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THE EFFECT OF INFLAMMATION ON THE DISPOSITION OF ACEBUTOLOL AND PROPRANOLOL ENANTIOMERS

BY

MICHELINE PIQUETTE-MILLER

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

IN

Pharmaceutical Sciences (Pharmacokinetics)

Faculty of Pharmacy and Pharmaceutical Sciences

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Spring 1994

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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 EFFECT OF INFLAMMATION ON THE DISPOSITION OF ACEBUTOLOL AND PROPRANOLOL ENANTIOMERS** submitted by **Micheline Piquette-Miller** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Pharmaceutical Sciences (Pharmacokinetics).**

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"To laugh often and much; to win the respect of intelligent people and the affection of children; to earn the appreciation of honest critics and endure the betrayal of false friends; to appreciate beauty, to find the best in others; to leave the world a bit better, whether by a healthy child, a garden patch or a redeemed social condition; to know even one life has breathed easier because you have lived. This is to have succeeded."

Ralph Waldo Emerson

To my husband Dean and my son Nathan, whose presence is a constant reminder of what is truly important in life.

ABSTRACT

β-blockers are often prescribed to hypertensive patients also suffering from inflammatory disease states such as arthritis. Pathophysiological changes which may alter drug disposition such as altered plasma protein concentrations and reduced organ function are often apparent in these subjects. The influence of inflammation on the stereospecific pharmacokinetics of two extensively metabolized β-blockers, acebutolol and propranolol was studied. Stereospecific HPLC assays involving chiral derivatization with S-(+)-1-(1-naphthyl)ethyl isocyanate were developed for these drugs.

In healthy volunteers, slightly but significantly higher plasma concentrations of the S enantiomer of acebutolol and the R enantiomer of its metabolite, diacetolol were found. The oral clearance of acebutolol, and renal excretion of diacetolol, were stereoselective in favor of the R enantiomer. Renal clearance values of acebutolol and diacetolol are significantly decreased with increasing age. Age-related changes in the enantiomer ratios of acebutolol and diacetolol suggest an influence of aging on stereoselective pathways. Clearance of S-acebutolol, was slightly but significantly decreased in arthritic patients.

As the minor decrease in the metabolism of S-acebutolol may be more apparent in active disease, we tested this possibility in the rat. The pharmacokinetics of acebutolol enantiomers were delineated in female rats and found to be comparable to human pharmacokinetics. Significant three to ten fold increases in the initial plasma

concentrations of acebutolol were observed in arthritic rats. Enantiomers were equally affected. Thus the intrinsic clearance of acebutolol is non-stereoselectively decreased in adjuvant arthritis.

Adjuvant arthritis had a minimal effect on the disposition of acebutolol, thus further studies were continued using the ß-blocker, propranolol. Adjuvant arthritis did not significantly influence the stereoselective pharmacokinetics of propranolol after iv doses. On the other hand, after oral doses, clearance was significantly decreased in adjuvant arthritis. Although significant for both enantiomers, this effect was more pronounced on the less active R enantiomer. The enantiomeric ratio in plasma was, therefore, significantly altered.

Arthritic-induced changes in drug disposition may be related to the severity of inflammation and hence minimized or suppressed in subjects receiving non-steroidal antiinflammatory agents. This possibility was investigated by examining the pharmacokinetics of propranolol in ketoprofen-treated and control arthritic rats suffering from differing degrees of inflammation. A significant correlation between disease severity (arthritic index) and the AUC of R- and S-propranolol was observed. Furthermore, treatment with ketoprofen significantly reduced the AUCs of propranolol in line with the clinical improvement of arthritic scores. Therefore, increased plasma concentrations of propranolol in arthritis are related to the degree of inflammation and reduction of joint swelling by ketoprofen treatment diminishes arthritic-induced changes in propranolol disposition.

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LIST OF ABBREVIATIONS AND SYMBOLS

AA	Adjuvant-induced arthritis
AC	Acebutolol
AAG	α_1-acid glycoprotein
AI	Arthritis Index
AUC	Area under the plasma concentration-time curve
$AUC_{0\text{-}t}$	Area under the plasma concentration-time curve from 0 hours to the last measured concentration
$AUC_{0\text{-}\infty}$	Area under the plasma concentration-time curve from 0 hours to infinity
β	Terminal elimination rate constant
°C	Degrees celcius
C	substrate concentration
CL_{bile}	Biliary clearance
CL_{cr}	Creatinine clearance
CL/F	Body clearance after oral doses
CL_{int}	Intrinsic clearance
CL_{oral}	Oral clearance
CL_{nr}	Non-renal clearance
CL_{renal}	Renal clearance
CL_{sys}	Systemic clearance
cm	Centimeters

