_{0-24} of conjugates. (see Figure 2-4: Mean plasma ketoprofen conjugate levels (AUC0-24) for non-arthritic (NA) and arthritic (A) subjects. *X = significant difference S-ketoprofen vs. R-ketoprofen; *Significant difference for S-ketoprofen for 150 mg dose (NA) vs. (A).*) However, this reached significance only for S-ketoprofen at the 150 mg dose ($p = 0.007$). The AUC_{0-24} of conjugated S-ketoprofen was significantly greater than that of R-ketoprofen in the non-arthritic subjects for both doses, and in the arthritic subjects for the 150 mg dose.

The renal clearance of conjugated ketoprofen enantiomers ($CL_{R(conj)}$) exhibited stereoselectivity, with $CL_{R(conj)}$ of R-ketoprofen consistently greater than for S-ketoprofen for arthritic and non-arthritic subjects for both doses. (see Figure 2-5: Mean renal clearance of ketoprofen conjugates for non-arthritic (NA) and arthritic (A) subjects. * Significant difference for S-ketoprofen for 150 mg dose (NA) vs. (A).) This difference did not, however, reach significance in any subgroup due to large intersubject variations. As well, the $CL_{R(conj)}$ of both ketoprofen enantiomers was reduced in arthritic subjects as compared with the non-arthritic group for both doses; this was significant between groups for S-ketoprofen at the 150 mg dose ($p = 0.006$).

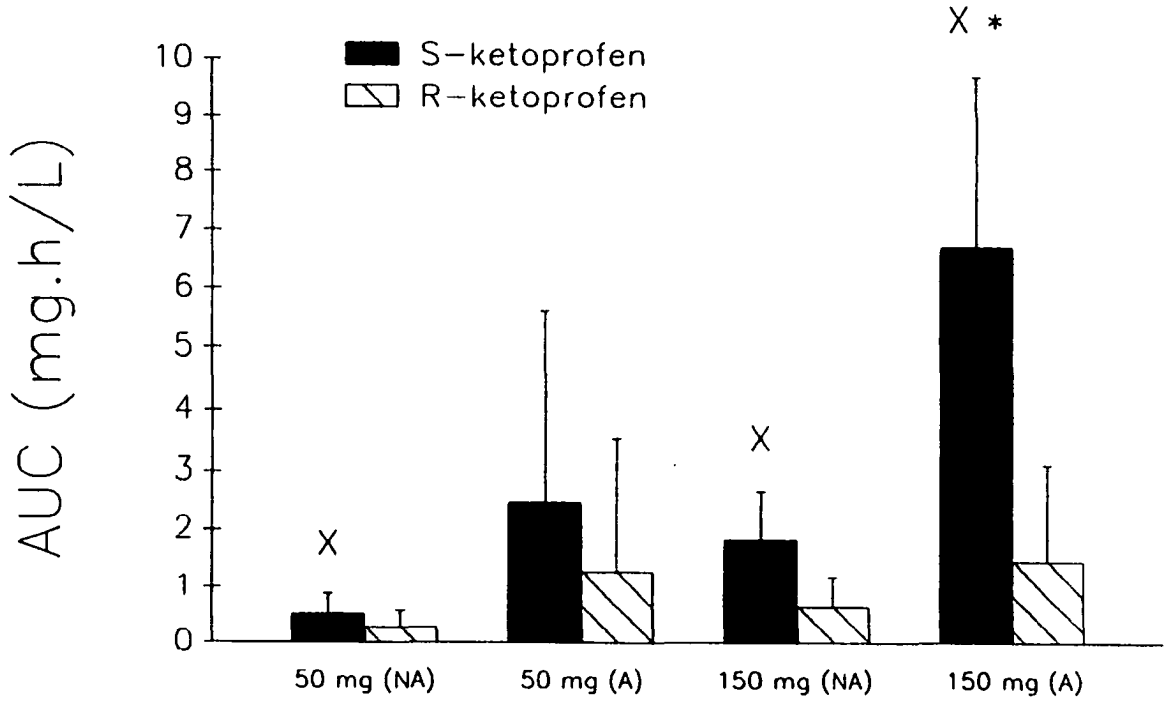


Figure 2-4: Mean plasma ketoprofen conjugate levels (AUC0-24) for non-arthritic (NA) and arthritic (A) subjects. **X = significant difference S-ketoprofen vs. R-ketoprofen; *Significant difference for S-ketoprofen for 150 mg dose (NA) vs. (A).**

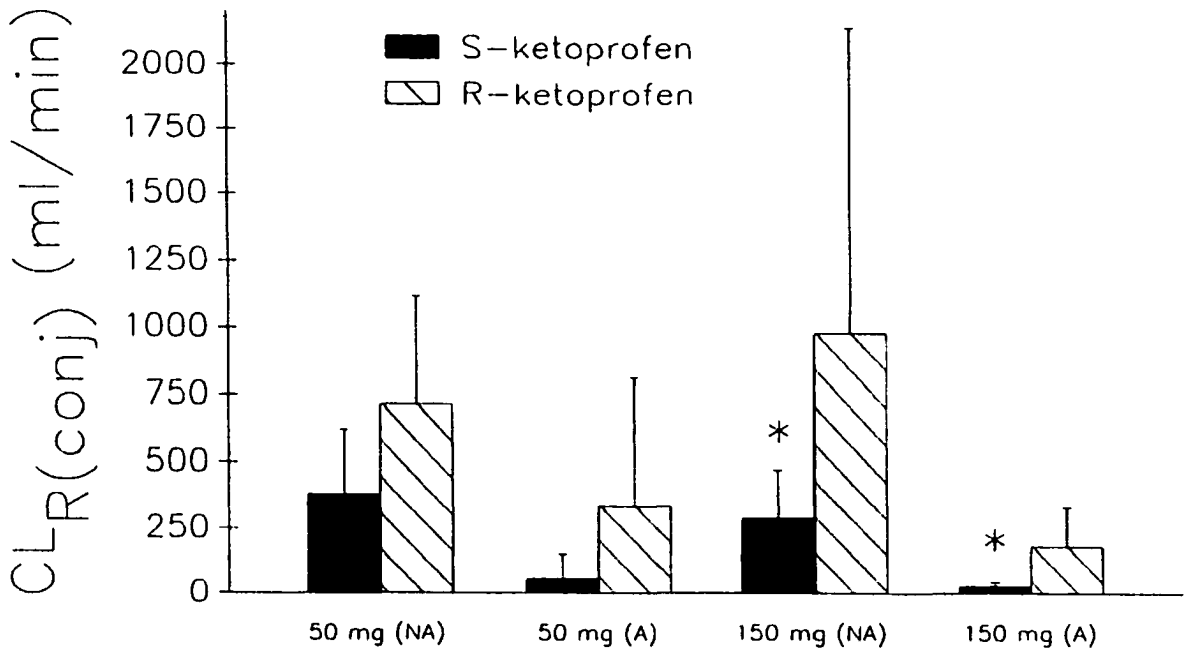


Figure 2-5: Mean renal clearance of ketoprofen conjugates for non-arthritic (NA) and arthritic (A) subjects. * Significant difference for S-ketoprofen for 150 mg dose (NA) vs. (A).

2.5 DISCUSSION:

The significant accumulation of ketoprofen in elderly subjects described by Advenier *et al.* (1983) and Dennis *et al.* (1985) has not been a consistent finding by other investigators (Foster *et al.* 1988) and has not been reflected clinically by excessive or cumulative toxicity in this age group (Le Loet 1989). The possibility that these discordant results were due to dose-dependent disposition kinetics as proposed earlier (Foster *et al.* 1988) is unlikely, as shown by the lack of difference between 50 mg and 150 mg doses in our study. The linear relationship between AUC and dose of oral ketoprofen has been previously demonstrated in a review article (Jamali & Brocks 1990) and our results are consistent with those reported therein.

The values recorded for AUC for our subjects were consistent with those observed for other non-arthritic elderly subjects and also with those reported for young subjects in previous studies (Jamali & Brocks 1990). This may indicate 1) an extent of absorption similar to those reported in young adults and/or 2) a reduced absorption along with a reduced clearance of the drug in the elderly arthritic patients with reduced renal function. The second suggestion in supported by the observation that in our study the mean percent excretion of total ketoprofen dose was only up to 21% in the arthritic subjects, while in the non-arthritic subjects up to 72% was excreted. Prior studies have found urinary excretion values of 70% to 85% in young adults, and significantly reduced values of 50-65% in elderly subjects (Jamali & Brocks 1990). Similarly, in patients with renal dysfunction, only 32.9% of the dose has been found in the urine (Stafanger *et al.* 1981). Furthermore, T_{max} was delayed to 4 hours in a number of our subjects. This may also be consistent with a trend towards reduced gastrointestinal motility and delayed

gastric emptying with increasing age (Greenblatt *et al.* 1982). Reduced urinary excretion of conjugated ketoprofen in the elderly has also been attributed to a compensatory enhanced biliary excretion (Levy 1979). However, neither our data nor the available information in the literature offer any clear explanation as to the exact mechanism involved in the reduced renal excretion of conjugated ketoprofen in the elderly patients.

It has been shown that ketoprofen kinetics were altered by impaired renal function in a study of patients with a reported mean CL_{Cr} of 37 ml/min (Stafanger *et al.* 1981). In that study significantly reduced CL/F, and increased $t_{1/2}$ were seen in the renal failure patients compared to normal volunteers. A significant correlation between CL/F and CL_{Cr}, with corresponding reductions in urinary excretion of conjugated ketoprofen, was also identified. Foster *et al.* (1989) observed a positive linear relationship between the cumulative urinary excretion of conjugated ketoprofen enantiomers and CL_{Cr} (r^2 = 0.94 for S and 0.97 for R) in a study of cholecystectomy patients. The elderly subjects whom were examined by Foster *et al.* (1988) had a mean CL_{Cr} of 50.9 ml/min, but did not exhibit significant ketoprofen accumulation. They did note, however, an elevated AUC of glucuroconjugates, consistent with reduced urinary excretion of conjugated ketoprofen, compared to young adult subjects with a mean CL_{Cr} of 95.6 ml/min. In our study, only small amounts of conjugated ketoprofen enantiomers were present in the plasma of our non-arthritic elderly subjects, compared to the amount of intact ketoprofen present. (see Fig. 2-4) This relationship is similar to that reported by Upton *et al.* (1980) using a non-stereospecific approach in young adults (50 mg dose: C_{max}, 4.25±1.71 and 0.47±0.17 (mg/L) for ketoprofen and ketoprofen conjugates, respectively).

In this study, although none of our subjects had severe renal impairment, there was a significant reduction in CL_{Cr} in our arthritic compared to non-arthritic subjects; and the former

group had significantly greater concentrations of conjugated ketoprofen in their plasma (see Fig. 2-4). In none of the studies that identified kinetic alterations in the elderly (Advenier *et al.* 1983, Dennis *et al.* 1985) were plasma conjugated ketoprofen levels determined. It has been shown that *in vitro* hydrolysis of ester glucuronides in biological fluids can occur (Upton *et al.* 1980). A plausible explanation for the observed increases in AUC in these two studies may have been inadvertent hydrolysis of plasma ketoprofen glucuroconjugates prior to analysis. It is unclear whether the authors who reported accumulation of ketoprofen in elderly subjects (Advenier *et al.* 1983, Dennis *et al.* 1985) and patients with renal dysfunction (Stafanger *et al.* 1981) had considered the implications involved in measuring unchanged ketoprofen. The results of the present work coupled with those reported previously suggest that indeed, within the recommended therapeutic dosage range, the renal clearance of conjugated ketoprofen rather than that of unchanged ketoprofen is affected by renal function, and possibly by aging and arthritic conditions.

Data on the effects of aging, chronic NSAID use, and the presence of underlying rheumatic diseases on the specific tubular secretory pathways important in NSAID and NSAID-glucuronide excretion are lacking. Hence, it is difficult to definitively separate the effects of renal impairment and the presence of rheumatic disease in our arthritic subjects. The impact of rheumatic disease (most frequently RA) on NSAID kinetics has been examined in the past in a number of different drugs, with results obtained varying from no influence to a significant modifying effect (Crock *et al.*1982, Grindel *et al.* 1979, Eriksson *et al.* 1989, Alvan *et al.* 1975). However, our arthritic subjects were a heterogeneous group, with several different rheumatic disorders unrelated in their pathophysiology. As such, a uniform disease-specific effect on kinetic drug disposition is unlikely.

Characteristic of our entire arthritic group of patients, though, was the chronic use of NSAIDs. It is known that NSAIDs, by inhibiting renal prostaglandin synthesis, can produce a variable degree of renal impairment through renal hemodynamic alterations. This may lead to reductions in renal plasma flow and solute excretion, including organic anions such as NSAIDs (Toto 1991). This would result in reduced $CL_{R(conj)}$ and elevated plasma glucuroconjugate levels, as was found in our arthritic subjects. The extent of these alterations would be dependent on a number of variables, including the nature of any co-existent renal or pre-renal disease; or concomitant medications (Toto 1986). It has been stated that this occurs only in the presence of pre-existing risk factors such as renal disease or volume-contracted states such as uncompensated congestive heart failure, cirrhosis, nephrosis, or diuretic usage (Toto 1991). However, these adverse effects have been noted in patients lacking any such risk factors (Unsworth *et al.* 1987), and in middle-aged and elderly subjects with clinically inapparent mild renal insufficiency or compensated CHF (Whelton *et al.* 1990, Guwitz *et al.* 1990). The time required for renal function to return to baseline after stopping the NSAID varied from 3 to 28 days in these studies. As our patients' washout period prior to entering the study was only 2 to 5 days, it is possible that some residual renal impairment was present and contributed to the observed lower CL_{Cr} as compared to the non-arthritic subjects. Therefore it appears that NSAID use itself may influence the renal clearance of its conjugated metabolites. However, in the previous study by Foster *et al.* (1988) of young arthritic adults with previous exposure to NSAIDs, negligible concentrations of conjugated ketoprofen were found in plasma. Similarly, the finding of only small concentrations of conjugated ketoprofen in the plasma of our non-arthritic subjects (CL_{Cr} 91.4 ml/min), may indicate that age *per se* does not have any substantial effect on the kinetics of either unchanged or conjugated drug. These observations indicate that

a combination of factors such as NSAID use, aging, and reduced renal function, may be involved in reducing renal clearance of conjugated ketoprofen.

The AUC_{0-24} of ketoprofen conjugates demonstrated stereoselectivity in every study subgroup. The observed stereoselectivity of the renal conjugate clearance ($CL_{R(conj)}$) may explain the enantiomeric differences in plasma conjugate AUC values in individual subjects and groups. R-ketoprofen $CL_{R(conj)}$ was uniformly greater than S-ketoprofen $CL_{R(conj)}$ for all subgroups. The small size of each group and the large intra-group variability contributed to the failure for this difference to reach statistical significance. That this process is at least partially an active secretory pathway is confirmed by observing $CL_{R(conj)}$ values in excess of the subject's CL_{Cr}.

In a study previously carried out in our laboratory (Foster *et al.* 1988), the S:R ratios of conjugated ketoprofen excreted in urine were found to be 1.30 ± 0.11 and 1.34 ± 0.06 for young and elderly arthritic subjects, respectively. In our study, S:R ratios of 1.60 ± 0.25 and 1.65 ± 0.27 for arthritic and non-arthritic subjects were seen respectively, for the 50 mg dose. One possible explanation for this discrepancy may have been the difference in ketoprofen formulation administered, as the presence of rheumatic disease and/or renal dysfunction was common to both studies. Foster *et al.* (1988) administered ketoprofen in capsular form, and in the present study enteric-coated tablets were used.

It has been well documented that some 2-arylpropionic acid NSAIDs undergo a unidirectional R to S metabolic inversion (Jamali 1988). The extent of this chiral inversion varies from negligible (*e.g.*, flurbiprofen) to extensive (*e.g.*, ibuprofen and fenoprofen). The inversion appears to take place both systemically and presystemically in the gut wall during absorption (Jamali *et al.* 1988, Jamali *et al.* 1992). For ibuprofen, this process is partially

dependent on the rate of absorption, as a longer residence time at the presystemic site of inversion (*i.e.*, intestine) may result in a greater extent of inversion (Jamali *et al.* 1988, Jamali *et al.* 1992). The S:R ratios of greater than one in urine observed after administration of readily absorbed capsules of ketoprofen (no lag time for absorption), has been shown to reflect an approximately 10% inversion (Foster *et al.* 1988). The similarities between plasma enantiomer ketoprofen concentrations despite such large differences in urine excretion S:R ratios is not unexpected. These results are compatible with a prior study in which administration of R-ketoprofen alone resulted in a 9% to 12% R to S inversion, negligible amounts of S-ketoprofen in plasma, and 7.7% excretion of total dose in urine as S-ketoprofen enantiomer (Jamali *et al.* 1990). It is likely that after ingestion of enteric coated tablets (0.5-4 h lag time for absorption) a longer time is allowed for presystemic inversion in the gut. This preliminary observation requires further study, as pathologically or pharmacologically induced functional alterations in gastrointestinal physiology may impact on the final observed enantiomer ratio.

Our results, in a pharmacokinetic evaluation of the target disease group, indicate that within the recommended therapeutic dosage regimen, significant accumulation of ketoprofen does not occur in elderly subjects in the presence or absence of rheumatic disease and moderate renal dysfunction. However, these conditions appear to be associated with reduced renal clearance of conjugated ketoprofen. The stereoselectivity of this process has been demonstrated, with greater accumulation of S-ketoprofen plasma conjugates as a consequence.

There does not appear to be sufficient evidence to support the clinical strategy of reducing ketoprofen dosage based on age *per se*. Some evidence exists for dose modification based on renal function (Stafanger *et al.* 1981), but further studies are necessary to define the expected kinetic alterations in the presence of renal impairment.

3 THE INFLUENCE OF RENAL FUNCTION ON THE PHARMACOKINETICS OF UNCHANGED AND ACYL-GLUCUROCONJUGATED KETOPROFEN ENANTIOMERS

3.1 ABSTRACT

To study the effect of renal dysfunction on the pharmacokinetics of ketoprofen (KT), and the possibility of saturation of clearance upon increasing dose, single 50 and 100 mg doses of racemic ketoprofen were administered in a cross-over fashion to 9 patients with varying levels of renal function(CL_{CR} 6 ml/min to 110 ml/ min). The stereospecific disposition kinetics of ketoprofen enantiomers and their acyl-glucuronide conjugates (KT_{conj}) were determined in plasma and urine for 24 hrs post-dose. Significant trends were found towards reduced ketoprofen oral clearance (CL_O) and terminal elimination rate constant (β) with decreased renal function. Stronger correlations were observed between ketoprofen pharmacokinetic indices and renal clearance of KT_{conj} (CL_{Rconj}) after both doses. CL_{Rconj} was reduced with diminished renal function.