C_{max}	Maximum plasma concentration after oral doses
DC	Diacetolol
ESR	Erythrocyte sedimentation rate
ΣX_{bile}	Cumulative excretion in bile
ΣXu	Cumulative urinary excretion
F	Absolute bioavailability
F_{gut}	Availability from gastrointestinal tract
F_{liver}	Availability after first pass through liver
Fig.	Figure
g	Centrifugal force X *g*
g	Grams
h	Hours
HCl	Hydrochloric Acid
HPLC	High performance liquid chromatography
id	Intraduodenal
ip	Intraperitoneal
iv	Intravenous
kg	Kilograms
l	Litres
M	Molar
μg	Micrograms
μl	Microlitres
μM	Micromolar

mg	Milligrams
min	Minutes
ml	Millilitres
mM	Millimolar
N	Normal
NADP$^+$	β-Nicotinamide adenine dinucleotide phosphate, oxidized form
NaOH	Sodium hydroxide
NEIC	Naphthylethylisocyanate
NSAID	Non-steroidal anti-inflammatory drug
PR	Propranolol
Q_{liver}	Hepatic blood flow
r	Pearson correlation coefficient
R_s	Resolution factor
s	Seconds
SD	Standard deviation
SEM	Standard error of the mean
t_{max}	Time to maximal plasma concentration
$t_{1/2}$	Elimination half-life
U	Units
UDPGA	Uridine 5'diphosphoglucuronic acid
Vd	Volume of distribution
Vd/F	Volume of distribution after oral doses
y	Years

1. INTRODUCTION

Dosage guidelines in patient population groups are commonly extrapolated from pharmacokinetic experimentation in young healthy subjects. However, it is a well established fact that drug disposition is often altered in disease states. Consequently increased toxicity or reduced efficacy may be apparent in certain patients. Rheumatic diseases are estimated to affect up to 11% of the world population (Harris, 1981). The high prevalence of these conditions underlies the importance of considering the effect of inflammation on the pharmacokinetics of xenobiotics. Pathophysiological changes such as altered plasma proteins, and reduced organ function are frequently apparent in these subjects.

To date, pharmacokinetic studies in arthritic patients have mainly dealt with drugs used for the treatment of inflammation such as the non-steroidal antiinflammatory agents (NSAIDs). Many times little or no change in their disposition as compared to healthy subjects are seen. These agents, however are primarily bound in plasma to albumin, concentrations of which are only slightly diminished in arthritis. Notwithstanding, the incidence of other diseases are the same as or greater in arthritic patients than in non-arthritic subjects. For instance, due to disease or therapy-induced changes, the prevalence of cardiovascular disorders is increased in arthritic patients (Mustonen *et al.*, 1993). Several studies have also shown increased blood pressure due to NSAID therapy in hypertensive patients (Minuz *et al.*, 1990). Many drugs used in the treatment of cardiovascular disease, such as the β-adrenergic blockers are bound to the acute phase reactant, α_1-acid glycoprotein (AAG), levels of which are greatly elevated in inflammatory conditions (Belpaire *et al.*, 1982). Furthermore, acknowledged pathophysiological changes in rheumatoid diseases have been found to significantly alter the pharmacokinetics of these agents (Schneider *et al.*, 1981).

Although the literature contains many detailed reports on the pharmacokinetics of the ß-blockers in inflammation, various factors have not been adequately addressed. For instance, to date these investigations have all utilized non-stereospecific methods. The majority of ß-blockers are commercially available as racemates, however, antihypertensive activity mainly resides in the S enantiomer. Moreover, the pharmacokinetics of a number of these agents have been reported to be stereoselective. Therefore, it is essential to utilize stereospecific analytical methods as disease-induced disposition changes may not affect each enantiomer equally. Patient factors such as disease severity and therapeutic intervention may also play a role in inflammation induced changes in drug disposition. As inflammation severity influences liver production of the acute phase proteins, this in turn likely affects plasma concentrations of xenobiotics. Therefore, it is imperative to examine the role that disease activity exerts on circulating levels of the active drug moiety, prior to developing dosage adjustment guidelines for individual arthritic patients.

The focus of the studies incorporated in this thesis was to further explore the initial findings of altered disposition of the ß-blockers in inflammation. Our primary objective included investigation of the stereoselective nature of the disposition of acebutolol and propranolol and the influence inflammation may exert on the pharmacokinetics of their individual enantiomers. In order to ascertain the underlying mechanisms governing elevated plasma concentrations in human arthritis, the rat was tested as an animal model. Subsequently, the importance of disease severity and influence of drug therapy on inflammation-induced pharmacokinetic changes were examined.

Previous research in this area will be presented before describing the specific objectives of the pharmacokinetic studies.