Following both 50 and 100 mg doses, reduced renal function resulted in significantly lower AUC (mean S/R, 0.87 and 0.83, respectively) of (S)-ketoprofen but higher AUC (mean S/R, 3.4 and 5.2, respectively) and cumulative urinary excretion (mean S/R, 2.1 and 2.2, respectively) of (S)-KT_{conj} as compared with the antipode. CL_O remained constant after

This chapter published in part: Br J Clin Pharmacol 42: 163 – 169, 1996

increasing the dose, indicating linearity in the pharmacokinetics of KT despite reduced clearance. CLr_{conj}, however, was significantly reduced after the 100 mg dose suggestive of saturation of the urinary clearance and existence of a compensatory pathway. Renal impairment reduces renal clearance of KT_{conj} and this appears to be the rate-limiting step for clearance of ketoprofen. The observed stereoselectivity in the urinary excretion of the KT_{conj} may indicate increased chiral inversion due to a longer residence time with renal dysfunction. Dose reduction of rac-ketoprofen is indicated only for patients with $CL_{CR} < 20$ ml/min.

3.2 RATIONALE

Ketoprofen (KT) is a nonsteroidal anti-inflammatory drug (NSAID) that is used in the management of rheumatic disorders. The principal metabolic pathway for ketoprofen is the formation of acyl-glucuronide conjugates (KT_{conj}) and subsequent renal elimination (Jamali & Brocks 1990). Acyl-glucuronidated ketoprofen is unstable, and upon retention in the body secondary to renal dysfunction may be cleaved and yield the parent drug (Upton *et al.* 1982). Rheumatic disorders are frequently encountered in patients with renal disease, and many patients receiving NSAIDs for various forms of arthritis have some degree of renal impairment (Skeith *et al.* 1993).

In the past, the effect of renal dysfunction on the disposition kinetics of ketoprofen, a chiral drug administered as the racemate, has been studied in a non-stereoselective manner (Stafenger *et al.* 1981, Halstenson *et al.* 1992) or without quantification of glucuronide accumulation (Stafenger *et al.* 1981, Halstenson *et al.* 1992, Sallustio *et al.* 1988, Hayball *et al.* 1993). Stafanger *et al.*

(1981) demonstrated in five patients with varying degrees of renal insufficiency, that increases in the elimination $t_{1/2}$ of ketoprofen correlated with decreases in renal function. Total oral clearance (CL_O) was reduced in comparison with normal controls. Cumulative urinary excretion of ketoprofen and KT_{conj} was also reduced in the presence of renal impairment. Halstenson *et al.* (1992) reported similar findings and recommended dosage adjustments for ketoprofen in patients with a glomerular filtration rate < 50 ml/min/1.7m^2. Sallustio *et al.* (1988), on the other hand, did not find an association between CL_{CR} and ketoprofen disposition kinetics.

Hayball *et al.* (1993) described the pharmacokinetics of ketoprofen enantiomers in 15 patients with a range of CL_{CR} from 26 to 159 ml/min. This study concluded that renal failure is associated with an decresed clearance of (S)-ketoprofen, presumably due to regeneration of parent aglycone arising from the hydrolysis of the accumulated KT_{conj}. These authors, however, did not measure plasma KT_{conj} levels. Hence, the the pharmacokinetics of KT_{conj} in the presence of renal impairment to date have not been fully characterized.

Two non-stereoselective studies had observed a substantial accumulation of ketoprofen, with increased AUC and $t_{1/2}$, in elderly subjects (Advenier *et al.* 1983, Dennis *et al.* 1985). More recently, stereospecific investigations have demonstrated that the pharmacokinetics of ketoprofen in the elderly are similar to young adults, but with greater amounts of KT_{conj} enantiomers in the plasma of arthritic elderly subjects with mildly impaired renal function (Foster *et al.* 1988, Skeith *et al.* 1993). Plausible explanations for the discrepancy between the earlier observations (Advenier *et al.* 1983, Dennis *et al.* 1985) and the more recent ones (Foster *et al.* 1988, Skeith *et al.* 1993) may be the hydrolysis of conjugates to parent drug during storage or processing, the larger doses administered in the earlier studies, and/or differences in the degree of renal impairment.

We report for the first time in this study a stereospecific pharmacokinetic evaluation of ketoprofen and KT_{conj} in the presence of renal dysfunction. As saturable disposition may result in reduced clearance, the effect of increased dosage was also evaluated by administering 50 mg and 100 mg doses.

3.3 MATERIALS AND METHODS

3.3.1 Patient Study

This project was conducted according to the principles of the Declaration of Helsinki, with institutional ethics committee approval. Eight patients (five female and three male, age 57 $\pm$ 16 years, total body weight 77 $\pm$ 16 kg) with a past or current diagnosis of renal disease (CL_{CR} 6 - 72 ml/min ; mean 43 $\pm$ 20 ml/min) and one 34 year old 93 kg female patient with early diabetic nephropathy who had efficient renal function (CL_{CR} 110 ml/min), were recruited for the study. All except one subject were receiving concurrent medications, and these were continued, except for diuretics which were discontinued one day before the days of the pharmacokinetic studies. None of the subjects were using NSAIDs prior to the study (see Table 3-1: Patient Characteristics). Seven of these subjects received in a randomized cross-over fashion doses of 50 mg and 100 mg of racemic ketoprofen (molar equivalent conversion factor, 3.93) in the form of 50 mg enteric-coated tablets (Orudis E-50; Rhone-Poulenc Pharmaceutical, Montreal, Canada). Two subjects received single ketoprofen doses only, one of 50 mg (CL_{CR} 110 ml/min) and one of 100 mg (CL_{CR} 28 ml/min). There was a 7 - 14 day washout period between studies in

those subjects receiving both doses. After an overnight fast, subjects received their ketoprofen tablet(s), along with 200 ml of water. No food intake was allowed for 2 hours after dosing. Venous blood was collected *via* an indwelling catheter inserted into a forearm vein into heparanized tubes at 0, 0.25, 0.50, 0.75, 1, 2, 3, 4, 6, 8, 12, 15, 18, 21, and 24 hours post-dose. After collection of blood samples, plasma was separated by centrifugation. Urine was collected for 0-6, 6-12, 12-18, and 18-24 hour periods post-dose. All samples were stored in acid-rinsed containers at -20° C prior to analysis. All subjects had their renal function assessed by a 24 hour creatinine clearance determination during each study period.

3.3.2 Assay

Plasma and urinary concentrations of KT and KT_{conj} enantiomers were measured by a reverse-phase stereospecific HPLC method (Palylyk & Jamali 1991). Sample volume used for analysis was 1.0 ml for plasma and 0.1 ml for urine. Calibration curves prepared using plasma and urine showed consistent linearity ($r^2 > 0.995$) between peak area ratios (drug:internal standard) and concentrations. The minimum quantifiable concentration of the assay was 50 ng/ml extracted from 1.0 ml of sample. Both plasma and urine samples were assayed before and after alkaline hydrolysis and the difference between these two values constituted the concentration of KT_{conj} enantiomers.

3.3.3 Data Analysis

Ketoprofen enantiomer concentrations were plotted *vs* time. The area under the plasma concentration-time curve to 24 hours (AUC_{0-24}) was calculated by the trapezoidal rule for both intact and conjugated KT. The apparent elimination rate constant (ß) was calculated from the log-linear terminal phase of the curve. Oral clearance and apparent volume of distribution were calculated from $\mathbf{CL_O = Dose/(AUC_{0-24} + C_{last}/\beta)}$ and $\mathbf{Vd = CL_O/ß}$, respectively, where $\mathbf{C_{last}}$ is the last measurable plasma drug concentration. Both CL_O and Vd were corrected for total body weight. Renal clearance of KT_{conj} $\mathbf{(CLr_{conj})}$, was assessed as $\mathbf{\Sigma X_{Uconj}/AUC_{conj}}$, where $\mathbf{\Sigma X_{Uconj}}$ is the cumulative urinary excretion and $\mathbf{AUC_{conj}}$ is the AUC of KT_{conj}.

The statistical significance of differences between groups was evaluated using Student's paired or unpaired t-test where appropriate. Correlations between groups was assessed using Pearson's correlation coefficient. The level of significance was set at $\alpha = 0.05$. Values are expressed as mean ± standard deviation. The patient with CL_{CR} of 110 ml/min who only received the 50 mg dose was not included in the calculation of the means.

3.4 RESULTS

As shown in Table 3-1: Patient Characteristics, the study subjects had a wide range of renal function from severely impaired (6 ml/min CL_{CR}) to normal (110 ml/min CL_{CR} in a patient

with early diabetic nephropathy). In the seven subjects that received both doses of KT, there were only negligible differences in CL_{CR} between study periods.

Table 3-1: Patient Characteristics

Patient	Sex	Age, Years	Weight, kg	CL_{CR} ml/min	Medications
1	F	64	56.3	6	prednisone, nifedipine
2	F	68	57	18	allopurinol, ranitidine
3*	F	59	96	28	nil
4	M	36	76.3	32	insulin, nifedipine, lisinopril
5	F	39	69.3	34	nifedipine
6	M	41	98	36	propranolol, allopurinol
7	M	65	87.7	46	nadolol, lisinopril, phenytoin
8	F	82	76	72	prednisone
9**	F	34	93	110	lisinopril

- 100 mg ketoprofen dose only, ** 50 mg ketoprofen dose only

Representative plasma concentration-time curves for KT and KT_{conj} are shown in Figure 3-1 for a patient with severe renal dysfunction (CL_{CR} of 6 ml/min) and for a patient with minimal renal impairment (CL_{CR} of 72 ml/min).

Table 3-2 and Table 3-3 list pharmacokinetic indices after 50 and 100 mg doses, respectively. Negligible concentrations of KT or KT_{conj} were found in plasma or urine 24 hours post-dose following either dose.

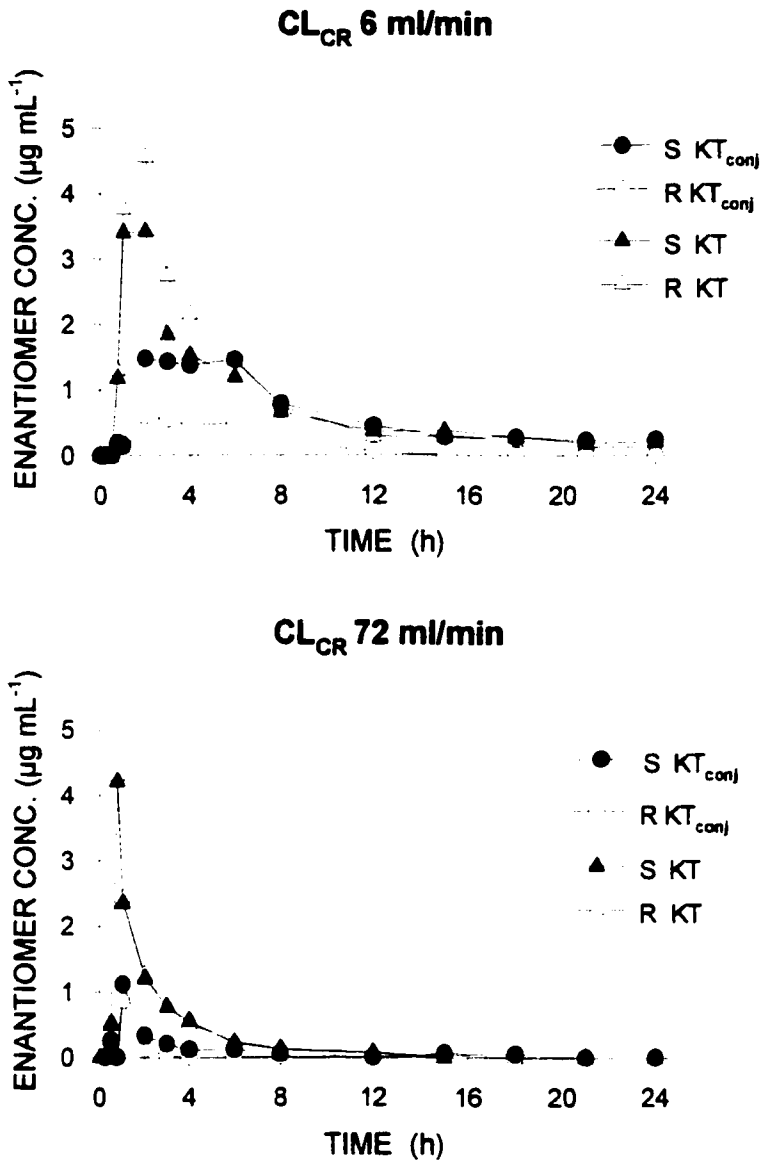


Figure 3-1: Concentration-time profiles for ketoprofen and acyl-glucuronidated ketoprofen for 2 patients with different renal function (6 ml/min and 72 ml/min CL_{CR}).

Table 3-2: Pharmacokinetic indices of enantiomers of ketoprofen and glucuronidated ketoprofen after single 50 mg racemic dose.

Patient		1	2	4	5	6	7	8	9	Mean ± SD[a]	p[a,b]
Cl_{cr}, mL/min		6	18	32	34	36	46	72	110	44.3 ± 20.9	
T_{max}, h	S	0.75	0.75	2.00	1.00	3.00	3.00	2.00	2	1.79 ± 0.98	
	R	0.75	0.75	2.00	1.00	3.00	3.00	2.00	2	1.79 ± 0.98	
C_{max}, µg/mL	S	3.20	6.99	1.28	2.16	1.41	1.58	1.04	1.06	2.52 ± 2.10	0.006
	R	3.46	7.47	1.51	2.33	1.58	1.96	1.05	1.18	2.77 ± 2.21	
	S/R	0.92	0.94	0.85	0.93	0.89	0.81	0.99	0.9	0.90 ± 0.06	
$AUC_{0\text{-}24}$, (µg/mL)h	S	14.2	16.5	3.61	5.75	4.17	3.76	2.89	2.75	7.27 ± 5.63	0.017
	R	16.3	18.8	4.51	7.07	4.4	5.76	2.62	1.86	8.49 ± 6.37	
	S/R	0.87	0.88	0.8	0.81	0.95	0.65	1.1	1.48	0.87 ± 0.14	
Clo, mL/min/kg	S	0.53	0.44	1.52	1.05	2.04	1.26	1.9	1.63	1.25 ± 0.62	0.124
	R	0.46	0.39	1.22	0.86	0.97	0.82	2.1	2.41	0.97 ± 0.57	
t½, h	S	14.4	8.4	3.1	3.3	2.18	3.26	3.1	2.92	5.39 ± 4.47	0.096
	R	9.42	4.5	1.68	4.48	2.09	2.93	1.3	1.76	3.77 ± 2.80	
Vd, L/kg	S	0.66	0.33	0.41	0.30	0.19	0.36	0.52	0.42	0.40 ± 0.15	0.016
	R	0.38	0.15	0.18	0.33	0.18	0.21	0.24	0.37	0.24 ± 0.09	
AUC_{conj}, (µg/mL)h[f]	S	4.91	9.31	2.56	4.59	2.13	4.14	0.69	1.14	4.05 ± 2.77	d
	R	c	1.98	c	1.36	1.09	1.12	c	0.97	1.39 ± 0.41	
	S/R	d	4.70	d	3.38	1.95	3.70	d	1.18	3.43 ± 1.14[e]	
$\Sigma xu_{(conj)}$, mg[f]	S	4.16	13.9	21.2	18.4	21.7	20	5.94	13.8	15.04 ± 7.31	0.002
	R	1.34	8.1	10.1	10.1	10.5	13.2	2.97	10.3	8.04 ± 4.32	
	S/R	3.10	1.72	2.10	1.82	2.07	1.52	2.00	1.34	2.05 ± 0.51	
$CL_{R(conj)}$, mL/min/kg	S	0.25	0.44	1.82	0.97	1.74	0.92	1.89	2.17	1.15±0.68	d
	R	d	1.20	d	1.79	1.65	2.24	d	1.91	1.72 ± 0.43[e]	

a) Pateint 9 was not included due to her normal renal function; b) Paired Student t-test S *vs* R enantiomer; c) Below assay sensitivity; d) Not determined due to low values for (R)-enantiomer; e) The true mean substantially higher as values could not be determined for those with very rapid CL for (R)-enantiomer; f) parent drug equivalent.

Table 3-3: Pharmacokinetic indices of enantiomers of ketoprofen and glucuronidated ketoprofen after single 100 mg racemic dose.

Patient		1	2	3	4	5	6	7	8	Mean ± SD	p[a]
Cl_{cr}, mL/min		6	18	28	32	34	36	46	72	34 ± 20	
T_{max}, h	S	2	1	1	2	1	2	2	0.75	1.47 ± 0.57	
	R	2	1	1	3	1	3	3	0.75	1.84 ± 1.03	
C_{max}, µg/mL	S	3.42	5.26	1.75	1.34	5.02	1.82	1.72	4.21	3.07 ± 1.61	0.0037
	R	4.55	5.93	1.97	1.82	5.43	2.18	2.17	4.3	3.54 ± 1.69	
	S/R	0.75	0.89	0.89	0.74	0.92	0.83	0.79	0.98	0.85 ± 0.09	
$AUC_{0\text{-}24}$, (µg/mL)h	S	18.4	23.1	7.17	4.46	13.7	10.6	6.75	6.47	11.3 ± 6.59	0.0088
	R	21.4	29.0	7.41	7.59	15.9	11.8	9.91	6.7	13.7 ± 7.95	
	S/R	0.86	0.80	0.97	0.59	0.86	0.90	0.68	0.97	0.83 ± 0.13	
Clo, mL/min/kg	S	0.81	0.63	1.21	2.46	0.88	0.8	1.41	1.7	1.24 ± 0.61	0.0730
	R	0.7	0.51	1.17	1.45	0.76	0.72	0.96	1.64	0.99 ± 0.40	
t½, h	S	8.74	6.84	6.71	4.19	3.48	4.37	3.88	2.64	5.11 ± 2.08	0.0769
	R	8.15	5.97	5.16	3.01	3.53	3.33	4.31	3.01	4.56 ± 1.80	
Vd, L/kg	S	0.62	0.38	0.71	0.89	0.27	0.31	0.48	0.39	0.51 ± 0.22	0.0380
	R	0.49	0.26	0.52	0.38	0.23	0.21	0.36	0.43	0.36 ± 0.12	
AUC_{conj}, (µg/mL)h[f]	S	20.3	8.81	7.28	7.51	11.9	4.94	8.59	3.36	9.09 ± 5.21	0.0011
	R	2.35	b	1.09	1.01	4.06	1.09	3.52	0.86	2.00 ± 1.33	
	S/R	8.65	c	6.68	7.44	2.92	4.53	2.44	3.91	5.22 ± 2.38	
$\Sigma xu_{(conj)}$, mg[f]	S	5.61	11.5	11	23.3	22.1	23.2	27.9	19.8	18.1 ± 7.73	0.0002
	R	1.49	6.29	3.85	9.41	10.1	14.5	18.3	12.8	9.59 ± 5.61	
	S/R	3.77	1.83	2.87	2.48	2.18	1.6	1.52	1.54	2.22 ± 0.79	
$CL_{R(conj)}$, mL/min/kg	S	0.084	0.38	0.26	0.68	0.45	0.80	0.611	1.30	0.57 ± 0.37	0.0267
	R	0.19	c	0.62	2.05	0.61	2.27	0.99	3.28	1.43 ± 1.12	

a) Paired Student t-test S *vs* R enantiomer. b) Below assay sensitivity; c) Not determined due to low values for (R)-enantiomer; e) The true mean substantially higher as values could not be determined for those with very rapid CL for (R)-enantiomer. f) parent drug equivalent.