1.1. Background

1.1.1. Inflammation

Inflammation is the characteristic response of the body to tissue injury. Detailed descriptions of inflammation are found in relevant therapeutic textbooks such as the Textbook of Rheumatology (Ed. Kelly *et al.*, 1981) and Anderson's Pathology (Mari, 1990) in which the following information was obtained. Whenever tissue is injured, a series of events referred to as the inflammatory response is activated in an attempt to limit tissue damage by noxious agents. The causative agents involved in tissue injury and thereby evoking the inflammatory response include diverse factors such as bacteria and other microorganisms as well as injury resulting from trauma, extreme temperatures and chemicals. According to its duration, inflammation is classified as acute or chronic. Inflammation is referred to as acute when it lasts days or less than a few weeks. Some examples include viral and bacterial infections such as typhoid fever and diphtheria, and hypersensitivity reactions. The persistent presence of such causative infections, hypersensitivity or noxious agents will often result in a chronic condition even after the initial acute process subsides. Inflammation is classified as chronic when its duration is prolonged (ie. months, years). Generally, periodic episodes of necrosis and tissue regeneration, resulting in an "acute flare" of inflammation is involved in chronic conditions. Specific examples of chronic inflammatory conditions include rheumatoid arthritis, tuberculosis, syphilis, Reiter's syndrome and Crohn's disease.

The main events comprising of the acute inflammatory response include vasodilation, exudation of plasma and emigration of neutrophils into the injured tissue. In chronic conditions there is a subsequent accumulation of macrophages, lymphocytes and plasma cells at the site of inflammation which is accompanied by necrosis and proliferation of tissue cells. Infiltration of macrophages to the site of inflammation is also apparent. However, although this is transient in acute conditions, a persistent proliferation of macrophages is seen in chronic conditions. The cellular release of hormone-like proteins called cytokines, from activated macrophages, in turn acts upon the liver causing an increase in the concentration of liver-derived plasma proteins- the acute phase proteins. Some of the acute phase proteins include fibrinogen, serum amyloid A, C-reactive protein, α_1-antichymotrypsin, α_1-antitrypsin, α_1-acid glycoprotein, haptoprotein and the complement components. The acute phase proteins play a role in inflammation and healing. Furthermore, changes in the serum levels of acute phase proteins may be used to assess disease activity and therapeutic intervention.

Animal models of inflammation have been extensively utilized by pharmacologists to study drug therapy. An acute inflammatory response is often elucidated by subcutaneous injection of chemicals such as turpentine into the rat or rabbit (Whitehouse, 1988). Inflammation activity and successful therapeutic intervention is evaluated by measurement of physical indices such as paw swelling or by determining concentrations of the acute phase proteins. However, difficulties arise when information obtained in these studies are extrapolated to the intervention of diseases associated with chronic inflammation. Beck and Whitehouse (1974) found a differential effect of acute and chronic inflammation on drug disposition. Although results from the study were mainly discussed in terms of their implications towards the experimental design of drug screening in adjuvant-induced arthritis, the question was raised as to the systemic effect of disease on drug distribution and metabolism. Therefore an animal model which is commonly employed is rat adjuvant arthritis. Injection of an adjuvant prepared from several species of *Mycobacterium* induces a chronic inflammatory disease resembling rheumatoid arthritis (Whitehouse, 1988).

1.1.2. Drug Disposition

Interest in the area of altered drug disposition in inflammation was generated when it was observed that various chronic inflammatory conditions in humans such as Crohn's disease, coeliac disease and rheumatoid arthritis were associated with large increases in drug plasma concentrations after oral doses of propranolol (Schneider *et al.*, 1976) and oxprenolol (Kendall *et al.*, 1979). These findings were not restricted to chronic conditions as plasma oxprenolol concentrations were also found to be significantly raised in patients suffering from viral infections (Kendall *et al.*, 1979).

When single doses of oxprenolol were given to a group of healthy volunteers on various occasions, mild infections with a concurrent elevation of their erythrocyte sedimentation rates (a non-specific indicator of inflammation) led to a 75-100% increase in the area under the plasma concentration time curve (AUC) in two subjects (Kendall *et al.*, 1979). Possible causes of the inflammation-induced increases in drug concentration include increased absorption, altered distribution and/or a reduction in elimination.

<u>1.1.2.1. Absorption</u>. It is thought that changes in intestinal integrity due to the disease or drug therapy may influence the absorption of xenobiotics (Mielants *et al.*, 1991). Altered absorption, however, has not been observed to be a major factor in inflammation-induced changes in drug disposition. One report by Kirch *et al.* (1982), noted a decrease in the absorption of atenolol in patients suffering from an acute respiratory infection. Nevertheless, the proposed change in atenolol absorption requires confirmation, as total urinary recoveries of atenolol were unchanged. Indeed, as elimination of atenolol is primarily through the renal route, and renal clearances were significantly increased from normal values, the reduction in atenolol AUC is probably due to changes in clearance rather than absorption. *In vivo* investigations of another β-blocker, propranolol, comparing perfused jejunum from healthy and treated rats, failed to find any significant effect of adjuvant arthritis on oral absorption of the drug (Key *et al.*, 1986). Altered absorption in inflammation has not been reported for other drugs.

1.1.2.2. Distribution/Protein Binding. Inflammatory conditions are associated with altered plasma protein concentrations. Hypoalbuminaemia, which is often observed in arthritic subjects, may affect protein binding of acidic drugs, such as the NSAIDs. In patients with rheumatoid arthritis, the pharmacokinetics of the highly protein bound NSAID, naproxen, were found to be significantly altered due to an arthritic-induced reduction in plasma albumin concentrations (Van Den Ouweland *et al.*, 1987). Van Den Ouweland *et al.* (1987) observed signficantly increased free fractions of naproxen in arthritic patients which resulted in both a 60% increase in the volume of distribution and a 40% increase in the apparent clearance of naproxen.