Renal dysfunction was associated with the presence of substantial concentrations of KT_{conj} enantiomers in plasma with the S enantiomer being predominant. (R)- KT_{conj} exhibited a substantially faster renal clearance that its antipode so that in four of the 50 mg and one of the 100 mg treatments, the plasma concentration was below the sensitivity of the assay. The urinary excretion consisted of KT_{conj} with negligible unchanged drug. The C_{max} and AUC_{0-24} of the unchanged R enantiomer was significantly higher than the S enantiomer after both doses in patients with renal impairment.

In general, renal dysfunction resulted in trends towards higher KT plasma concentrations. For example, the AUC_{0-24} values for the patients with the lowest renal function were at least two times greater than those with the best renal function. Significant correlations between pharmacokinetic indices and renal function, however, were not always observed after both doses. Correlations observed between the oral clearance (CL_O) of the ketoprofen enantiomers and CL_{CR} following a) the 50 mg dose: (R)-ketoprofen, $r = 0.94$, $P < 0.001$; (S)-ketoprofen, $r = 0.62$, $P < 0.104$ b) the 100 mg dose: (R)-ketoprofen, $r = 0.69$, $P < 0.058$; (S)-ketoprofen, $r = 0.46$, $P = 0.255$ (see Figure 3-2). Correlations observed between enantiomer conjugate renal clearance of acyl-glucuronidated ketoprofen (CLr_{conj}) and CL_{CR} following a) the 50 mg dose: (R)-ketoprofen, $r = 0.20$, $P < 0.64$; (S)-ketoprofen, $r = 0.78$, $P = 0.02$ b) the 100 mg dose: (R)-ketoprofen, $r = 0.78$, $P < 0.018$; (S)-ketoprofen, $r = 0.92$, $P < 0.001$ (see Figure 3-3).

Following the 50 mg dose, only AUC ($P < 0.037$), CL_O ($P < 0.001$) of the R enantiomer, and CL_{conj} of the S enantiomer ($P < 0.024$) were significantly correlated with

Cl_{CR}. Significant associations were, however, found between many of the pharmacokinetic indices of unchanged KT enantiomers and CLr_{conj} ($P < 0.002$ - 0.04 for C_{max}, AUC, CL_O, $t_{1/2}$ and β) after the 50 mg dose. Among the pharmacokinetic indices calculated following the 100 mg dose, β, $t_{1/2}$, AUC_{conj} and CLr_{conj} of both enantiomers and R-ΣXu_{conj} were significantly correlated with CL_{CR} ($P < 0.001$- 0.048). Further, β and $t_{1/2}$ of the unchanged KT enantiomers were significantly correlated with CLr_{conj} for the 100 mg dose ($P < 0.004$ - 0.05).

There were no significant differences in the pharmacokinetic indices of unchanged ketoprofen enantiomers calculated following the two adminstered doses (see Table3-2 and 3-3). The mean percent cumulative urinary excretion of both enantiomers following the 100 mg dose, however, was reduced approximately to one half of that observed after the 50 mg dose. This was accompanied by a proportional decrease in CLr_{conj}.

A significant difference between enantiomers was observed with respect to their V/F after both doses. No significant differences were seen for t_{max} values between enantiomers or between doses for either enantiomer.

The S:R ratio of KT and KT_{conj} AUC as well as that of ΣXu_{conj} exhibited some degree of association with CL_{CR} for both doses. This parameter, however, reached statistical significance only for AUC of KT ($r = 0.80$; $P < 0.017$) after tha 50 mg dose and for ΣXu_{conj} following the 100 mg dose ($r = -0.71$, $P < 0.048$).

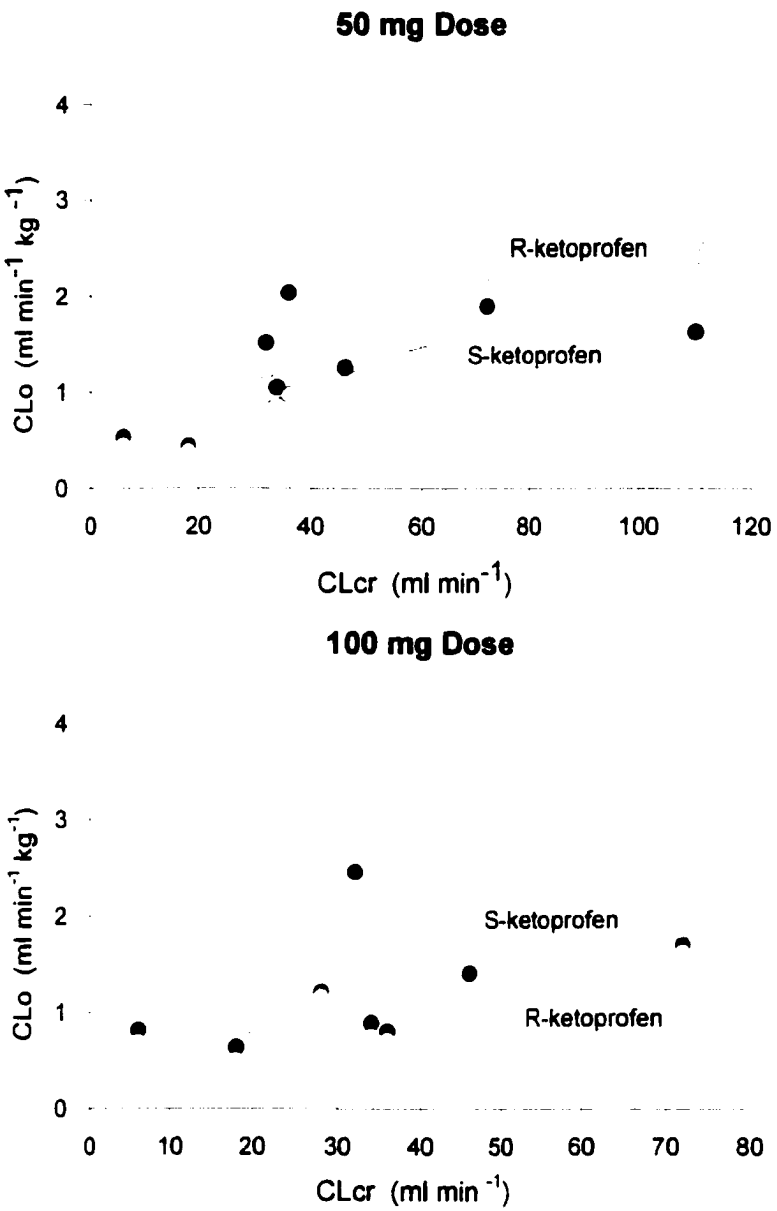


Figure 3-2: Correlation between oral clearance (CLo) of ketoprofen enantiomers and creatinine clearance (CLcr) following single 50 mg and 100 mg doses of racemic ketoprofen (o R ketoprofen, • S ketoprofen).

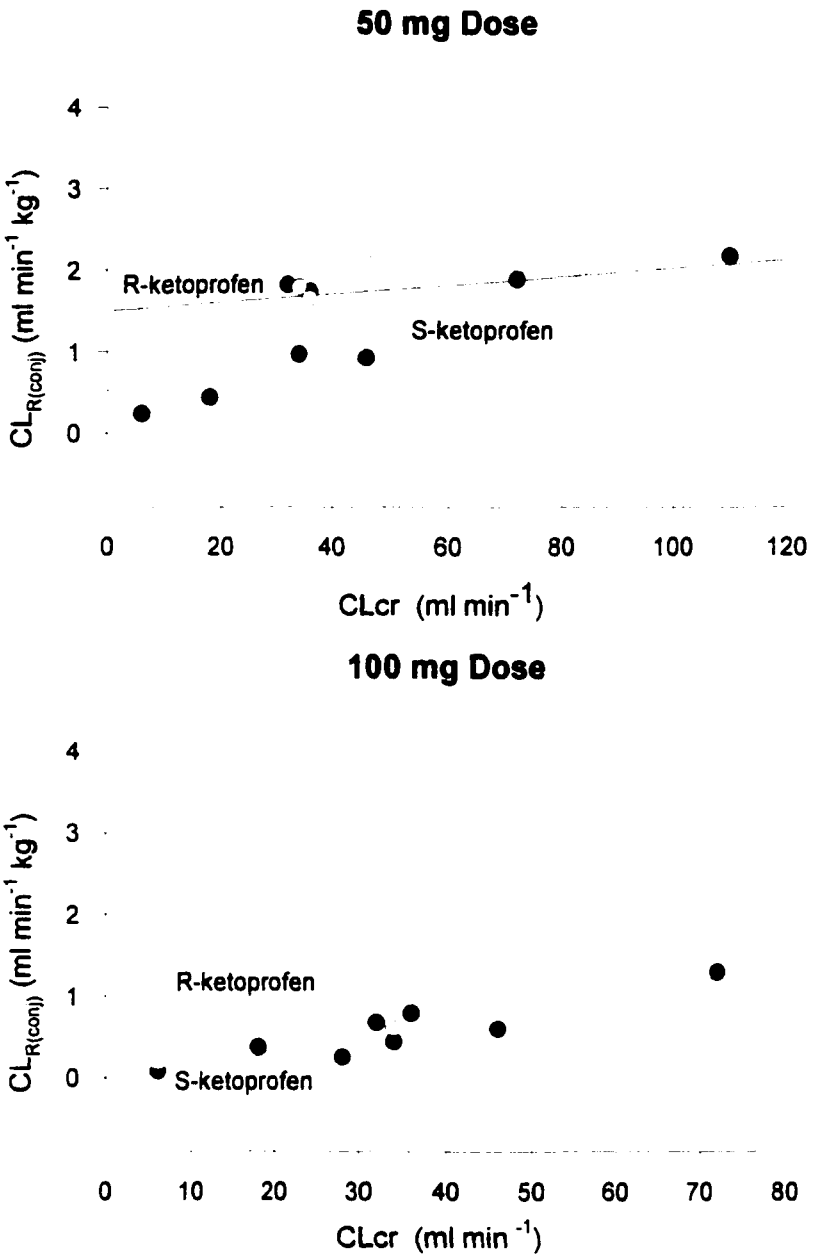


Figure 3-3: Correlation between enantiomer conjugate renal clearance of acyl-glucuronidated ketoprofen (CLrconj) and creatinine clearance (CLcr) following 50 mg and 100 mg doses of racemic ketoprofen (o R ketoprofen, • S ketoprofen).

3.5 DISCUSSION

The observed reduction in CL_O (see Figure 3-2) and β with diminished renal function is not unexpected since Hayball *et al.* (1993) have already shown a positive association between the reciprocal of AUC and CL_{CR}. Our observation, therefore, is also in agreement with that of Stafanger *et al.* (1981). Similarly, in elderly subjects, the renal clearance of the KT_{conj} enantiomers has been shown to be diminished, but to a lesser extent in patients with healthy renal function (Foster *et al.* 1988, Skeith *et al.* 1993) as compared with those with moderate renal dysfunction (Skeith *et al.* 1993).

Although Hayball *et al.* (1993) did not measure the plasma concentration of KT_{conj} enantiomers, they suggested that an inhibition of clearance of these metabolites and their subsequent hydrolysis to the parent enantiomers may be responsible for decreased CL_O in renal dysfunction. Similarly, in this study which includes an assessment of the disposition kinetics of both KT enantiomers and their major metabolites in the presence of varying levels of renal dysfunction, we have observed substantial concentrations of the metabolites in plasma (see Figure 3-1). Ketoprofen clearance is mainly dependent upon the formation and subsequent renal elimination of KT_{conj} (Jamali & Brocks 1990). This metabolite is rapidly cleared by young subjects with healthy renal function so that very little KT_{conj} is found in plasma (Jamali & Brocks 1990). A substantial concentration of KT_{conj}, however, has been observed in elderly subjects (Foster *et al.* 1988, Skeith *et al.* 1993).

The observed positive association of CLr_{conj} with CLcr (see Figure 3-3) supports the dependency of acy-glucuronide clearance on renal function. These metabolites are, however,

unstable as they are prone to hydrolysis, yielding the parent KT enantiomers. This suggests that glucuroconjugation of KT is, indeed, a reversible process. In healthy subjects, the formed conjugates are efficiently eliminated before substantial cleavage takes place. In the presence of kidney dysfunction, as the clearance of the KT_{conj} is reduced and the residence time of the metabolite is increased, more opportunity is provided for hydrolysis to the parent drug.

An indication of a reversible metabolism is equal terminal t½ values for the drug and the metabolite (Jamali *et al.* 1988). In this study the slopes of the terminal elimination phase of the plasma concentration-time curves, in general, appeared parallel for unchanged and conjugated KT. Due to fluctuations in the plasma concentrations of KT_{conj}, however, we could only accurately calculate the elimination t½ of (S)- KT_{conj} in two patients who had the lowest CL_{CR} (6 and 18 ml/min) (see Table 3-3) following the 100 mg dose: 10.2 hrs and 6.84 hrs for (S)-KT_{conj} *vs* 8.74 hrs and 6.75 hrs for KT, in patients 1 and 2, respectively. Nevertheless, in the majority of the patients, the plasma concentration of the drug and its metabolite fluctuated in a parallel fashion. Despite this argument, there must be other metabolic processes occurring, as the proportion of AUC_{conj} to AUC of parent drug actually increased as the patients' renal function increased (See Table 3-2 and 3-3), which is the inverse expected with only reversible metabolism as the sole explanation.

The correlation between CL_O and CL_{CR} was only significant for the R enantiomer after the 50 mg dose (see Figure 3-2). As depicted in Figure 3-3, however, a significant positive association was found between CLr_{conj} and CL_{CR} after both doses and for both enantiomers except for the R enantiomers after the 50 mg dose for which the R-KT_{conj} plasma concentration was often below the assay sensitivity. Hence, due to the limited number of data points, the

correlation could not be accurately determined. The association between CLr_{conj} and CL_{CR} may indicate the dependency of KT clearance on renal function indirectly through CLr_{conj}. Further, after the 50 mg dose, almost all of the pharmacokinetic indices calculated for the unchanged enantiomers were significantly correlated with CLr_{conj}. This may reflect the dependency of KT pharmacokinetics on the renal clearance of its metabolite.

This explanation, however, does not seem to hold valid for the 100 mg dose as, among the calculated indices, only β and $t_{1/2}$ were significantly correlated with CLr_{conj}. The difference between the two examined doses may be explained by the observed reduced CLr_{conj} after the 100 mg dose (see Tables 3-2 and 3-3). In light of the observed linear pharmacokinetics, based upon consistency of oral clearance between the two doses, the cumulative urinary excretion of KT_{conj} following a 100 mg dose was expected to be twice as much as that of a 50 mg dose. This, however, was not the case as the percent of the dose excreted in urine and thereby CLr_{conj} were significantly less after the 100 mg dose as compared with the 50 mg dose. A reduced CLr_{conj} despite a constant CL_O may indicate that, following administration to renally impaired patients of relatively high KT doses, a non-renal clearance pathway becomes more predominant to compensate for renal dysfunction. It has previously been suggested that in renal dysfunction, the biliary excretion or emergence of another metabolic pathway may assume more importance (Foster *et al.* 1989). Our data appear to indicate that such compensatory mechanisms may be more operational after higher doses. After 50 mg doses of racemic KT to cholecystectomy patients with CL_{CR} of 67±18 ml/min, Foster *et al.* (1989) noticed only less than 2% of the dose excreted in bile and as KT_{conj}. The patients reported presently, however, had more severe renal dysfunction and the reduced CLr_{conj} was detected at a higher dose level than that used by Foster

et al. (1989). Based upon the data presented herein, the compensatory role of the biliary pathway following higher doses administered to patients with relatively severe renal impairment, may hold plausible.

It should be noted that two patients with the lowest CL_{CR} (6 and 18 ml/min) (see Figure 3-1) exhibited rather long terminal $t_{1/2}$ for both enantiomers (4.5 - 14.2 hrs). Our 24 hr sample collection period, therefore, appears insufficient for accurate estimation of $t_{1/2}$ or complete urine collection. Nevertheless, the dose-dependency of the renal clearance of conjugated KT despite linear oral clearance of the parent drug can be suggested with confidence since an equal collection period was used after both doses, and the observation was consistent in all patients despite inter-subject variations in $t_{1/2}$.

Previous reports on the pharmacokinetics of KT in both young and elderly subjects indicate lack of stereoselectivity in plasma concentration-time courses (Jamali & Brocks 1990). In the present study, on the other hand, a small but significant stereoselectivity in plasma concentrations in favour of (R)-KT was noticed following both doses (see Tables 3-2 and 3-3). This stereoselectivity which seems to be positively associated with CL_{CR} may be explained as follows: a stereoselective clearance *via* acyl-glucuronidation or other pathways may become more important in renal dysfunction due to a tighter binding to plasma protein of (R)-KT as compared with its antipode; stereoselective protein binding in aged patients (68 ± 9 years) with moderate renal dysfunction (CL_{CR} 78 ± 32 ml/min) (Hayball *et al.* 1993), but not in healthy young subjects (Hayball *et al.* 1991), has been reported. Alternatively, in renal dysfunction, the plasma concentration of the unchanged (R)-KT may be increased due to a more efficient back hydrolysis of (R)- KT_{conj} than its antipode (Hayball *et al.* 1993). This is, however, unlikely as

the R enantiomer of KT_{conj} has substantially faster renal clearance than its antipode (see Tables 3-2 and 3-3). The plasma concentration of (R)- KT_{conj} was below the assay's detection limit in three patients following the 50 mg dose and in one after the 100 mg dose, indicating very rapid clearance as compared with the S isomer. Consequently, a rapid removal of the circulating conjugated enantiomer may provide less opportunity for hydrolysis. Another explanation for a smaller than unity of S/R plasma concentration may be an inhibition of another pathway of the R enantiomer clearance, namely chiral inversion to (S)-KT which is reported to account for clearance of approximately 10% of the dose in healthy subjects (Jamali *et al.* 1990). This, although plausible, seems to be in contrast with the observed two fold greater urinary excretion of the (S)-KT_{conj} as compared with (R)-KT_{conj}. Indeed, one may argue that a consequence of the observed lower clearance of (R)-KT may be a greater extent of chiral inversion due to a longer systemic residence time of the substrate. Nevertheless, the data generated in this study are not conclusive to provide a precise explanation for the observed stereoselectivity in plasma KT concentration-time course.

A substantial stereoselectivity in the plasma concentrations of KT_{conj} but in the opposite direction to that observed for the unchanged ketoprofen was noted, particularly in patients with lower CL_{CR} (see Tables 3-2 and 3-3). The plasma concentration of the (S)-KT_{conj} was relatively high compared with (R)-KT_{conj}. Once formed, (R)-KT_{conj} is cleared rapidly. (S)-KT_{conj} , on the other hand, appears to have a rather slow clearance in the presence of renal dysfunction. This is important as hydrolysis of (S)- KT_{conj} results in elevation of plasma (S)-KT which is believed to possess the main antiinflammatory effect of the racemic dose (Jamali & Brocks 1990).

In correlating renal function with various pharmacokinetic indices we empirically chose linear relationships due to the lack of more suitable alternatives. The association between the variables, however, may be more complicated as reflected by the fact that some of our regression lines did not result in the expected intercepts (e.g. the origin in Figure 3-2).

This study is the first report on the influence of varying degrees of renal failure on the pharmacokinetics of KT and its glucuroconjugated metabolite. Our results suggest that the observed alterations of KT disposition previously reported are principally attributable to impaired renal clearance of glucuroconjugated KT in a stereoselective fashion. Subsequently, hydrolysis of the conjugate back to the aglycone results in increased concentration of the intact drug in plasma. Further, despite the observed dose independency of oral clearance, renal clearance of KT_{conj} appeared to be reduced upon the elevation fo the dose to 100 mg suggestive of saturation fo the renal route and compensation by alternative pathways. These observed changes in KT disposition appear to become significant only with moderately severe renal failure (CL_{CR} < 20 ml/min), and dosage adjustments appear to be required only in the presence of this degree of renal impairment.

4 EFFECT OF DIFFERENT FORMULATIONS ON KETOPROFEN PHARMACOKINETICS AND CHIRAL INVERSION

4.1 ABSTRACT

In order to assess pharmacokinetic variability as measured in subjects dosed consecutively with different formulations of ketoprofen, six healthy male subjects received each of the ketoprofen formulations: 1) three 50 mg enteric-coated (EC) tablets (Orudis® E-50; Rhone-Poulenc Pharmaceutical, Montreal, Canada) 2) a 150 mg sustained-release (SR) capsule (Oruvail® 150; Rhone-Poulenc Pharmaceutical, Montreal, Canada) 3) 150 mg ketoprofen in solution. To further assess whether alterations in gastrointestinal motility would significantly alter the ketoprofen pharmacokinetics observed with the above formulations, each subject received 1) metoclopramide (Maxeran®) 20 mg orally 1 hour prior to the ketoprofen 2) propantheline (Probanthine®) 30 mg orally in a similar fashion.

Pharmacokinetic analysis was based on urine drug concentration data only. Excretory rates were maximal for the ketoprofen solution, with the S-enantiomer being excreted more rapidly than the R-enantiomer for all formulations studied. After the solution, the descending order of excretory rates were: EC with metoclopramide, EC, EC with propantheline, SR with metoclopramide, and the SR formulation alone with the slowest rate. There was no significant difference between formulations for cumulative urinary excretion of the different ketoprofen enantiomers.

The mean urinary S:R ratios were significantly greater for the EC formulation in the 8 - 12 hour ($p = 0.021$) and the 12 - 24 hour ($p = 0.022$) collections, in comparison to the solution and the Oruvail® SR formulations. This may be attributable to differences in intestinal site of absorption for the different formulations. Considerations such as different formulations' effects on active drug pre-systemic metabolism, in this case due to varying levels of intestinal mucosal enantiomeric inversion, and therefore enantiomeric bioavailability, are important in addition to other pharmaceutic parameters including speed of onset and duration of effect.

4.2 RATIONALE

There have been numerous formulations of NSAIDs developed for clinical use. As the specific formulation of a compound can significantly influence the absorption and possibly the disposition of that compound in the body, it is important to accurately document these pharmacokinetic variations produced by changes in formulation. This information may allow more effective dosing recommendations for NSAIDs in defined clinical situations.

The 2-arylpropionic acid NSAIDs, including ketoprofen, are generally administered as racemic mixtures of the active S-enantiomer and the inactive R-enantiomer. It is known that some inversion from the r- to the S- enantiomer occurs during absorption of these compounds, and that the site of this enantiomeric inversion is partially restricted in the gastrointestinal (GI) tract, occurring more efficiently in the jejunum and ileum (Jamali et al 1988, Sattari & Jamali 1994). Other studies have also

indicated a systemic contribution to this inversion in humans for ibuprofen (Avgerinos et al. 1991), but results have shown that such pre-systemic inversion is both drug and species specific (Ahn et al. 1991b, Simmonds et al. 1980). It is postulated that differences in formulation of the same compound that would result in differences in exposure to this site of maximal inversion (by varying the site of absorption and the residence time in the GI tract) would then lead to alterations in the ratio of the enantiomers entering the general circulation. These differences in disposition of the enantiomers might, if significant, lead to differences in efficacy or toxicity between administered formulations. This study was designed to assess such pharmacokinetic variability as measured in subjects dosed consecutively with different formulations of ketoprofen, a commonly used NSAID.

4.3 METHODS

4.3.1 Study Subjects and Formulations

Six healthy male subjects between the ages of 25 and 45 were enrolled in the study. Each subject received each of the ketoprofen formulations with at least 48 hours washout between formulations. These consisted of 1) three 50 mg enteric-coated tablets (Orudis® E-50; Rhone-Poulenc Pharmaceutical, Montreal, Canada) 2) a 150 mg sustained-release capsule (Oruvail® 150; Rhone-Poulenc Pharmaceutical, Montreal, Canada) 3) 150 mg ketoprofen in solution (1.875 gm racemic ketoprofen powder dissolved in 1 N NaOH made up to 1.0 liter, final ph 9.0, 80 ml dose containing 150 mg ketoprofen ingested followed by 200 cc water).

4.3.2 Gastrointestinal Motility Effects

To further assess whether alterations in gastrointestinal motility would significantly alter the ketoprofen pharmacokinetics observed with the above formulations, by modifying the exposure of the drug to the anatomical site of maximal inversion, each subject received 1) metoclopramide (Maxeran®) 20 mg orally 1 hour prior to each of the enteric-coated and sustained-release formulations at the same dose of 150 mg ketoprofen 2) propantheline (Probanthine®) 30 mg orally 1 hour prior to the enteric-coated tablets at a dose of 150 mg ketoprofen.

4.3.3 Dissolution Studies

To assess the dissolution profiles of each of the studied commercial ketoprofen formulations, the Orudis® E-50 tablets and the Oruvail® 150 capsules were studied at pH 8.0. These conditions were chosen to illustrate the dissolution profile of the individual formulations at a pH compatible with the human small bowel environment, the major site of ketoprofen absorption and inversion. The buffer used for dissolution for pH 8.0 was Sorenson's phosphate buffer consisting of KH_2PO_4 (3.7 mg/100 ml) and Na_2HPO_4 (96.3 mg/100ml) in water, final pH measured 8.07. The individual formulations were placed in a USP dissolution apparatus utilizing the beaker method with rotating basket assembly at 37 degrees Celsius at 100 rpm and samples obtained at intervals defined below.

4.3.4 Sample Collection

4.3.4.1 Dissolution Samples

For the Oruvail® 150 dissolution study at pH 8.0, 5 ml samples were collected in triplicate, filtered, and the removed volume was replaced by buffer. Samples were collected at 0.25, 0.50, 1, 2, 3, 5, 8, 12, and 24 hours. For the Orudis® E-50 dissolution study at pH 8.0, 3 ml samples were collected in triplicate, filtered, and the removed volume was replaced by buffer. Samples were collected at 5, 10, 15, and 30 minutes, as well as 1, 2, 3, 5, 8, 12, and 24 hours. All of the dissolution samples were frozen at -20 degrees Celsius until analysis.

4.3.4.2 Urine Samples

Pharmacokinetic analysis was based on urine drug concentration data only. For each of the studied formulations each subject ingested the specified dosage in the morning with 200 ml of water after an overnight fast. No food was allowed for 2 hours post-dose. Urine was collected at timed intervals of 0 - 4 hrs, 4 - 8 hrs, 8 - 12 hrs, and 12 -24 hours. A second study using Oruvail® was carried out with dosing at bedtime with urine collections of 0 - 12 and 12 - 24 hrs. Urine volumes were accurately measured and recorded and sample aliquots of each collection were stored at -20 degrees Celsius prior to analysis.

4.3.5 Assay

Ketoprofen enantiomer concentrations were measured by a reverse-phase stereospecific high-pressure liquid chromatography method (Foster and Jamali 1987). Dissolution sample volume was 0.100 ml, and samples were acidified, extracted into chloroform, and then dried completely prior to derivatization and analysis. All urine sample volumes were 0.100 ml or 0.200 ml. Urine samples were assayed after alkaline hydrolysis to obtain total enantiomer concentrations consisting of conjugated and unconjugated drug.

4.3.6 Data Analysis

For the dissolution studies ketoprofen enantiomer concentrations were plotted versus time for each of the enteric coated and sustained release formulations.

For the urine excretion studies urinary excretion rates **(dX_u/dt)** were estimated by collecting all urine for fixed intervals as described above, determining the concentration of ketoprofen in the urine, and multiplying this concentration by the incremental volume of urine collected to determine the amount of drug excreted **(X_u).** This amount was divided by the collection time to calculate the excretion rate (μg/hr) **(dX_u/dt).** This was plotted against time at the midpoint of each collection interval (t_{mid}). Linear least-squares regression analysis of a semilogarithmic plot of this excretion rate versus time (t_{mid}) data yielded the slope equal to -K/2.303, derived from the relationship **$\log dX_u/dt = \log k_e X_0 - Kt/2.303$**. Elimination $t_{1/2}$ (hr) was calculated from the relationship **$t_{1/2} = 0.693/K$** for each plot.

The sigma-minus method was also employed for each urine collection. The amount of unchanged drug to be excreted (**A.R.E.**) after each collection interval was calculated by subtracting total urine excretion at the end of each interval from cumulative urine excretion ($X_u^\infty - X_u$). A semilogarithmic plot of A.R.E. versus time was produced for each total collection and analysis in the same manner as described above derived from the relationship $\log (X_u^\infty - X_u) = \log X_u^\infty - kt/2.303$ yielded the pharmacokinetic variables K and elimination $t_{1/2}$. Summation plots were produced using mean values from the urine collection data from each of the 6 individuals for each formulation.

Urinary ketoprofen enantiomer S:R ratios were determined for each collection period for all subjects and all formulations. Analysis of samples collected from the night dosing of Oruvail® were used only for determination of S:R ratios. Mean S:R values for each collection period for individual formulations were determined using aggregate data from all subjects. These were plotted versus time for each formulation to observe time-dependent alterations and to note differences between formulations.

4.3.7 Statistical Analysis

Statistical comparison of relevant pharmacokinetic variables was carried out between formulations using Student's paired or unpaired t test and by one way ANOVA, as deemed appropriate. Least-squares linear regression analysis and the Pearson's correlation coefficient of the regression slopes were used to assess the significance of the

relationships between various factors and indices. All tests were conducted with a level of confidence set at $\alpha = 0.05$. Data are expressed as mean $\pm$ standard deviation.

4.4 RESULTS

4.4.1 Dissolution Studies

The dissolution studies indicated a more rapid release of drug from the enteric-coated (Orudis® EC-50) formulation (see Figure 4-1: Dissolution profile of enteric-coated 50 mg ketoprofen formulation at pH 8.0) than from the sustained-release (Oruvail® 150) formulation (see Figure 4-2: Dissolution profile of sustained-release 150 mg ketoprofen formulation at pH 8.0), although both studies showed a rapid dissolution under those conditions. Essentially complete drug recovery was achieved with each formulation.

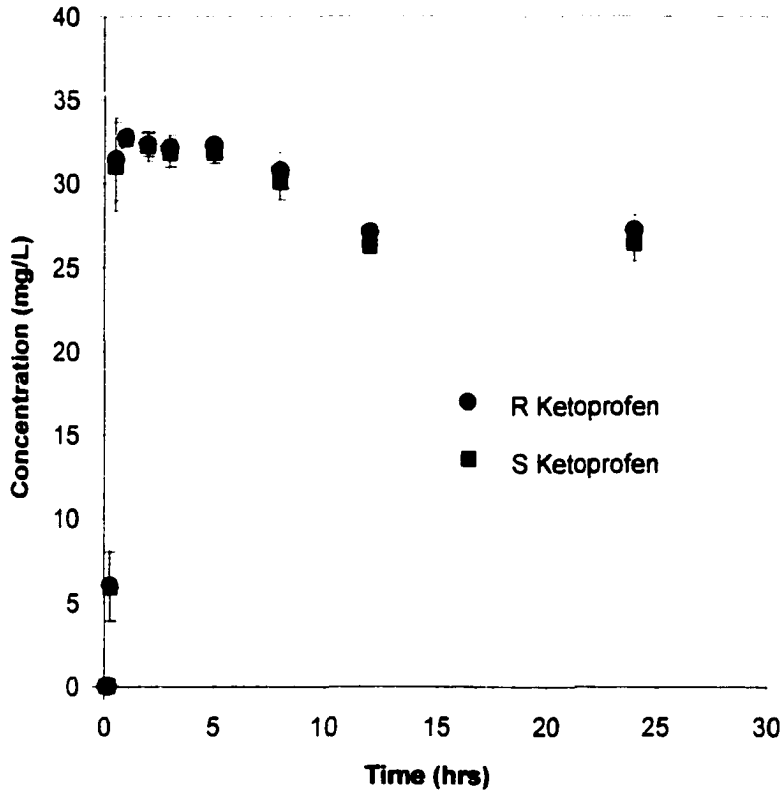


Figure 4-1: Dissolution profile of enteric-coated 50 mg ketoprofen formulation at pH 8.0

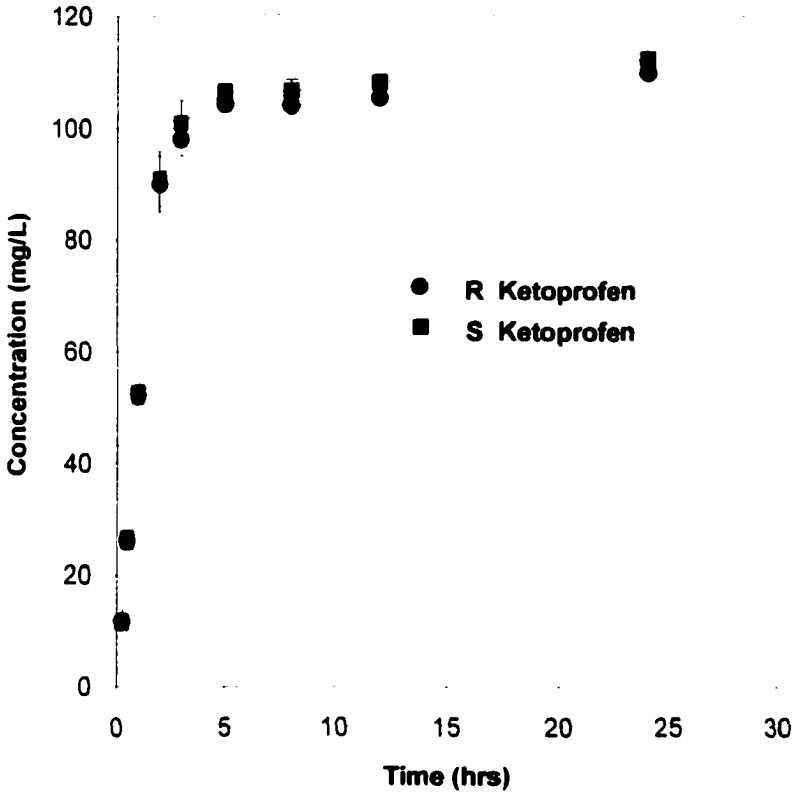


Figure 4-2: Dissolution profile of sustained-release 150 mg ketoprofen formulation at pH 8.0

4.4.2 Urine Excretion Pharmacokinetic Analysis

Analysis of the ketoprofen enantiomer urine excretion data indicated that the excretory rates were maximal for the ketoprofen solution, with the S-enantiomer being excreted more rapidly than the R-enantiomer for all formulations studied. After the solution, the descending order of excretory rates consisted of enteric-coated with metoclopramide, enteric-coated, enteric-coated with propantheline, sustained-release with metoclopramide, and the sustained-release formulation alone with the slowest rates (see Table 4-1: Urinary ketoprofen enantiomer excretion rates (mg/hr).

Differences between formulations and between enantiomers is illustrated by the urinary excretory rate *vs* time plots (see Figure 4-3 and Figure 4-4).

Table 4-1: Urinary ketoprofen enantiomer excretion rates (mg/hr).

Formulation	S-KT(mean)	S-KT (SD)	R-KT(mean)	R-KT (SD)	T_{mid}
Oruvail	0.58	0.38	0.54	0.36	2
	3.82	1.28	3.24	1.20	6
	3.14	1.28	2.51	1.02	10
	1.43	0.44	1.09	0.35	18
EC	4.01	1.67	3.59	1.53	2
	5.42	2.94	4.01	2.16	6
	1.71	0.44	1.17	0.23	10
	0.36	0.19	0.22	0.11	18
Solution	8.96	0.48	7.64	0.77	2
	1.61	0.36	1.31	0.52	6
	0.49	0.17	0.37	0.12	10
	0.10	0.02	0.08	0.02	18
EC-Maxeran	6.59	1.10	5.28	0.67	2
	4.36	0.46	3.11	0.53	6
	0.89	0.17	0.58	0.13	10
	0.17	0.05	0.10	0.03	18
EC-Probanthine	4.02	2.12	3.24	1.69	2
	5.25	2.34	3.93	1.66	6
	3.49	2.83	2.62	2.29	10
	0.41	0.28	0.26	0.19	18
Oruvail-Maxeran	1.90	1.08	1.63	0.85	2
	5.05	1.92	4.01	1.56	6
	2.59	0.87	1.96	0.68	10
	0.62	0.19	0.45	0.15	18

EC = Enteric-Coated

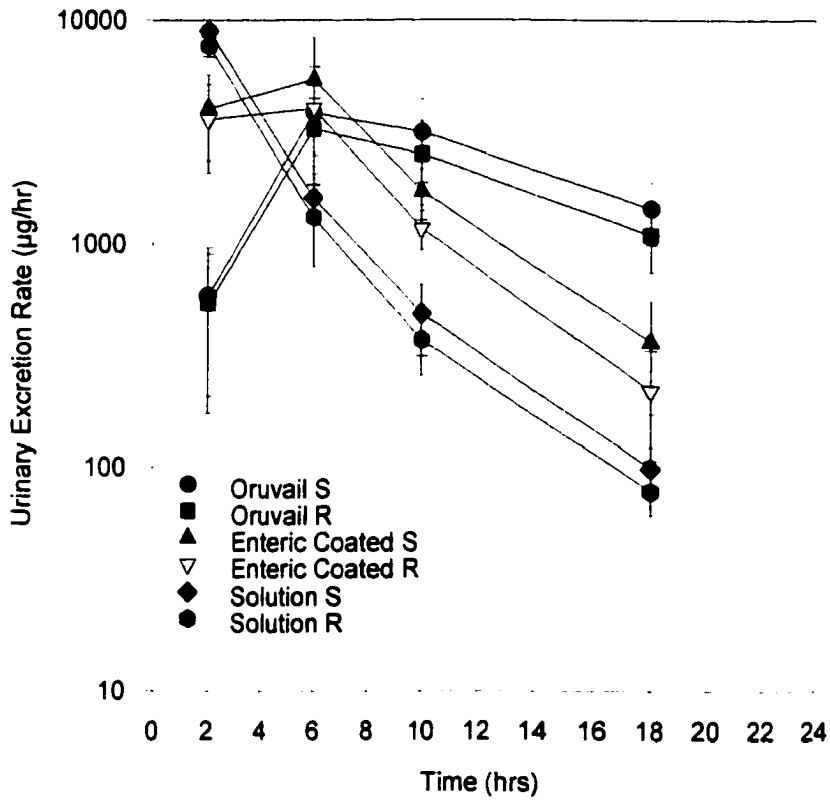


Figure 4-3: Urine excretion rates for Oruvail®, EC and solution.