Plasma concentrations of the acute phase protein, α_1-acid glycoprotein (AAG) are also greatly increased in inflammatory conditions. Basic drugs such as the ß-blockers are mainly bound to this protein, and therefore protein binding of these agents are often increased in diseases such as arthritis. Indeed, significant correlations between AAG and percentage of free drug have been found for the β-blockers and other basic drugs in disease (Table 1-I).

While plasma concentrations of the extensively metabolized, highly protein bound ß-blockers, oxprenolol and propranolol, were observed to be dramatically elevated in inflammatory conditions (Schneider *et al.*, 1976; Kendall *et al.*, 1979), plasma concentrations of the highly metabolized, poorly protein bound ß-blocker, metoprolol, were relatively unchanged (Schneider *et al.*, 1981). As the protein binding characteristics of these ß-blockers are distinct from one another, this raises the question as to whether increased plasma concentrations are a result of changes in hepatic enzyme activity or protein binding. Investigation of propranolol disposition after iv administration, similarly detected elevated plasma concentrations and AUC in Crohn's and rheumatoid arthritic patients (Waller *et al.*, 1981). Higher plasma concentrations and smaller distribution volumes could be accounted for by the increased binding to serum proteins in these subjects. Interpretation of this data led many to believe that increased protein binding to AAG in inflammation was the primary factor involved in altered drug disposition.

To delineate the cause of inflammation-induced changes observed in humans, subsequent studies were conducted in rats. It was noted that similar to humans, plasma concentrations of the ß-blocker, propranolol were elevated in adjuvant-induced arthritis (AA) in rats after oral and intravenous doses (Bishop *et al.*, 1981). The question of the involvement of protein binding in arthritis was thus also examined in the rat. One investigator suggested that based on his experimental findings in AA, elevated blood concentrations of propranolol could be attributed to increased protein binding rather than to changes in hepatic metabolism or blood flow (Walker *et al.*, 1986). Nevertheless, changes in the proportion of free drug could not account for observed increases in plasma concentrations of propranolol, metoprolol and antipyrine in turpentine-treated rats (Belpaire *et al.*, 1989). As both antipyrine and metoprolol are negligibly bound to serum protein, increases in their plasma concentrations were solely due to altered metabolism whereas higher circulating concentrations of propranolol could be explained by both a reduction of metabolism and increased protein binding.

1.1.2.3. Drug Metabolism. The impairment of drug metabolism in experimental models of inflammation has been well established. One of the first reports of inflammation induced changes in drug disposition was published by Morton & Chatfield in 1970. Following induction of adjuvant arthritis in rats, liver microsomal oxidative enzyme activity and cytochrome P-450 levels were greatly reduced. *In vivo* studies also demonstrated decreased paracetamol glucuronidation and increased phenobarbitone toxicity in arthritic rats, resulting from an impaired liver metabolism. Prabhu *et al.* (1972) noted that sleeping times with hexobarbital, an indirect measurement of drug metabolism, were increased in rats with chronic yeast-induced tissue inflammation. On the other hand, sleeping times for the non-metabolized barbiturate, barbital, were unchanged. Recent studies have also found significant reductions in hepatic enzyme activity and cytochrome P-450 content resulting in altered metabolic clearances of hexobarbital and tolbutamide in other experimental models of inflammation (Ishikawa *et al.*, 1991; Parent *et al.*, 1992).

More recently, the role of individual inflammatory mediators in altered drug disposition has been addressed. In response to tissue injury, the cytokines, specifically interleukins 1 and 6, are involved in increasing acute phase protein production and inhibiting synthesis of drug metabolizing enzymes in liver cells. Reports indicate a repressive effect of interleukin 1 on drug metabolizing enzymes (Ferrari *et al.*, 1992, 1993). Furthermore, incubation of hepatocytes with interleukin 1 has been found to decrease cytochrome P-450 in a dose-dependent manner (Sujita *et al.*, 1990).

Plasma concentrations of the β-blocker propranolol were dramatically elevated in AA in rats after oral and iv doses. Decreases in the distribution volumes and higher circulating levels of propranolol after iv administration could be explained by increased protein binding. However, the approximately 5 fold increase in the systemic bioavailability of propranolol in AA after oral doses was felt to reflect changes in hepatic enzyme activity. This was supported by the observation that the *in vitro* biotransformation of propranolol as well as that of antipyrine and lidocaine were significantly reduced in the liver of rats with turpentine-induced inflammation (Chindavijak *et al.*, 1987a). In the isolated perfused liver of turpentine treated rats, the hepatic clearance of propranolol was also decreased, however clearance differences between healthy and treated rats did not reach significance (Chindavijak *et al.*, 1987b).