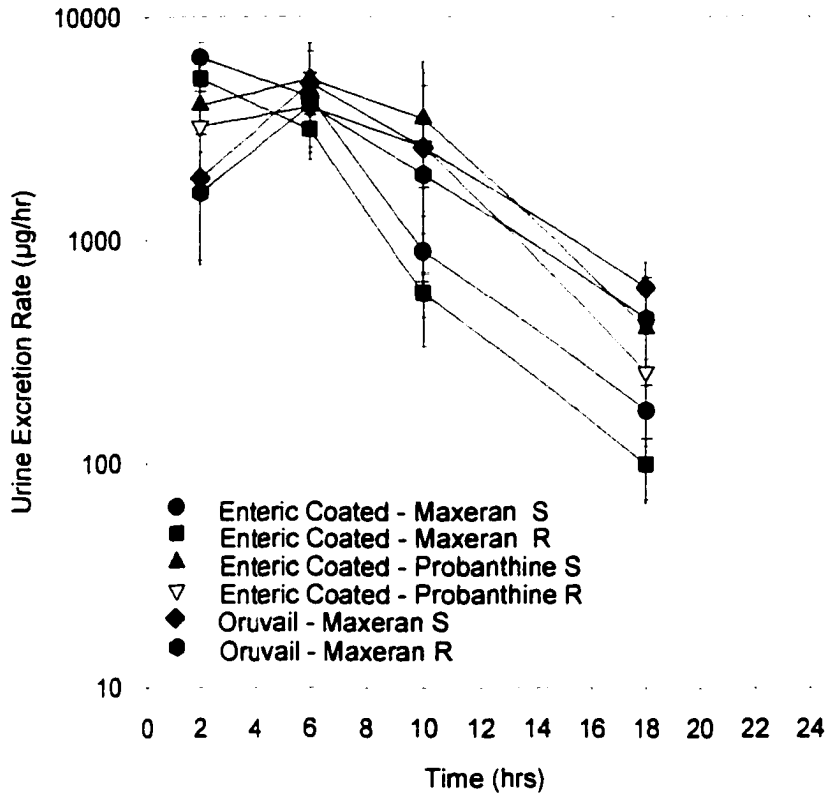


Figure 4-4: Urine excretion rates for EC-metoclopramide, EC-propantheline and SR-metoclopramide.

The concurrent use of propantheline with the enteric-coated formulation had a variable effect on different subjects. Two of the subjects had no ketoprofen excretion in the first 4 hour urine collection and one of these subjects had no excretion in the first 8 hours of collection. Both of these individuals excreted the majority of measured drug in urine in the last 12 hour collection.

There was no significant difference between formulations for cumulative urinary excretion of the different ketoprofen enantiomers (SeeTable 4-2). It can be seen that the majority of the solution was absorbed in the first 4 hours, with a combination of gastric and small bowel absorption.

Table 4-2: Cumulative excretion of ketoprofen enantiomers for solution, sustained-release and EC (mg) [mean(SD)].

Time (hr)	Solution S	Solution R	Sustained-Release S	Sustained-Release R	Enteric-Coated S	Enteric-Coated R
0 – 4	35.8 (1.9)	29.8 (3.1)	2.33 (2.1)	2.15 (1.6)	16.1 (0.7)	14.4 (0.6)
4 – 8	6.4 (1.4)	5.3 (2.1)	15.2 (0.5)	13.0 (0.5)	21.7 (1.2)	16.0 (0.9)
8 - 12	1.9 (0.7)	1.5 (0.5)	12.6 (0.5)	10.0 (0.4)	6.8 (1.8)	4.7 (0.9)
12 – 24	1.2 (0.3)	0.9 (0.2)	17.2 (0.5)	13.1 (0.4)	4.4 (2.3)	2.6 (1.4)
0 - 24	45.4 (1.7)	37.5 (3.7)	47.4 (4.0)	38.3 (4.2)	46.3 (6.3)	35.3 (3.8)

Plotting of the A.R.E. data derived using the sigma-minus method illustrated as well the differences between formulations and enantiomers (see Figure 4-5: A.R.E. plots for Oruvail®, EC and solution.).

The effect on urinary excretion of the ketoprofen enantiomers by the addition of motility-modifying agents is illustrated by their respective A.R.E. plots. As expected, metoclopramide produced more rapid excretion of drug with both enteric-coated and sustained-release formulations. The addition of propantheline to the enteric-coated formulation delayed the excretion (see Figure 4-6: Enteric-coated A.R.E. plots and Figure 4-7: Oruvail® A.R.E. plots).

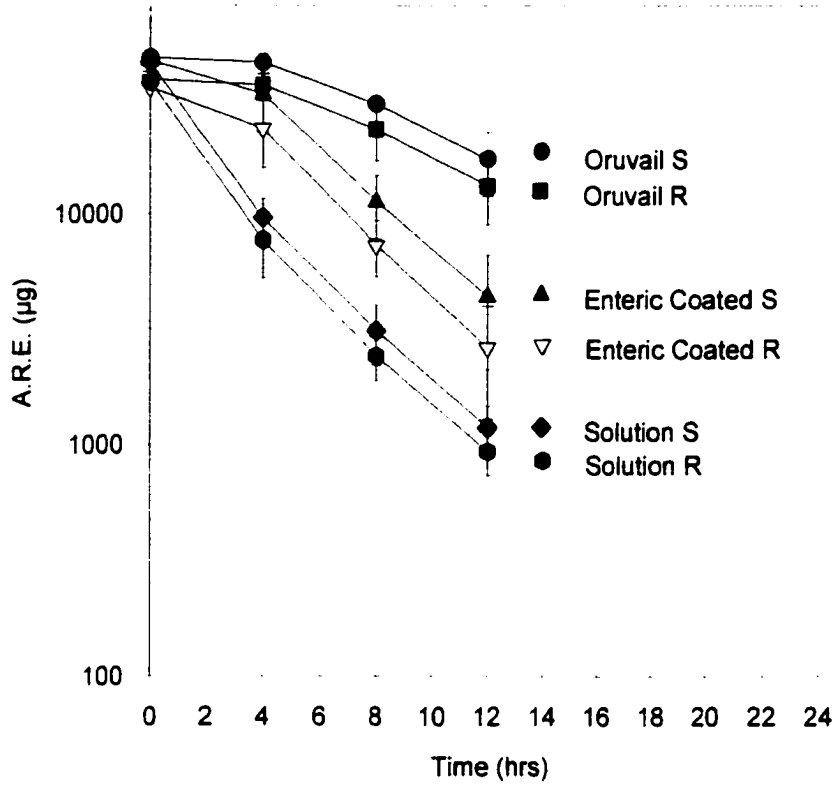


Figure 4-5: A.R.E. plots for Oruvail®, EC and solution.

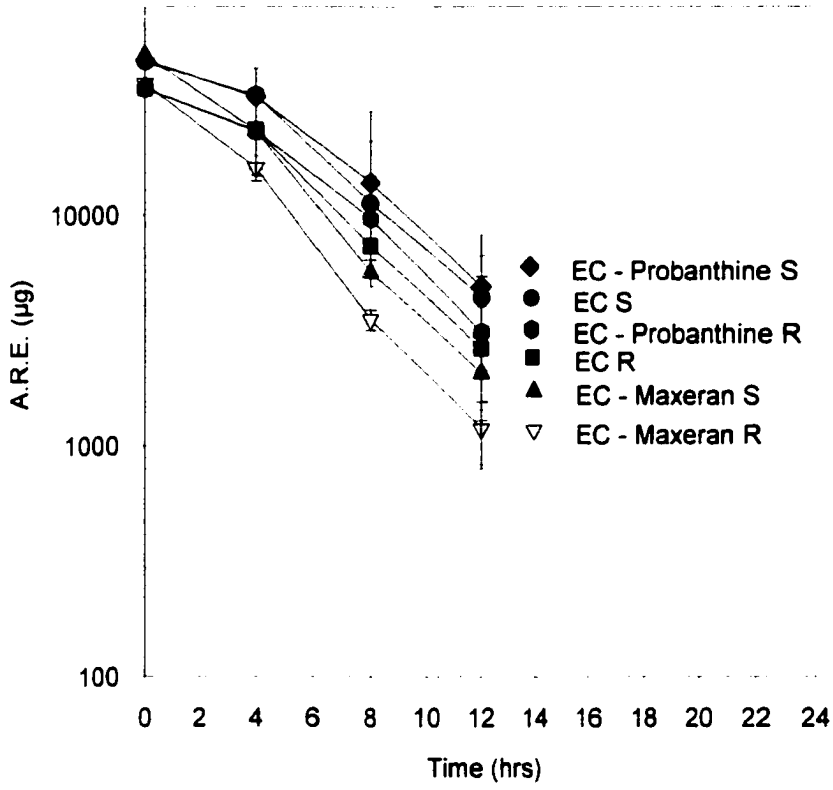


Figure 4-6: Enteric-coated A.R.E. plots

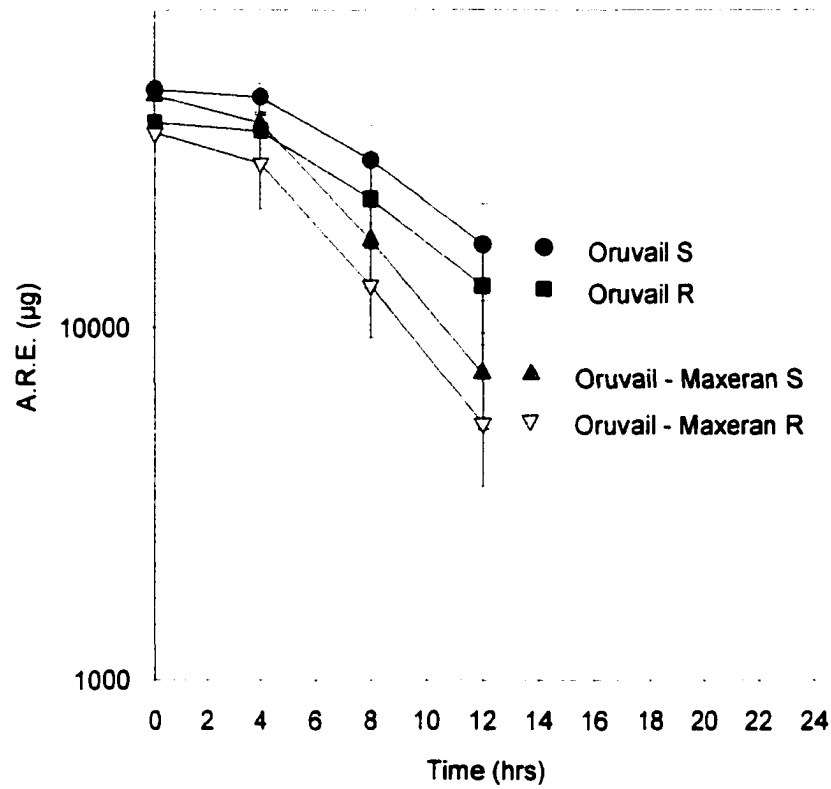


Figure 4-7: Oruvail® A.R.E. plots

The ratios between the amounts of ketoprofen S-enantiomer and R-enantiomer excreted in each collection for each formulation were determined. The mean urinary S:R ratios were significantly greater for the EC formulation in the 8 - 12 hour ($p = 0.021$) and the 12 - 24 hour ($p = 0.022$) collections, in comparison to the solution and the sustained-release formulations (see Table 4-3 and Figure 4-8).

Table 4-3: Ketoprofen urine collection S:R enantiomer ratios for different formulations [mean(SD)]

Time (hr)	Solution	SR	EC	EC-Met.	EC-Prop.	SR-Met.
0 - 4	1.21 (.10)	1.12 (.06)	1.13 (.10)	1.24 (.07)	1.24 (.04)	1.14 (.09)
4 - 8	1.31 (.24)	1.20 (.12)	1.36 (.07)	1.42 (.12)	1.32 (.10)	1.28 (.07)
8 - 12	1.30 (.13)	1.26 (.05)	1.45 (.10)	1.55 (.13)	1.42 (.15)	1.33 (.07)
12 - 24	1.28 (.26)	1.29 (.07)	1.64 (.08)	1.74 (.16)	1.65 (.16)	1.43 (.20)
0 - 24	1.22 (.11)	1.25 (.08)	1.33 (.13)	1.33 (.07)	1.33 (.05)	1.28 (.07)

EC = enteric-coated, Met. = metoclopramide, Prop. = propantheline, SR = sustained-release

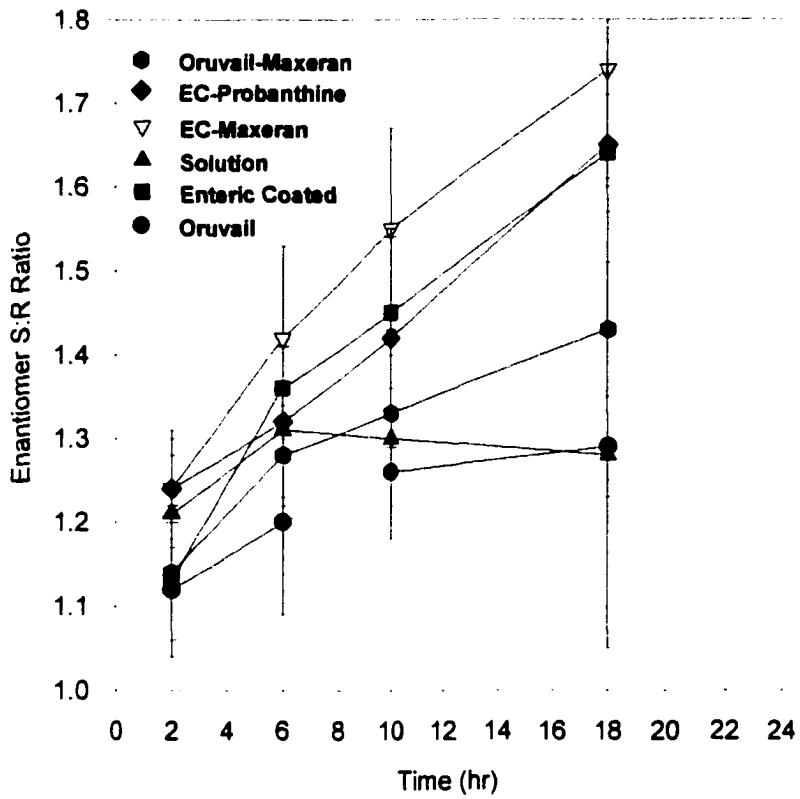


Figure 4-8: Urinary ketoprofen S:R enantiomer ratios for all formulations

Terminal elimination $t_{1/2}$, derived from the A.R.E. plots for each formulation, were similar for the solution (S - 2.64 hr, R - 2.64 hr) and the enteric-coated formulation enantiomers (S - 3.01 hr, R - 2.76 hr), but prolonged for the sustained-release formulation enantiomers (S - 5.23 hr, R - 5.02 hr).

4.5 DISCUSSION

A variety of different formulations have been devised for NSAIDs to date. These have been developed for a number of clinical rationales including improvement in compliance, tolerability and sustained efficacy, as well as innovative marketing iniatives. The use of NSAID solutions is not practical but other rapidly effective formulations are available for specific clinical indications, including parenteral ketorolac (Toradol®) and the sodium salt (eg naproxen [Anaprox®]) or the potassium salt (eg diclofenac [Voltaren Rapide®]) of different NSAIDs. Enteric-coating of NSAIDs theoretically reduces the direct gastric irritation of these compounds resulting in reduced gastrointestinal complaints and improved tolerability. Sustained-release formulations developed for numerous NSAIDs improve compliance and allow for more sustained clinical efficacy.

The patient's clinical substrate can significantly influence the efficacy and tolerability of a particular NSAID formulation. The presence of clinical or pharmaceutical factors that influence gastrointestinal motility can markedly impact on a particular NSAID formulation's effectiveness. For example, the presence of gastroparesis from diabetic neuropathy or the concurrent use of medications with anticholinergic effects eg tricyclic antidepressants can significantly reduce the rate of absorption and delay clinical effects.

The presence of increased bowel transit rates or limited bowel surface area eg. diarrheal states or the 'short bowel syndrome' from intestinal bypass surgery or bowel resections can restrict the utility of certain NSAIDs such as sustained-release formulations. The choice of a particular formulation of an NSAID by a clinician takes into consideration some or all of the above factors, as well as cost considerations and availability. This study has indicated that other considerations such as different formulations' effects on active drug pre-systemic metabolism and therefore enantiomeric bioavailability might need to be integral to these decisions.

It is known that 2-arylpropionic acid NSAIDs including ketoprofen undergo a variable extent of chiral inversion (unidirectional R- to S- in humans). This inversion process occurs primarily in the liver and is mediated through the formation of the R-enantiomer CoA thioester which is then racemized and hydrolyzed by a stereoselective epimerase (Knihinicki et al 1991). This process also occurs in the gastrointestinal tract where it constitutes a form of pre-systemic metabolism. It has been demonstrated for regular-release ketoprofen that approximately 10% of the administered dose undergoes this metabolic process. (Foster & Jamali 1988) As the desired pharmacological cyclo-oxygenase inhibitory activity for ketoprofen is attributable only to the s-enantiomer, any alterations in absorption, distribution or elimination resulting in altered S:R enantiomeric ratios could produce clinically important effects.

To date the sites of this metabolic process for this group of NSAIDs have been localized partly to the gut. Jamali et al (1988) have demonstrated that this process occurs in the gut wall during absorption, and that there is a site-specificity in the gastrointestinal

tract, with the jejunum and the ileum being the most active areas. A rapidly absorbed ibuprofen suspension was compared to an intravenous dose in humans and it was concluded that there is no presystemic inversion with that particular oral delivery (Hall et al. 1993). Drug formulations that deliver a greater proportion of the administered dose to this mucosal site of maximum inversion capacity are likely to be characterized by the greatest S:R enantiomeric ratios. Utilizing intravenous dosing as well as conventional and sustained-release ibuprofen microspheres in the rat model, it has been shown that the amount of S/R inversion is negligible systemically in that model, and is dependent on the extent of drug delivery to the site of maximal inversion as well as the specific formulation, with their varying absorption profiles (Adeyeye & Chen 1997).

This study was designed to observe any alterations in rates of drug elimination and any differences in enantiomeric S:R ratios in excreted drug in urine due to differences only in the administered formulation of ketoprofen. A solution of ketoprofen was expected to be rapidly absorbed, largely from the gastric mucosa, with further absorption in the proximal small bowel. As expected, the enantiomeric excretory rates were greatest for the solution in the early 0 - 4 hr collection. Enteric-coated ketoprofen was resistant to dissolution in the acidic gastric pH and underwent rapid disintegration and dissolution at the alkaline pH of the small bowel. The sustained-release formulation delivered drug into the gut lumen at a slowed rate, resulting in drug release throughout the small and large intestine, likely to a large extent at sites where little chiral metabolic inversion takes place. As predicted, the EC formulation enantiomeric excretory rates were less than those from the solution but greater than the sustained-release formulation (see Figure 4-3: Urine

excretion rates for Oruvail®, EC and solution.). The *in vitro* dissolution studies confirmed these formulations' pharmaceutical characteristics.