Very recently, the influence of iv administration of the inflammatory mediator, interleukin 1ß on the pharmacokinetics of propranolol was investigated in the rat (Vermeulen *et al.*, 1993). Administration of interleukin 1ß caused an enantioselective increase in plasma concentration of propranolol, mainly due to a decrease in the intrinsic metabolism of the R enantiomer. Protein binding was also altered in the interleukin treated rats; elevated AAG levels were associated with decreases in the free fraction of propranolol enantiomers.

1.1.2.4. Renal Excretion. Abnormalities of renal function are frequently noted due to disease and/or therapy-induced changes in rheumatoid arthritis (Mody *et al.*, 1987). Furthermore, administration of NSAIDs can produce a marked reduction of renal function through inhibition of renal prostaglandin synthesis (Downie, 1991). In fact, renal disease has been attributed to be one of the more common causes of death in patients with rheumatoid arthritis (Laakso *et al.*, 1986). However, few studies have examined the effect of inflammation on the renal clearance of drugs. Nevertheless, inflammatory bowel disease was not found to influence the plasma concentration profiles of the renally excreted ß-blocker, practolol (Parsons *et al.*, 1976). The plasma concentration time profiles in these coeliac patients were observed to follow similar patterns to that of normal subjects.

1.1.3. Disease Activity

In humans, a combination of altered protein binding and liver biotransformation is likely responsible for inflammation-induced changes in drug disposition. However, discrepancies exist as to the influence of inflammation on the pharmacokinetics of these agents. For example, although a four fold increase in the AUC of metoprolol is observed in turpentine-induced inflammation in rats (Belpaire *et al.*, 1989), inflammatory disease does not significantly influence metoprolol disposition in humans (Schneider *et al.*, 1981). It is important to note the differences in disease activity in the two species. Often in experimental models of inflammation there is a pronounced and extensive involvement of the joints. On the other hand, arthritic patients who participate in pharmacokinetic experiments frequently are modestly afflicted. Furthermore, inflammation in these subjects is often controlled by drug therapy. Perhaps one of the most important questions that should be raised from apparent discrepancies between results obtained from animal and human arthritic subjects is whether there is a connection between altered drug clearance and disease severity. Such a relationship has been implied from studies which have shown an association between increased plasma concentrations of propranolol with erythrocyte sedimentation rates (ESR). After oral administration of propranolol to rheumatoid arthritic patients, separation of the subjects based on their ESR values appropriately divided the groups into those with high and low plasma concentrations (Schneider & Bishop, 1979); arthritic patients with elevated ESRs (>20 mm/hr) were found to have higher than normal propranolol concentrations whereas those with near normal sedimentation rates (<20 mm/hr) did not differ from healthy controls. Despite this finding, relationships between various biochemical and physical indices of inflammation were not significantly correlated with plasma concentrations of propranolol. On the other hand, Waller *et al.* (1982) observed a positive correlation between the ESR and AUC of propranolol after intravenous administration.

Interpretation of results obtained in rats suggest that clinical improvement of inflammation through effective drug therapy may also have implications on drug disposition. Adjuvant-induced arthritis in rats is associated with a significant decrease in the clearance of the anti-rheumatic agent, cyclosporin (Pollack *et al.*, 1989). However, continuous administration resulted in both an improvement of arthritic scores as well as a return of cyclosporin pharmacokinetic parameters to that of controls (Pollack *et al.*, 1989). Beck and Whitehouse (1974) also found a protective effect of inflammation induced changes in both joint swelling and drug metabolism with the antiinflammatory agents dexamethasone and corticosterone. However, it may be misleading to extrapolate clinical findings with all antiinflammatory therapies as the improvement of inflammation with the NSAIDs, indomethacin and phenylbutazone did not influence liver metabolism (Beck and Whitehouse, 1974; Di Pasquale *et al.*, 1974). Furthermore, pretreatment of adjuvant administered rats with the antiinflammatory steroid agent, pregnenolone-16α-carbonitrile, had a protective effect on drug metabolic activity of the animal yet only had a slight effect on the severity of the disease (Kourounakis and Hadjipetrou-Kourounakis, 1984).

1.2 Rationale for the study of the pharmacokinetics of acebutolol and propranolol enantiomers in inflammation

Beta-adrenoceptor antagonists are frequently used in the treatment of cardiovascular disorders such as hypertension (Cruickshank and Prichard, 1988). Many hypertensive patients also have other common diseases such as arthritis. Pathophysiological changes such as elevated AAG concentrations and reduced organ function which are often apparent in arthritic subjects have been reported to influence the disposition kinetics of various drugs (Schneider *et al*, 1981; Belpaire *et al.*, 1982; Ferrari *et al.*, 1993). Although inflammation-induced changes in drug disposition are thought to result primarily from altered protein binding, physiological changes have mainly influenced the pharmacokinetics of extensively metabolized agents such as propranolol (Schneider *et al.*, 1981). Nevertheless, hepatic enzyme activity is also reduced in inflammation, and thus reduced intrinsic clearance may play a more vital role than originally thought. Hence, we studied the effect of arthritis on the pharmacokinetics of two ß-blockers, propranolol and acebutolol which reportedly undergo extensive first-pass metabolism yet differ in their degree of protein binding. The influence of inflammation on circulating concentrations of the minimally protein bound acebutolol, as compared to that of the highly serum bound propranolol, provides a clue as to the relative importance of modifications in protein binding and intrinsic clearance. Furthermore, various factors including stereoselectivity, disease severity, and drug therapy, all of which possibly influence the circulating levels of the active drug moiety in individual subjects, have not been addressed and should be examined prior to making decisions on dosage adjustment in arthritis.

1.3. Hypotheses

1. Adjuvant arthritis in the rat is a suitable animal model to study inflammation-induced changes in disposition of acebutolol enantiomers.
2. The pharmacokinetics of acebutolol enantiomers are altered in arthritis due to decreases in intrinsic clearance.
3. The pharmacokinetics of propranolol enantiomers are altered in adjuvant arthritis in rats due to:

   a. Decreased intrinsic metabolism

b. Increased plasma protein binding

4. Arthritis-induced drug disposition changes may be stereoselective.

5. Altered drug disposition in arthritis is related to disease severity.

1.4. Specific objectives, and their rationale

1.4.1. Establish suitable stereospecific methods for quantification of acebutolol and propranolol enantiomers.

A number of publications have addressed the importance of separation, identification, and quantification of racemic drugs (Jamali *et al.*, 1989; Walle *et al.*, 1988; Ariens *et al.*, 1988; Krstulovic, 1989; Evans *et al.*, 1988). Indeed, a good deal of literature has surfaced regarding the pharmacokinetics of an important class of racemic drugs used in the treatment of hypertension, the ß-blockers. This attention stems from the fact that, with the exceptions of timolol and penbutolol, all of the β-blockers are manufactured and administered as racemic mixtures (Riddell *et al.*, 1987). Generally, the β-blocking activity has been ascribed mainly to the S-(-)-enantiomer (Sankey *et al.*, 1984). In addition to the knowledge that the enantiomers differ pharmacologically, it is also known that some of the enantiomers of various β-blockers possess quite different pharmacokinetics (Pflugmann *et al.*, 1987; Olanoff *et al.*, 1984; Walle *et al.*, 1983; Von Bahr *et al.*, 1982). Furthermore, physiological changes in diseases such as arthritis may not equally influence the pharmacokinetics of the individual enantiomers. For these reasons, it is apparent that the disposition of the individual enantiomers must be delineated.

1.4.1.1. Develop a stereospecific method of analysis for acebutolol

The cardioselective β-blocker, (±)-Acebutolol (AC) was introduced in France and the United Kingdom in the mid 1970's (De Bono *et al.*, 1985). It is indicated in the treatment of hypertension and ventricular arrhythmia. Acebutolol is extensively metabolized to an active metabolite, diacetolol, upon first-pass through the liver. Measurement of both acebutolol and diacetolol enantiomers is essential as both contribute to therapeutic activity. Although several methods have been employed to measure acebutolol and diacetolol (Steyn, 1976; Meffin *et al.*, 1976, 1977b; Guentert *et al.*, 1979; Buskin *et al.*, 1982), these methods all utilize non-stereospecific methods. Initially, Sankey *et al.* (1984) were able to separate acebutolol and diacetolol enantiomers with the use of the optically pure derivatizing reagent, S-(-)-N-trifluoroacetylprolyl chloride (TPC). However, commercial TPC is contaminated with 4 to 15% of the R-(+)-enantiomer and rapidly racemizes upon storage (Gulaid *et al.*, 1985). Alternatively, derivatization with either R-(+)-1-phenylethyl isocyanate (R-PEIC) (Gulaid *et al.*, 1985), or S-(-)-PEIC (Hsyu and Giacomini, 1986) have been used to derivatize other β-blocking drugs, including acebutolol. However, these methods, utilizing reversed-phase high pressure liquid chromatography (HPLC) and fluorescence detection, lack sensitivity. Hence, the use of these reported methods for pharmacokinetic studies is limited. For this purpose we developed a stereospecific HPLC assay for the simultaneous determination of acebutolol and diacetolol enantiomers which would be suitable for pharmacokinetic studies of acebutolol in humans and rats.

1.4.1.2. Develop a stereospecific method of analysis for propranolol

Although propranolol is commercially available as a racemate, antihypertensive activity of S-(-)-propranolol is about 100 times greater than the R-(+)-isomer (Rahn *et al.*, 1974). Stereoselective differences in the pharmacokinetics of propranolol enantiomers have been reported in humans and rats (Silber *et al.*, 1982; Lalonde *et al.*, 1988; Guttendorf *et al.*, 1991). Therefore examination of individual enantiomer disposition in pharmacokinetic studies is imperative.