The enteric-coated ketoprofen formulation would theoretically deliver a greater proportion of drug to the site of maximal inversion in the small bowel than either other formulation resulting in greater S:R enantiomeric ratios. This was in fact observed, with the EC formulation S:R ratios highest for total sample collection (1.33 *vs* 1.22 [solution] and 1.25 [Oruvail®]) and for each interval collection except the 0 - 4 hr collection. In this early collection the solution had a higher S:R ratio (1.21) than the EC (1.13) likely due to more rapid delivery of drug in the solution to the lower small bowel sites of maximal inversion, due to the rapid emptying of fluids from the stomach that normally occurs.

Further modifications in the rate at which ingested drug would be exposed to different sites in the gastrointestinal tract were accomplished by the use of gut motility-modifying medications. Metoclopramide has prokinetic actions reducing residence time in the stomach and speeding transit through the small bowel. Propantheline is an anticholinergic agent that slows gut motility generally, resulting in prolonged gastric retention and slowed bowel transit times. Metoclopramide would be theorized to move available drug to the site of maximal enantiomeric inversion more quickly but to possibly reduce the overall amount of inversion at that site by its enhanced gut motility. As predicted the addition of metoclopramide to the EC formulation was observed to increase the S:R enantiomeric ratios for each collection period in comparison to the EC formulation alone. However, there was not a significant difference in the observed S:R

ratio for complete sample collection between the two formulations (1.33 for each formulation).

Propantheline would be theorized to slow the arrival of the drug to the small bowel sites of maximal inversion, but would produce more or less inversion dependent on where in the gut the maximal absorption occurred. Reduced S:R ratios would be identified if most of the available drug was absorbed prior to reaching the jejunum because of slowed small bowel transit. This study observed reduced S:R ratios for each of the 4 - 8 and 8 - 12 hr collections when propantheline was added to the EC formulation, in comparison to the EC formulation alone. The overall collection S:R ratios were identical however (1.33 for each).

This study has demonstrated that the use of different formulations of a drug can lead to not only differences in rate of absorption and delivery of active drug but also to differences in pre-systemic drug metabolism (enantiomeric inversion) and to potentially the amount of active drug available. Dependent on the particular drug, the formulations available and the underlying clinical substrate, the choice of formulation prescribed should be recognized as potentially impacting on overall clinical efficacy, beyond simply dosing schedules and compliance factors.

5. PHARMACODYNAMICS OF IBUPROFEN IN RHEUMATOID ARTHRITIS

5.1 Abstract

Twelve adult patients with mild to moderately active rheumatoid arthritis were provided with four different racemic ibuprofen doses sequentially in a randomized double-blind fashion in a capsule formulation. Each dose was taken for two consecutive weeks with weekly assessments. These consisted of 200 mg, 400 mg, 800 mg, and 1200 mg doses every 8 hours. Clinical and laboratory and compliance assessments were done weekly for 9 weeks. Each subject had their trough enantiomeric ibuprofen plasma levels measured at each assessment. Each subject at one of their weekly assessments in a randomized fashion underwent an eight hour pharmacokinetic study.

Overall, S-ibuprofen enantiomeric concentrations correlated better with measured clinical and laboratory variables than total ibuprofen concentration or R-ibuprofen enantiomeric concentrations or dose. The observed correlations between the plasma ibuprofen concentrations and the number of swollen joints were more significant for S-ibuprofen ($r = -0.265$, $p = 0.007$) than for total ibuprofen ($r = -0.250$, $p = 0.011$) or R-ibuprofen ($r = -0.206$, $p = 0.038$), while the relationship with dose ($r = -0.060$, $p = 0.550$) was not significant. The relationships were similar for the number of tender joints,patient global assessment, clinician global assessment, pain previous week, pain previous day, pain

day of assessment, morning stiffness, Health Assessment Questionnaire (HAQ), ESR and C-reactive protein. None of these variables except patient global assessment showed a significant relationship with the administered dose of ibuprofen. In contrast to the above described efficacy variables, the assessment of drug-induced toxicity correlated in an inverse manner, maximal with dose. Our results support the premise that measurement of plasma active enantiomer NSAID levels is a valid and practical alternative to measurement of effect compartment (synovial fluid or synovium) levels, a more invasive methodology.

5.2 Rationale

Arthritis and nonarticular inflammatory and rheumatic disorders are the most prevalent clinical conditions affecting individuals of all origins. The nonsteroidal antiinflammatory drugs (NSAIDs) are among the most widely utilized classes of medications worldwide. These agents are also believed to be one of the most toxic classes of medications prescribed (Fries et al. 1991). A rational validated approach to dosing guidelines for NSAIDs, taking into consideration clinical characteristics of the patients and their concurrent pathologies, as well as an accurate knowledge of the pharmacokinetics and pharmacodynamics of the prescribed agents, optimally will limit drug toxicities.

A dose - effect or concentration - effect relationship has been clinically demonstrated for some classes of medications, with subsequent dosing guidelines formulated and widely applied. These include several antirheumatic drugs, in relation to efficacy and/or toxicity, such as sulfasalazine, methotrexate, antimalarials and cyclosporin. There have been a number of studies performed to date that have attempted to document a

dose - effect pharmacodynamic relationship for different NSAIDs. Fewer studies have included NSAID concentration data or have attempted to correlate plasma or synovial fluid drug concentration or other pharmacokinetic variables with observed effects.

Some of the NSAIDs in use are administered as racemic mixtures of active S-enantiomer and inactive R-enantiomer. These include most of the 2-arylpropionic acid NSAIDs including ketoprofen, ibuprofen, tiaprofenic acid and flurbiprofen. Naproxen is administered as the single active enantiomer. The disposition of these administered racemates includes a variable degree of enantiomeric inversion from the inactive R-enantiomer to the active S- enantiomer resulting in disparate and variable ratios of these enatiomers available to exert their pharmacological effects. With ibuprofen this bioinversion of the R- to S- enantiomer can be extensive (57.6%) (Cheng et al. 1994). To date there is limited information correlating clinically relevant enantiomeric concentrations and observed clinical effects in patients using these medications for their rheumatic disorders. Some of the previously published data that has attempted to demonstrate a concentration - effect pharmacodynamic relationship has been obtained using non-stereospecific methodology that limits their validity.

The intent of this study was to determine if an enantiomeric concentration - effect pharmacodynamic relationship could be demonstrated with ibuprofen administered to a group of patients with the active inflammatory disorder rheumatoid arthritis. To date most pharmacodynamic studies with NSAIDs have been carried out in rheumatoid arthritis as it is the most prevalent systemic inflammatory rheumatic disorder for which NSAIDs are prescribed. Individual doses of ibuprofen were administered for a sufficient duration

to achieve a pharmacokinetic and clinical steady state prior to pharmacokinetic sampling and clinical assessment.

5.3 Methods

5.3.1 Patient Selection

This study was conducted according to the principles of the Declaration of Helsinki, with prior institutional ethics approval. Twelve adult patients with mild to moderately active rheumatoid arthritis were recruited. All patients were receiving disease remittive agents in a stable fashion with no changes in therapy for at least 3 months prior to enrollment, and no changes were allowed during the study. Most patients were using NSAIDs prior to enrollment, and these were stopped at least one week prior to their baseline assessment. None of the patients were receiving oral corticosteroids, and none had received any corticosteroid injections for at least 3 months prior to the study. No oral or injectable corticosteroids were administered during the course of the study. None of the patients had a hypersensitivity to or had reported any significant adverse effects from NSAID use previously. All patients were medically stable with no other significant cardiac, renal or hepatic disease. All patients provided informed consent prior to entering the study.

All patients with severe active disease, a recent change in remittive agents, recent corticosteroid use, unstable or serious comorbidities, a history of lack of NSAID efficacy or reported NSAID intolerance, or cognitive or linguistic limitations were excluded from the study.

5.3.2 Drug Dosage Regimen

5.3.2.1 Ibuprofen

Patients were provided with four different racemic ibuprofen doses sequentially in a randomized fashion in a capsule formulation. Both the study patients and the study examiner were blinded as to the dose provided. Each dose was taken for two consecutive weeks with weekly assessments. These consisted of 200 mg, 400 mg, 800 mg, and 1200 mg doses taken every 8 hours with food. Patients documented the time of each dose and provided this information to the study examiner at each weekly interview. The first dose was provided at the baseline assessment after one week washout from their prior NSAID. Exactly enough study medication was provided at each weekly assessment for the following week. If the patient reported that their level of symptoms was intolerable on the current dose of study drug after the first week of that dose, despite using supplemental acetaminophen, they were provided with the next dose at that time in the usual randomized fashion.

5.3.2.2 Acetaminophen

All patients were provided with 325 mg acetaminophen tablets as escape analgesic medication for the duration of the study, to be used as required subjectively for arthritis symptom control. This was initiated at the start of their washout period from their prior NSAID use and was continued until study completion.

5.3.2.3 Compliance Measures

A documented pill count of ibuprofen capsules in the returned container was performed at each weekly assessment as a compliance measure. As well, plasma ibuprofen levels obtained at each visit confirmed that the patient had been taking their provided study medication.

5.3.3 Clinical Assessment

Each subject was interviewed and examined prior to enrollment to ensure compliance with study inclusion criteria. Subsequently each subject was assessed on a weekly basis for a maximum of nine weeks (baseline after one week of NSAID washout and two weekly assessments for each study ibuprofen dose). Assessments were performed by the same individual at usually the same time of day, and were always conducted prior to the next ibuprofen dosing interval.

Each clinical assessment included a documentation by the study examiner of the number of swollen joints, the number of tender joints, bilateral grip strength testing (performed three times with each hand in an alternating fashion with the maximum strength recorded for each hand), and the number of returned ibuprofen capsules. The examiner also recorded the clinician's global assessment of disease activity utilizing a nominal scale consisting of the following choices: none, mild, moderate, moderately severe, severe.

Each study subject at each assessment also completed a questionnaire in relation to their present disease state (based on the day of assessment and the previous week). Questionnaire outcome measures documented included the patient's global assessment of

disease activity utilizing the same scale as the clinician's global assessment and pain scales for the time of assessment, for the previous day, and for the previous week (10 cm visual analogue pain scales performed in triplicate at each visit). The duration of morning stiffness (in minutes to a maximum of 300 minutes), the number of analgesic acetaminophen tablets used in the last week, and the amount of gastrointestinal (GI) symptoms in the previous week (10 cm visual analogue scale) were each reported. The Health Assessment Questionnaire, a validated measure of functional capacity and subjective health status satisfaction in rheumatoid arthritis was also completed at each visit (Ramey et al. 1992). Each questionnaire was reviewed by the study examiner at the time of the assessment to ensure completion.

5.3.3.1 Sleep Assessment

As sleep disturbance is a frequent feature of active inflammatory arthritis (Drewes et al. 1998), an instrument to assess sleep characteristics was devised in the form of a questionnaire. This included documentation (in relation to the previous night) of the time required to get to sleep (minutes), the total duration of sleep time (minutes), and the number of times the patient was awake during the night for more than one minute. The questionnaire also included questions regarding the quality of the previous night's sleep in the form of 6.0 cm visual analogue scales for each measure. These measures included 1) deep to light sleep 2) long to short sleep 3) uninterrupted to interrupted sleep 4) restful to restless sleep 5) best to worst possible sleep. Patients were instructed to complete the sleep questionnaire immediately after awakening on the day of their assessments and these were reviewed each week by the study examiner.

5.3.4 Laboratory Assessment

Each subject on each day of their study assessments had blood drawn in the clinical laboratory for measurement of the erythrocyte sedimentation rate (ESR) and the C-reactive protein (CRP), both of which are nonspecific markers of inflammation that have been validated as useful outcome measures in rheumatoid arthritis (Wolfe 1997). All subjects had baseline laboratory testing to document normal hematologic, renal and hepatic function.

5.3.4.1 Cytidine Deaminase

With each assessment of the first six patients blood was drawn for measurement of plasma cytidine deaminase levels, a validated marker of inflammation in rheumatoid arthritis (Skeith et al. 1993).

5.3.5 Pharmacokinetic Studies

5.3.5.1 Weekly Samples

Each subject had two vials of blood (7 ml each) drawn at each assessment at the time of their trough ibuprofen blood level (immediately prior to the subsequent dosing interval). The plasma was removed after centrifugation from each vial and immediately frozen and stored at -70 degrees Celsius. Plasma ibuprofen enantiomer levels were measured in these samples at a later date using the stereospecific methodology described below.

5.3.5.2 Steady State Dosing Study

Each subject at the time of one of their weekly assessments in a randomized fashion participated in a formal eight hour pharmacokinetic study. Subjects were fasting apart from water after midnight and initiated each study at 0800 hrs. The patients were administered their ibuprofen dose (in the usual double blinded fashion) with 200 ml of water after the time 0 (trough level) sampling was carried out. Subsequent blood samples were obtained at 0.25, 0.50, 1.0, 2.0, 3.0, 6.0, and 8.0 hrs after dosing. The plasma was removed after centrifugation from each vial and immediately frozen and stored at -70 degrees Celsius. Plasma ibuprofen enantiomer levels were measured in these samples at a later date using stereospecific methodology. Pharmacokinetic parameters were determined for each patient study using Winnonlin and were adjusted for dose where indicated.

5.3.6 Assay

Plasma ibuprofen levels were determined using a stereospecific high pressure liquid chromatography (HPLC) technique as previously described (Wright et al. 1992).

5.3.7 Data Analysis

Pearson's correlation coefficient was determined for relationships between each of 1) ibuprofen dose 2) R-ibuprofen enantiomer concentration 3) S-ibuprofen enantiomer concentration 4) total ibuprofen enantiomeric concentration and each of the measured clinical and laboratory variables. Student's paired t test was used to evaluate the differences between individual enantiomer pharmacokinetic indices. All tests were conducted with a level of confidence set at alpha = 0.05. These evaluations were

determined for all patient assessments, as well as for second week assessment data only. For this latter analysis, if only a single week at a particular dose was received by a study subject, that first week assessment data was used.

5.4 Results

5.4.1 Study Subjects

The first 12 subjects interviewed fulfilled inclusion criteria and were enrolled in the study. The 10 female and 2 male subjects varied in age from 25 years to 66 years, with a mean disease duration of 6.08 ± 2.94 years. Eleven of the 12 subjects were receiving NSAIDs prior to the initial washout phase of the study, and each of the 12 subjects were taking disease remittive agents, each of which was continued unchanged throughout the study. Each subject completed the study. There were a total of 102 weekly patient assessments, with 6 of the drug dosage intervals consisting of a single week rather than two weeks administration. There were no significant toxicities reported by any study subject attributable to their study medications. Patient characteristics are listed in Table 5-1: Patient characteristics at time of enrollment.

Table 5-1: Patient characteristics at time of enrollment.

Patient	Sex	Age	Duration of RA (yrs)	Medications
1	F	25	2	chloroquine, naproxen
2	F	39	8	gold, methotrexate, piroxicam, diflunisal
3	F	66	10	d-penicillamine, diclofenac
4	F	43	5	gold, methotrexate, flurbiprofen
5	M	48	4	gold, diclofenac
6	F	49	8	chloroquine, no NSAID
7	F	63	12	chloroquine, diclofenac
8	F	54	5	chloroquine, naproxen
9	F	65	6	chloroquine, sulindac
10	F	31	3	salazopyrine, tiaprofenic acid
11	M	60	6	methotrexate, tiaprofenic acid
12	F	31	4	methotrexate, diclofenac

5.4.2 Assessment Variables

5.4.2.1 Clinical Assessment

The correlations between individual clinical variables and each of 1) dose, 2) R-ibuprofen enantiomer concentration, 3) S-ibuprofen enantiomer concentration, and 4) total enantiomeric ibuprofen concentration were determined for the complete data and for the second week data (if second week data was missing, first week data was used). Although there were only minor differences between results from these two separate data sets, the complete data results showed greater levels of correlation for most of the assessment variables for which a significant correlation was identified. Correlation coefficients and their levels of statistical significance are listed in Table 5-2 : Assessment variable - concentration correlation coefficients for complete data (r values) and in Table 5-3: Statistical significance of assessment variable - concentration correlations (p values) respectively.

Table 5-2 : Assessment variable - concentration correlation coefficients for complete data (r values)

Assessed Variable	Number	Dose	R - ibuprofen conc.	S - ibuprofen conc.	Total ibuprofen conc.
Ibuprofen Dose (mg)	102	1.000	0.554	0.740	0.689
Number swollen joints	102	-0.060	-0.206	-0.265	-0.250
Number tender joints	102	-0.095	-0.329	-0.412	-0.392
Patient global assessment	101	-0.280	-0.429	-0.389	-0.417
Clinician global assessment	95	-0.079	-0.316	-0.400	-0.380
Pain over last week	101	-0.112	-0.257	-0.309	-0.298
Pain previous day	102	-0.086	-0.233	-0.295	-0.280
Pain at assessment	102	-0.125	-0.278	-0.342	-0.327
Grip strength right (mm Hg)	101	-0.064	0.077	0.196	0.155
Grip strength left (mm Hg)	101	0.016	0.071	0.187	0.147
Morning stiffness (minutes)	101	-0.070	-0.208	-0.318	-0.284
Returned pill count	91	-0.069	-0.147	-0.179	-0.172
Number analgesics used	98	-0.059	-0.233	-0.317	-0.293
HAQ	102	-0.056	-0.368	-0.378	-0.386
Sleep 1: minutes to fall asleep	100	0.021	0.051	0.012	0.028
Sleep 2: minutes of sleep	100	-0.094	-0.111	-0.058	-0.081
Sleep 3: number of awakenings	100	-0.072	-0.158	-0.152	-0.159
Sleep 4: sleep quality	100	0.027	0.182	0.141	0.162
GI complaints in last week	101	0.213	0.035	0.003	0.016
ESR (mm/hr)	94	0.072	-0.154	-0.222	-0.202
C-reactive protein (mg/L)	99	0.032	-0.190	-0.230	-0.222
Cytidine deaminase	52	0.052	-0.267	-0.288	-0.283

Table 5-3: Statistical significance of assessment variable - concentration correlations (p values)

Assessed Variable	Number	Dose	R - ibuprofen conc.	S - ibuprofen conc.	Total ibuprofen conc.
Ibuprofen Dose (mg)	102	< 0.001	< 0.001	< 0.001	< 0.001
Number swollen joints	102	0.550	0.038	0.007	0.011
Number tender joints	102	0.345	0.001	< 0.001	< 0.001
Patient global assessment	101	0.005	< 0.001	< 0.001	< 0.001
Clinician global assessment	95	0.449	0.002	< 0.001	< 0.001
Pain over last week	101	0.268	0.009	0.002	0.002
Pain previous day	102	0.388	0.018	0.003	0.004
Pain at assessment	102	0.210	0.005	< 0.001	0.001
Grip strength right (mm Hg)	101	0.525	0.442	0.049	0.121
Grip strength left (mm Hg)	101	0.874	0.483	0.061	0.143
Morning stiffness (minutes)	101	0.486	0.037	0.001	0.004
Returned pill count	91	0.513	0.166	0.089	0.103
Number analgesics used	98	0.637	0.060	0.009	0.017
HAQ	102	0.573	< 0.001	< 0.001	< 0.001
Sleep 1: minutes to fall asleep	100	0.838	0.612	0.908	0.784
Sleep 2: minutes of sleep	100	0.355	0.273	0.563	0.423
Sleep 3: number of awakenings	100	0.478	0.121	0.135	0.118
Sleep 4: sleep quality	100	0.791	0.070	0.162	0.108
GI complaints in last week	101	0.033	0.727	0.980	0.878
ESR (mm/hr)	94	0.489	0.139	0.032	0.051
C-reactive protein (mg/L)	99	0.753	0.059	0.022	0.027
Cytidine deaminase	52	0.716	0.056	0.038	0.042

The correlations between the administered ibuprofen dose and the enantiomeric and total ibuprofen trough plasma concentrations were highly statistically significant, as expected. Overall, S-ibuprofen enantiomeric concentrations correlated better with measured clinical and laboratory variables than total ibuprofen concentration or R-ibuprofen enantiomeric concentrations or dose. The observed correlations between the plasma ibuprofen concentrations and the number of swollen joints were more significant for S-ibuprofen ($r = -0.265$, $p = 0.007$) than for total ibuprofen ($r = -0.250$, $p = 0.011$) or R-ibuprofen ($r = -0.206$, $p = 0.038$), while the relationship with dose ($r = -0.060$, $p = 0.550$) was not significant. The relationships were similar for the number of tender joints:

S-ibuprofen ($r = -0.412$, $p < 0.001$), total ibuprofen ($r = -0.392$, $p < 0.001$), R-ibuprofen ($r = -0.329$, $p = 0.001$), dose ($r = -0.095$, $p = 0.345$).