Numerous methods involving chiral derivatization have been reported for the separation of propranolol enantiomers. Unfortunately, the applicability of these methods to our pharmacokinetic studies was limited as many lacked sensitivity for small sample sizes (Sedman & Gal, 1983; Schmitthenner *et al.*, 1989) or did not have an internal standard (Schaefer *et al.*, 1990). Furthermore, in rat samples, large differences in enantiomer concentration are present, often exceeding ratios of 10:1, thus high resolution of diastereoisomer derivatives is required. Reported methods utilizing derivatization with the optically pure reagents (+)-phenylethyl isocyanate (Langaniere *et al.*, 1989) and (-)-menthyl chloroformate (Prakash *et al.*, 1989), lacked sufficient resolution. The method of Linder *et al.* (1989), involving derivatization of propranolol with (R,R)-O,O-α-Diacetyl tartaric acid anhydride reportedly results in resolution factors between 4 and 6. However, the procedure was found to be unsuitable as it requires lengthy reaction times and resulting diastereomers lack stability. Therefore, we developed a stereospecific HPLC assay suitable for pharmacokinetic studies of propranolol in the rat.

1.4.2. Study the pharmacokinetics of acebutolol enantiomers in humans

In the human studies we sought to:

1.4.2.1. Delineate the pharmacokinetics of acebutolol enantiomers in healthy subjects

Although acebutolol is manufactured and subsequently administered as the racemate, beta-blocking activity resides mainly with the S-(-)-enantiomer (Walle *et al.*, 1988). As enantiomers of racemic compounds, including the beta-blockers, often exhibit different pharmacokinetics, it is necessary to discern the respective time-courses of each individual enantiomer. To date, however, pharmacokinetic studies have utilized non-stereospecific analytical methods. Consequently, the pharmacokinetics of acebutolol enantiomers in man remain unknown. Therefore the stereospecific pharmacokinetics of acebutolol were studied in a group of healthy, young volunteers.

1.4.2.2. Determine the effect of aging on the pharmacokinetics of acebuolol enantiomers

While the prevalence of arthritis increases with age, many altered physiological functions apparent in inflammation such as changed plasma protein ratios and reduced renal and hepatic function are also seen in aged subjects (Roubenoff, 1993). Progressive changes in these functions are seen with increasing age (Greenblatt *et al.*, 1982). Indeed, significant pharmacokinetic changes for a number of beta-blockers have been observed in elderly subjects (Fitzgerald, 1988; Ishizaki *et al.*, 1980; Gretzer *et al.*, 1986; Rigby *et al.*, 1985; Bastain & Marlow, 1989). Moreover, as stereoselective differences in the disposition of enantiomers have been reported for a number of beta-blockers (Jamali *et al.* ,1989; Walle *et al.*, 1988), age-related pathophysiological changes may also affect the stereoselective disposition of these agents. As it is possible that aging may obscure results obtained from arthritic subjects, the disposition of acebutolol enantiomers was examined in a group of healthy volunteers ranging from 60 to 75 years of age.

Although protein binding of acebutolol is minimal in humans, the relative binding of each enantiomer has not been determined. Therefore, the *in vitro* binding of acebutolol enantiomers was examined in plasma obtained from healthy young and aged subjects.

1.4.2.3. Establish effect of arthritis on disposition of acebutolol enantiomers

To date, there is no information which describes the pharmacokinetics of acebutolol in inflammation. Therefore, the pharmacokinetics of acebutolol enantiomers were compared between a group of arthritic patients and age matched controls.

1.4.3. Study the pharmacokinetics of acebutolol in the rat

In the rat studies we sought to:

1.4.3.1. Delineate the pharmacokinetics of acebutolol enantiomers in healthy rats.

Due to the limited availability of human arthritic subjects with active disease and not on concurrent drug therapy, we sought to develop an animal model for the study of the effect of arthritis on acebutolol disposition. Adjuvant arthritis in rats is an accepted model for rheumatoid arthritis (Whitehouse, 1973). Induction of arthritis results in a systemic disease, with joint inflammation and nodule formation, such as that found in rheumatoid arthritis. However, virtually nothing is known about the disposition of acebutolol in the rat. For this purpose we chose to delineate the pharmacokinetics of acebutolol enantiomers in the rat. Experiments were also performed to:

1.4.3.2 Elaborate on factors involved in the absorption profile of acebutolol

In humans and rats, fluctuations in plasma concentrations of acebutolol enantiomers in the form of multiple peaks were revealed after oral doses. Multiple absorption peaks have also been reported for other drugs and this phenomenon has been attributed to a multitude of causes such as intestinal absorption windows, changes in gastrointestinal motility or enterohepatic recirculation (Plusquellec *et al.*, 1987; Oberle & Amidon, 1987; Suttle *et al.*, 1992). Intravenous and oral doses of racemic acebutolol were administered to bile duct cannulated rats to determine if multiple peaks were due the recirculation of acebutolol after a first-pass elimination into bile. In addition, transfer of acebutolol across the intestinal mucosa was examined *in vitro* to test for the existence of absorption windows or active transport carriers.