Other clinical assessment variables that exhibited similar significant relationships included patient global assessment, clinician global assessment, pain previous week, pain previous day, pain day of assessment, morning stiffness, and Health Assessment Questionnaire (HAQ). None of these variables except patient global assessment showed a significant relationship with the administered dose of ibuprofen.

Grip strength assessments correlated significantly only for the right hand with S-ibuprofen concentration ($r = 0.196$, $p = 0.049$), although for the left hand the relationship approached significance ($r = 0.187$, $p = 0.061$). The number of ibuprofen capsules returned at each visit had no correlation with either dose or drug concentration. The number of analgesic acetaminophen tablets used related significantly to S-ibuprofen concentration ($r = -0.317$, $p = 0.009$) and total ibuprofen concentration ($r = -0.293$, $p = 0.017$), but not to R-ibuprofen ($r = -0.233$, $p = 0.060$) or to dose ($r = -0.059$, $p = 0.637$). None of the assessed sleep questionnaire variables exhibited a significant correlation with either dose or measured drug concentrations.

The level of GI toxicity correlated in a significant fashion only with administered dose, but not with any measured drug concentration. The observed ranking in the level of correlation was the opposite of that observed with all of the efficacy variables, with dose > R-ibuprofen > total ibuprofen > S-ibuprofen.

5.4.2.2 Laboratory Assessment

Similar to the clinical disease activity variables described above, the assessed laboratory measurements showed the highest correlation with S-ibuprofen enantiomer concentrations, and then total ibuprofen concentration, R-ibuprofen enantiomer concentration, and dose in descending order (see Table 5-2 and Table 5-3). The ESR correlated in a significant fashion only with S-ibuprofen ($r = -0.222$, $p = 0.032$), although the relationship with total ibuprofen approached significance ($r = -0.202$, $p = 0.051$). C-reactive protein levels correlated better with S-ibuprofen ($r = -0.230$, $p = 0.022$) and total ibuprofen ($r = -0.222$, $p = 0.027$) than with R-ibuprofen ($r = -0.190$, $p = 0.059$) or dose ($r = 0.032$, $p = 0.753$).

Cytidine deaminase levels, as measured in the first 6 study subjects, showed similar relationships with the greatest correlation with S-ibuprofen ($r = -0.288$, $p = 0.038$) and progressively less with total ibuprofen ($r = -0.283$, $p = 0.042$), R-ibuprofen ($r = -0.267$, $p = 0.056$) and dose ($r = 0.052$, $p = 0.716$).

5.4.3 Pharmacokinetic Data

The pharmacokinetic parameters derived from evaluation of the individual 8 hour steady state pharmacokinetic studies are listed in Table 5-4: Pharmacokinetic parameters for R-ibuprofen enantiomer and in Table 5-5: Pharmacokinetic parameters for S-ibuprofen enantiomer, including AUC/dose for each enantiomer. As the study subjects received their

ibuprofen doses in a blinded randomized fashion, the individual subjects received from 200 mg to 1200 mg for these pharmacokinetic evaluations.

There was no significant difference between enantiomers for Tmax, but Cmax was significantly greater for R-ibuprofen than S-ibuprofen (15.0 $\pm$ 4.3 vs. 13.3 $\pm$ 2.2, p = 0.043). The t1/2 for S-ibuprofen was significantly greater than for R-ibuprofen (2.88 $\pm$ 0.92 vs. 2.10 $\pm$ 1.18, p = 0.044). The AUC and the dose-adjusted AUC for S-ibuprofen were significantly greater than for R-ibuprofen (AUC: 60.9 $\pm$ 17.1 vs. 46.1 $\pm$ 13.9, p = 0.003; AUC/dose: 0.12 $\pm$ 0.07 vs. 0.09 $\pm$ 0.07, p = 0.029). The Vd/F was not significantly different between enantiomers, but CL/F was significantly greater for R-ibuprofen than S-ibuprofen (12.2 $\pm$ 5.6 vs. 8.1 $\pm$ 3.3, p = 0.003).

Representative time-concentration curves for both ibuprofen enantiomers are illustrated in Figure 5-1 for subject 12 (200 mg dose) and in Figure 5-2 for subject 4 (800 mg dose).

Table 5-4: Pharmacokinetic parameters for R-ibuprofen enantiomer

Subject	Dose (mg)	Tmax (hrs)	Cmax (mg/L)	Lambda z	t1/2 (hrs)	AUC	AUC/dose	Vd/F	CL/F
1	400	1	21.0	0.47	1.49	45.8	0.114	18.1	8.4
2	800	0.5	24.9	0.43	1.60	67.4	0.084	26.4	11.4
3	200	1	7.6	0.15	4.50	43.8	0.219	20.9	3.2
4	800	1	20.5	0.46	1.52	53.9	0.067	31.1	14.1
5	200	1	15.4	0.42	1.66	49.0	0.245	9.3	3.9
6	800	2	23.0	0.51	1.35	58.1	0.073	25.8	13.2
7	1200	3	12.7	0.14	5.06	69.5	0.058	75.4	10.3
8	800	1	13.0	0.28	2.47	40.2	0.050	63.2	17.7
9	800	2	17.4	0.48	1.46	58.3	0.073	41.7	19.9
10	200	0.5	16.0	0.43	1.63	25.9	0.130	17.7	7.5
11	800	2	9.6	0.31	2.25	42.4	0.053	54.6	16.8
12	200	1	11.4	0.51	1.37	31.6	0.158	12.2	6.2
	Mean	1.51	15.0	0.37	2.10	46.1	0.090	38.3	12.2
	SD	0.79	4.4	0.13	1.18	13.9	0.070	23.5	5.6

Table 5-5 : Pharmacokinetic parameters for S-ibuprofen enantiomer

Subject	Dose (mg)	Tmax (hrs)	Cmax (mg/L)	Lambda z	t1/2 (hrs)	AUC	AUC/dose	Vd/F	CL/F
1	400	1	16.8	0.24	2.90	56.0	0.140	25.3	6.0
2	800	0.5	19.4	0.16	4.48	63.1	0.079	57.5	8.9
3	200	1	6.3	0.17	4.13	40.4	0.202	18.6	3.1
4	800	1	16.7	0.36	1.93	62.1	0.078	33.6	12.0
5	200	1	12.3	0.26	2.67	50.5	0.252	13.0	3.4
6	800	2	16.2	0.24	2.88	67.2	0.084	40.4	9.7
7	1200	6	14.8	0.25	2.78	88.2	0.074	38.7	9.7
8	800	1	12.2	0.17	4.04	55.0	0.069	62.2	10.7
9	800	2	15.4	0.15	4.69	86.8	0.109	63.1	9.3
10	200	0.5	14.2	0.37	1.88	47.2	0.236	10.8	4.0
11	800	1	14.0	0.24	2.88	61.5	0.077	45.7	11.0
12	200	1	9.8	0.28	2.51	37.4	0.187	17.0	4.7
	Mean	1.87	13.3	0.24	2.88	60.9	0.120	37.0	8.1
	SD	1.68	2.2	0.07	0.92	17.1	0.070	19.6	3.3

Representative time-concentration curves for both ibuprofen enantiomers are illustrated in Figure 5-1 for subject 12 (200 mg dose) and in Figure 5-2 for subject 4 (800 mg dose).

Figure 5-1: Concentration-time profile (200 mg dose)

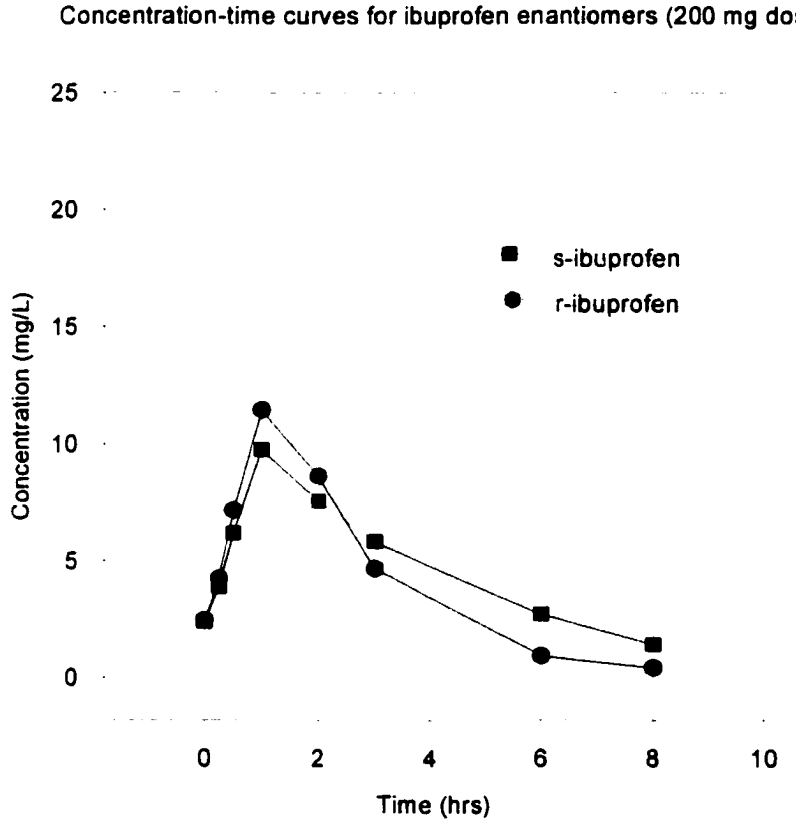
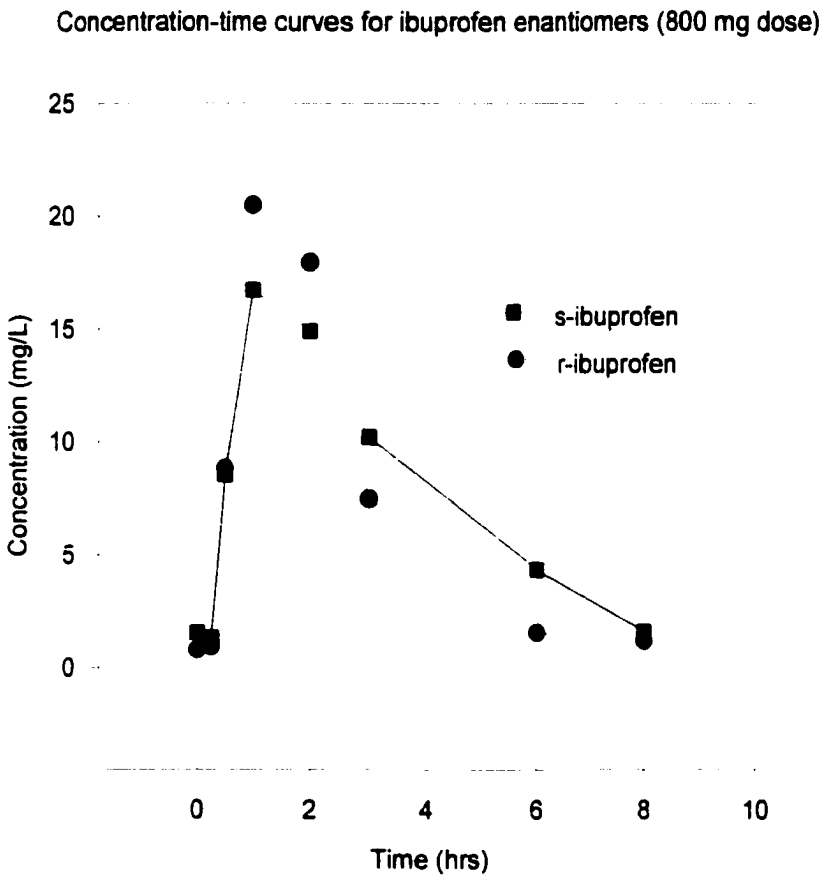


Figure 5-2: Concentration-time profile (800 mg dose)



The relationships between the administered ibuprofen doses and measured enantiomeric ibuprofen concentrations (with their corresponding r values) are illustrated in Figure 5-3 below. The significant correlations between number of tender and number of swollen joints with enantiomer concentration are shown in Figure 5-4. The patient global assessment of disease activity and CRP levels correlated with enantiomer concentration are shown in Figure 5-5, and morning stiffness and pain correlations with drug concentration are illustrated in Figure 5-6.

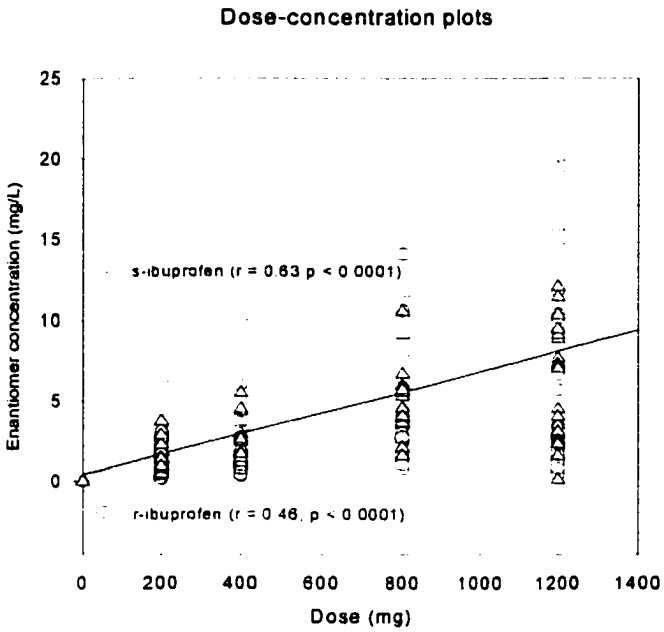


Figure 5-3: Ibuprofen dose - enantiomer concentration plots (Δ S-ibuprofen, o R-ibuprofen)

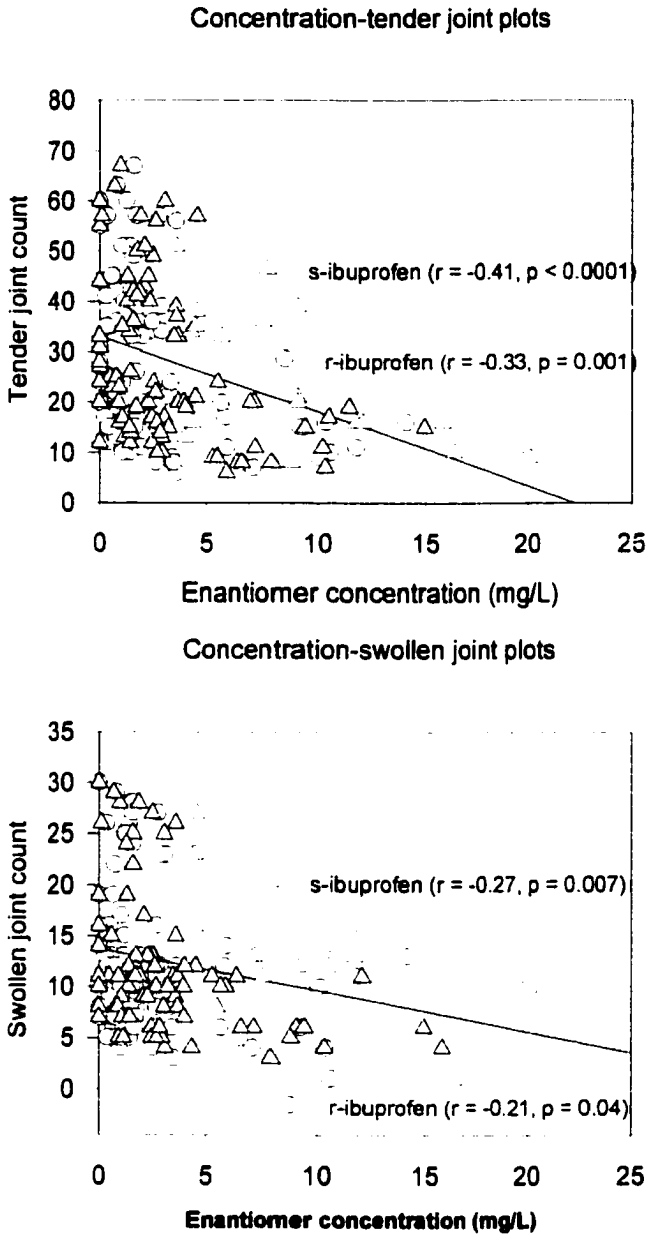


Figure 5-4: Joint count - enantiomer concentration plots (Δ S-ibuprofen, o R-ibuprofen)

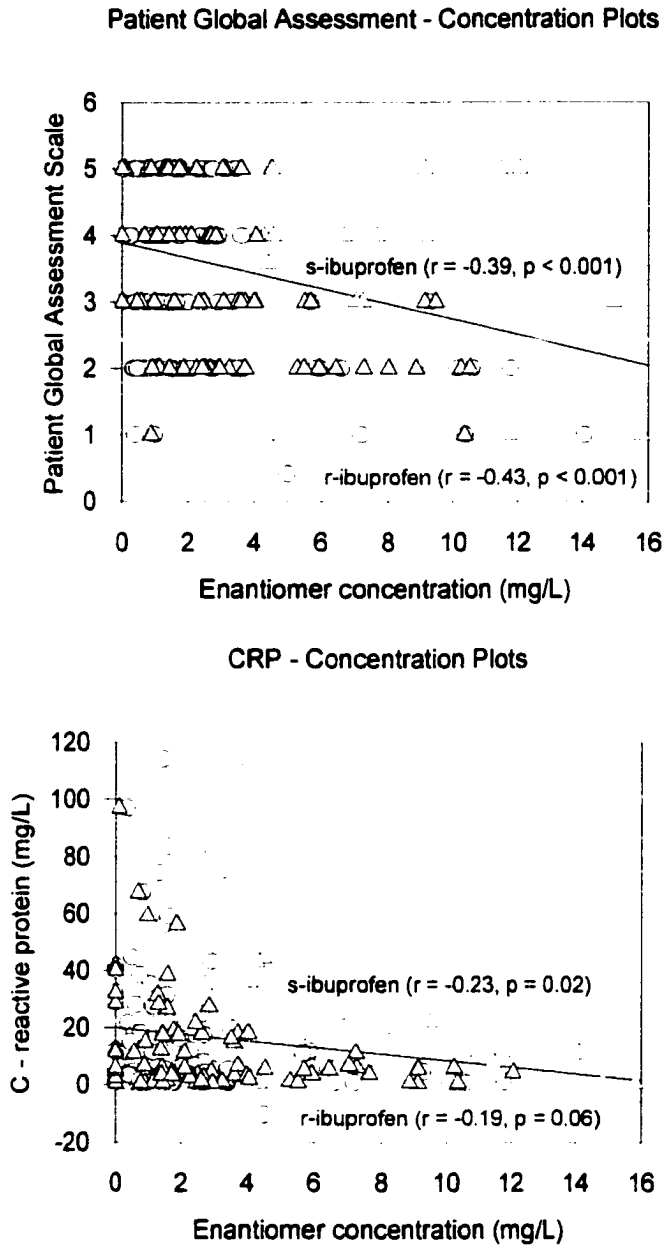


Figure 5-5: Patient global assessment and CRP - enantiomer concentration plots (Δ S-ibuprofen, o R-ibuprofen)

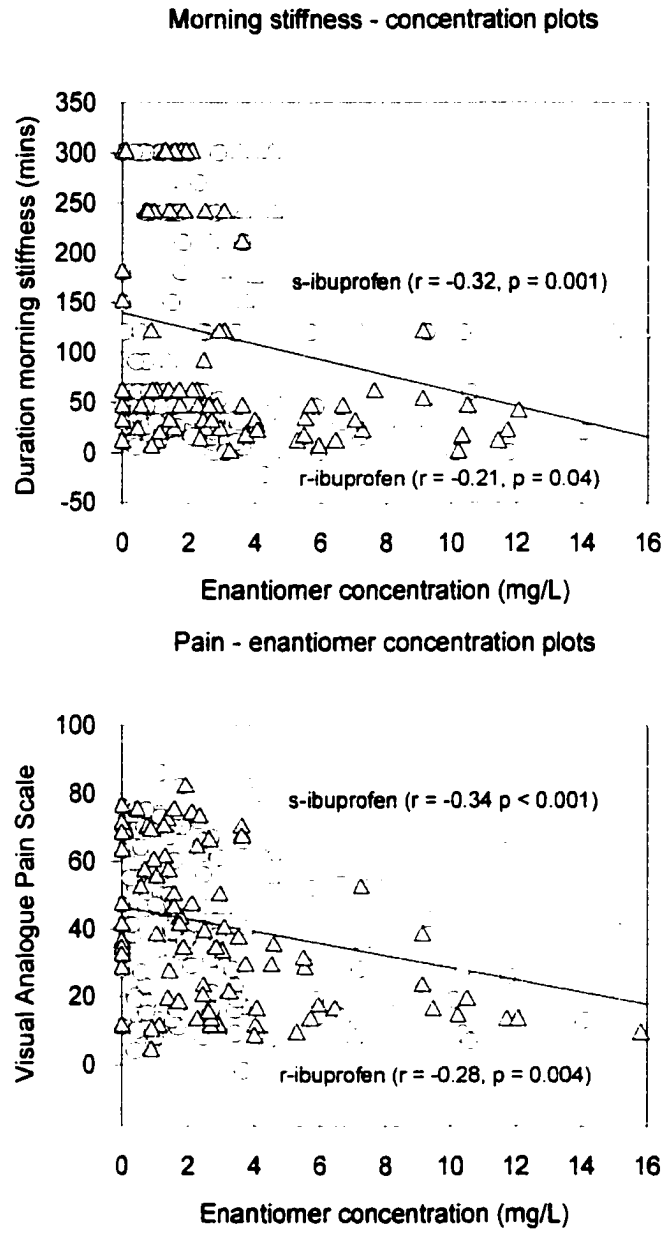


Figure 5-6: Morning stiffness and pain - enantiomer concentration plots (Δ S-ibuprofen, o R-ibuprofen)

5.5 Discussion

Ibuprofen is an arylpropionic acid nonsteroidal compound that is extensively utilized in patients of all ages for its analgesic, antipyretic and antiinflammatory actions. Ibuprofen is administered clinically as the racemate of R- enantiomer and S-enantiomer. Although ibuprofen's stereoselective pharmacokinetics have been accurately characterized, pharmacodynamic evaluations of its varied clinical effects have been inconsistent and in many studies have failed to define a consistent concentration-effect or dose-effect relationship.

There is a widely held clinical concept of NSAID 'responders' and 'non-responders' and evaluations of NSAID plasma levels in these two groups in arthritis has indicated that pharmacokinetic variability is a minor contributor to this differentiation in clinical response (Day & Brooks 1987). No pharmacokinetic differences were observed with ibuprofen (Capell et al. 1977), indomethacin (Barber et al. 1977) and flurbiprofen (Orme et al. 1981) between responders and non-responders. These studies did not evaluate enantiomeric disposition, however.

A large inter-individual variability in the ratio of S-ibuprofen to R-ibuprofen at steady state has been documented, and some authors believe that this accounts for the known lack of correlation between racemic (total) ibuprofen concentrations and therapeutic efficacy (Oliary et al. 1992). The steady state concentrations of both ibuprofen isomers fluctuates less in synovial fluid than in plasma, and the synovial fluid concentrations of the active S-enantiomer are significantly higher than the R-enantiomer. Some investigators have concluded that the binding of the ibuprofen isomers to albumin

and the serum-synovial fluid albumin ratio controls the steady state synovial fluid concentrations and thereby modifies the pharmacodynamics of this drug (Cox et al. 1991). In our study all patients had serum albumin and total protein concentrations in the normal range, but these were only measured at the time of enrollment.

It has been shown that an important factor in determining pain relief from an NSAID in rheumatoid arthritis is the degree of pain at commencement of therapy (Lee et al. 1973). Patients with minimal symptoms and patients with very severe pain and inflammation will note little response from NSAID use. For this reason patients included in our study had moderate levels of disease activity.

Pharmacodynamic studies of ibuprofen's analgesic effects have produced some evidence of a concentration-response and dose-response relationship. Nielsen et al. (1990) did not identify any difference between 400 mg and 800 mg doses of ibuprofen in volunteers with laser-induced pain. Kobal et al. (1994) did show a dose-related effect with these same dosages of ibuprofen in tonic and phasic pain. Schou et al. (1998) identified a positive analgesic dose-response relationship from 50 mg to 400 mg in 304 dental surgery patients. Single dose studies of 400 mg of ibuprofen in post-operative pain from dental surgery did not reveal any correlation between the level of pain relief and any measured pharmacokinetic variable (Jones et al. 1997).

A number of investigators have attempted to define a pharmacodynamic relationship for various NSAIDs in different types of arthritis with inconsistent results. Steady state pharmacokinetic sampling from 85 patients with osteoarthritis treated with piroxicam showed no correlation between free drug concentration and change in any of the clinical response variables after four weeks of therapy (Hundal & Rugstad 1993). No

relationship was found between piroxicam plasma or synovial fluid concentrations and any disease activity parameters after 10 days of therapy in patients with reactive arthritis (Hundal et al. 1993a).

Bertin et al. (1994) found equivalent concentrations of naproxen in synovial fluid and plasma in rheumatoid arthritis patients, with a greater free fraction in synovial fluid (0.14% vs. 0.11%). They identified a significant correlation between clinical efficacy and the free naproxen concentration in synovial fluid. A significant plasma concentration – effect relationship for both free and total naproxen and a number of clinical variables was established in rheumatoid arthritis patients by Day et al. (1982).

A weak correlation was reported between total AUC and several clinical variables for ibuprofen in rheumatoid arthritis patients, but no increased clinical response was noted for 2400 mg/day in comparison to 1600 mg/day total dose (Grennan et al. 1983). Pharmacokinetic measurements of the S-ibuprofen enantiomer were correlated with clinical response in 45 patients receiving either 1200 mg or 2400 mg of racemic ibuprofen per day for osteoarthritis. Ibuprofen dose correlated with S-ibuprofen AUC and trough concentrations but not with clinical outcome. The s-ibuprofen AUC correlated with a variety of clinical outcome measures (Bradley et al. 1992).

Laboratory measurements of inflammation are useful and validated tools for assessing the level of disease activity in rheumatoid arthritis. The ESR and CRP levels are measures of acute phase reactants and are reliable and widely employed for this purpose in clinical and research settings (Wolfe 1997). Cytidine deaminase is a cytoplasmic enzyme that is released from damaged neutrophils. A close correlation exists between synovial fluid cytidine deaminase activity and synovial fluid neutrophil count, and activity of this

enzyme has been shown to be an accurate measurement of acute synovial inflammation (Thompson et al. 1986). In this study each of the above laboratory measures indicated a significant correlation with S-ibuprofen enantiomer levels, and in a descending fashion correlated with total ibuprofen concentration, R-enantiomer levels and dose.

Our study indicated a highly significant correlation between the administered racemic ibuprofen dose and the measured trough steady state enantiomer levels, more for the S-enantiomer than the R-enantiomer. This was consistent with previous studies of ibuprofen pharmacokinetics (Bradley et al. 1992, Evans et al. 1990). The results of our steady state pharmacokinetic studies were consistent with previous published reports, except that in our study the S-ibuprofen t1/2 was significantly greater than for R-ibuprofen. Considerable inter-individual variability in observed pharmacokinetic indices was noted, and is consistent as well with prior published studies.

Similar to the observed relationships between measured laboratory variables and drug concentration or dose in this study, most of the assessed clinical variables identified a significant correlation with S-ibuprofen levels, and in descending order, variable levels of correlation with total ibuprofen, R-ibuprofen and dose. All types of clinical assessment variables, including subjective patient assessments, objective disease activity measurements, and functional evaluations (HAQ) exhibited this same significant correlation and ranking.

In contrast to the above described efficacy variables, the assessment of drug-induced toxicity correlated in an inverse manner. A significant relationship was identified for the relationship with administered dose only, and in a descending fashion R-ibuprofen, total ibuprofen, and then S-ibuprofen. Although there is limited evidence to date that

ingestion of the inactive r-enantiomer with administered NSAID racemates is responsible for added toxicities, the results in our study indicate that such a concept might be valid.

Sleep disturbance is a frequent complaint in rheumatoid arthritis (Drewes et al. 1998) and this study attempted to utilize variations in sleep characteristics as an additional clinical evaluation assessment. This strategy has been utilized in a number of clinical trials in rheumatoid arthritis patients, and in some studies sleep patterns correlated with drug efficacy and/or concentration levels (Caldwell 1994), but not in others (Lavie et al. 1991). It has been shown that the most accurate and sensitive sleep assessment methodology utilized in trials to date is the comprehensive sleep study (polysomnography). It is postulated that the demonstrated lack of correlation of sleep parameters in this study may be due to lack of accurate recall and reporting by patients, an invalid or insensitive questionnaire format, or the presence of confounding primary sleep disturbances unrelated to their arthritis or treatments.

As the concentration of active free S-enantiomer in synovial fluid is postulated to be the most important determinant of drug efficacy in inflammatory arthritis (not measured in this study), plasma S-enantiomer levels would be the most relevant available drug concentration in this study. Our results support this premise and confirm that measurement of plasma active enantiomer levels is a valid and practical alternative to measurement of effect compartment (synovial fluid or synovium) levels, a more invasive methodology of limited practicality.

In contrast to previous assumptions that a valid and definable concentration-effect pharmacodynamic relationship for NSAIDs is absent or tenuous, we have shown that measurements of the active S-enantiomer rather than total drug concentration results in a

highly significant correlation with many assessment variables of drug efficacy. Utilization of this methodology should allow for formulation of accurate, appropriate dosing strategies, particularly in high-risk patient populations.

6 CONCLUSION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely prescribed medications. They are also described as being the most toxic group of medications prescribed or utilized by the general population. Consensus recommendations have been developed to limit this tolerability, and some of these guidelines are based on clinical pharmacokinetic data. Optimal dosing to minimize this toxicity requires accurate information regarding the influence of various clinical and pathophysiologic conditions on the disposition and the efficacy of these drugs. As well, there has been difficulty to date in defining a relationship between measured drug concentrations or dose with observed clinical effects for NSAIDs. The 2-arylpropionic acid (2-APA) class of NSAIDs are chiral compounds, usually administered as the racemate, in which efficacy is mainly attributable to the S-enantiomer, and which are characterized by a variable degree of racemization of the R-isomer to the S-isomer. Given this, it is essential that only enantiospecific pharmacokinetic and pharmacodynamic evaluations be performed to obtain valid data.

An age-related accumulation of ketoprofen due to a reduced clearance has been reported in the elderly. Other studies have not observed these changes in the kinetics of unchanged ketoprofen, but have reported increased plasma levels and reduced urinary excretion of conjugated ketoprofen. We examined the effects of dose, renal function, and the presence of arthritis on the stereoselective kinetics of ketoprofen in 5 non-arthritic and 6 arthritic subjects. No significant differences in CL/F, AUC, $t_{1/2}$, Tmax or Cmax were found between groups or between doses, and values were similar to those previously

reported in young adults. Urinary ketoprofen conjugate S:R ratio was 1.6±0.25 and 1.65±0.27 for arthritic and non-arthritic subjects. Greater amounts of conjugated ketoprofen enantiomers were present in the plasma of the arthritic compared to non-arthritic subjects, likely due to reduced renal function in the arthritic subjects. Renal clearance of ketoprofen conjugates exhibited stereoselectivity (R > S), and was decreased in the arthritic group. In contrast to some earlier studies, significant accumulation of unchanged ketoprofen was not found to occur in elderly subjects in the presence or absence of rheumatic disease or moderate renal impairment. No dosage adjustment is necessary for ketoprofen based on age *per se*. Although this was a limited sample size, there does not appear to be any reason to reduce the dose of ketoprofen in arthritic patients in the absence of other significant comorbidities. The pharmacokinetics of ketoprofen, similar to prior published studies, were linear in the range of doses used in this evaluation.

To study the effect of renal dysfunction on the pharmacokinetics of ketoprofen (KT), and the possibility of saturation of clearance upon increasing dose, single 50 and 100 mg doses of racemic ketoprofen were administered in a cross-over fashion to 9 patients with varying levels of renal function, but not requiring dialysis. The stereospecific disposition kinetics of ketoprofen enantiomers and their acyl-glucuronide conjugates (KT_{conj}) were determined in plasma and urine for 24 hrs post-dose. Significant trends were found towards reduced ketoprofen oral clearance (CL_O) and terminal elimination rate constant (β) with decreased renal function. Stronger correlations were observed between ketoprofen pharmacokinetic indices and renal clearance of KT_{conj} (CLr_{conj}) after both doses. As expected, CLr_{conj} was reduced with diminished renal function.

Following both 50 and 100 mg doses, reduced renal function resulted in significantly lower AUC of S-ketoprofen but higher AUC and cumulative urinary excretion of S-KT_{conj} as compared with the antipode. CL_O remained constant after increasing the dose, again indicating linearity in the pharmacokinetics of KT despite reduced clearance. CLr_{conj}, however, was significantly reduced after the 100 mg dose suggestive of saturation of the urinary clearance and existence of a compensatory pathway. Renal impairment reduces renal clearance of KT_{conj} and this appears to be the rate-limiting step for clearance of ketoprofen. The observed stereoselectivity in the urinary excretion of the KT_{conj} may indicate increased chiral inversion due to a longer residence time with renal dysfunction. Dose reduction of ketoprofen is indicated only for patients with moderately severe renal impairment ie. $CL_{CR} < 20$ ml/min.

The choice of an appropriate NSAID formulation can at times result in increased efficacy and limit toxicities. The site in the gastrointestinal tract where NSAIDs are absorbed varies with different formulations and this has been shown to modify pharmacokinetic profiles, including the extent of enantiomeric inversion which occurs in the intestine as a form of pre-systemic metabolism for some NSAIDs. In order to assess pharmacokinetic variability as measured in subjects dosed consecutively with different formulations of ketoprofen, and specifically to assess the variability of enantiomeric inversion, healthy subjects received each of the ketoprofen formulations: 1) enteric-coated (EC) 2) a sustained-release (SR) capsule 3) the same dose of ketoprofen in solution. To further assess whether alterations in gastrointestinal motility, thereby modifying the time to and rate of drug delivery to sites of intestinal inversion, would significantly alter the

ketoprofen pharmacokinetics observed with the above formulations, each subject received 1) metoclopramide and 2) propantheline.

Our pharmacokinetic analysis, based on urine drug concentration data only, indicated that excretory rates were maximal for the ketoprofen solution, with the S-enantiomer being excreted more rapidly than the R-enantiomer for all formulations studied. This was expected as absorption was expected to begin in the stomach without the need for disintegration or dissolution. After the solution, the descending order of excretory rates were: EC with metoclopramide, EC, EC with propantheline, SR with metoclopramide, and the SR formulation alone with the slowest rate. There was no significant difference between formulations for the total amount of drug absorbed and excreted.

The mean urinary S:R ratios were significantly greater for the EC formulation in the later collection periods (8 - 12 hour and 12 - 24 hour), in comparison to the solution and the SR formulations. This may be attributable to differences in intestinal site of absorption for the different formulations. Considerations such as different formulations'effects on active drug pre-systemic metabolism, in this case due to varying levels of intestinal mucosal enantiomeric inversion, and therefore enantiomeric bioavailability, may be important in addition to other pharmaceutic factors in selection of the optimal formulation for an individual patient.

The inability of many investigators to define a significant pharmacodynamic concentration or dose-response relationship for different NSAIDs has produced numerous potential explanations. In the evaluation of chiral 2-APA NSAIDs, a failure to measure

enantiomer levels rather than total drug levels is one obvious limiting factor. The best correlations between plasma concentration data and effect have been produced for naproxen, which is administered (and measured) as solely the active S-enantiomer.

In order to determine if significant enantiospecific correlations were valic for the NSAID ibuprofen (for which total drug data to date has been equivocal), twelve adult patients with mild to moderately active rheumatoid arthritis were provided with varying doses of racemic ibuprofen sequentially in a randomized double-blind fashion. Clinical and laboratory and compliance assessments and trough (pre-dose) ibuprofen enantiomer plasma levels were done weekly, and clinical and laboratory efficacy and toxicity was correlated with drug levels.

Overall, S-ibuprofen enantiomeric concentrations correlated better with measured clinical and laboratory variables than total ibuprofen concentration or R-ibuprofen enantiomeric concentrations or dose. Many of these correlations for the active S-enantiomer were clinically and statistically very significant. In contrast to the above described efficacy variables, the assessment of drug-induced toxicity correlated in an inverse manner, maximal with dose, and in a descending fashion with R-ibuprofen, total concentration and then S-ibuprofen. Our results support the premise that measurement of plasma active enantiomer NSAID levels is a valid and practical methodology for these compounds. The observed efficacy/toxicity discrepancies also suggest that administration of the s-enantiomer alone would result in greater clinical safety for the achieved effect. These studies indicate that valid data is available from which clinical NSAID dosing regimens can be developed.

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