1.4.3.3. Determine the effect of adjuvant induced arthritis on the disposition of acebutolol enantiomers

To study the effects of inflammation on the disposition of acebutolol, the plasma concentrations of the enantiomers of acebutolol and their respective metabolites (diacetolol), were examined in healthy and arthritic rats after oral administration of acebutolol. In order to minimize stress from jugular vein cannulation and repeated sampling, which have been reported to influence protein binding and intrinsic clearance of drugs, only a single blood sample was obtained from each animal.

1.4.3.4. Establish the extent of plasma protein binding of acebutolol in healthy and arthritic rats

Although protein binding of acebutolol is minimal in humans, its extent is unknown in rats, particularly in the presence of arthritis which is known to elevate AAG (Belpaire *et al.*, 1982). Therefore the *in vitro* binding of acebutolol enantiomers in plasma obtained from arthritic rats was compared with that in controls.

1.4.4. Study the stereospecific pharmacokinetics of propranolol in rats

In the rat studies we sought to:

1.4.4.1. Determine the effect of adjuvant arthritis on the pharmacokinetics of propranolol enantiomers

Studies utilizing non-stereospecific methods have shown significantly higher than normal plasma concentrations of propranolol in rats with adjuvant-induced arthritis and turpentine-induced inflammation (Bishop *et al.*, 1981; Walker *et al.*, 1986; Belpaire *et al.*, 1989). As therapeutic activity is mainly dependent upon concentrations of the S-enantiomer, it is important to examine whether the observed changes in disposition kinetics of propranolol reflects those of the therapeutically more relevant enantiomer. Consequently the pharmacokinetics of propranolol enantiomers were studied in healthy and arthritic rats after oral and intravenous doses to determine the effect of adjuvant-induced arthritis on the stereoselective disposition of propranolol.

1.4.4.2. Demonstrate the effects of adjuvant arthritis on biotransformation of propranolol enantiomers.

Increased bioavailability of propranolol in experimentally induced inflammation may reflect changes in hepatic enzyme activity. Non-stereospecific studies have established a reduction in propranolol metabolism in the 9000X*g* supernatant liver fraction of turpentine-treated rats (Chindavijak *et al.*, 1987a). Therefore, to assess the influence of AA on the metabolism of the enantiomers of propranolol and its contribution to observed changes in propranolol bioavailability, the relative *in vitro* metabolism of propranolol enantiomers was compared in microsomal fractions obtained from arthritic and healthy rats.

1.4.4.3. Determine the influence of arthritis on the protein binding of propranolol enantiomers.

Elevated plasma propranolol concentrations in inflammation have been attributed to increased protein binding. A reduction in the free fraction of total (R+S) propranolol has been demonstrated in experimentally induced inflammation in rats (Walker *et al.*, 1986; Belpaire *et al.*, 1989). Therefore, the *in vitro* binding of propranolol enantiomers were compared in plasma obtained from arthritic and healthy rats.

1.4.4.4. Study the relationship between severity of inflammation and altered propranolol disposition in adjuvant arthritis

Experimental data in humans, which have shown an association between increased plasma concentrations of propranolol with ESR values (Schneider *et al.*, 1979, Waller *et al.*, 1981), imply that there may be a connection between altered drug clearance and disease severity. Nevertheless, the influence of disease severity has not been examined. Specifically, clearance of a drug such as propranolol may be more or less reduced in differing degrees of arthritis. Therefore, to investigate this possibility, the pharmacokinetics of propranolol enantiomers were examined in AA rats afflicted with various degrees of inflammation.

1.4.4.5. Determine the influence of ketoprofen therapy on the pharmacokinetics of propranolol in adjuvant arthritis

Interpretation of results obtained in arthritic rats suggest that clinical improvement of inflammation through effective drug therapy may also have implications on drug disposition (Pollack *et al.*, 1989; Beck & Whitehouse, 1974). As inflammation in arthritic patients is often controlled by drugs such as the NSAIDs, it is possible that arthritic-induced changes in drug disposition are minimized or suppressed in these subjects. To ascertain the influence of NSAID treatment, the pharmacokinetics of propranolol enantiomers were examined in non-treated and ketoprofen-treated AA rats suffering from various degrees of inflammation.

1.4.4.6. Study the influence of ketoprofen therapy on the protein binding of propranolol enantiomers

Because protein binding may be a factor in altered distribution of propranolol in inflammation, the *in vitro* binding of propranolol enantiomers was assessed in plasma obtained from non-treated and ketoprofen-treated arthritic rats.

Table 1-I. Relationship between the percentage of free drug and the concentrations of α_1-acid glycoprotein

Drug	% free drug versus AAG[†]	Reference
Alprenolol	-0.904 *	F.M. Belpaire *et al.* 1982
Oxprenolol	-0.895 *	" "
Pindolol	-0.917 *	" "
Propranolol	-0.897 *	" "
Timolol	-0.876 *	" "
Aprindine	-0.841 *	O. Teirlynck *et al.* 1982
Moxaprindine	-0.941 *	" "
Chlorpromazine	-0.690 *	K. Piafsky *et al.* 1978

[†] Pearson correlation coefficients

* Linear regression slope significantly different than zero ($p<0.05$).

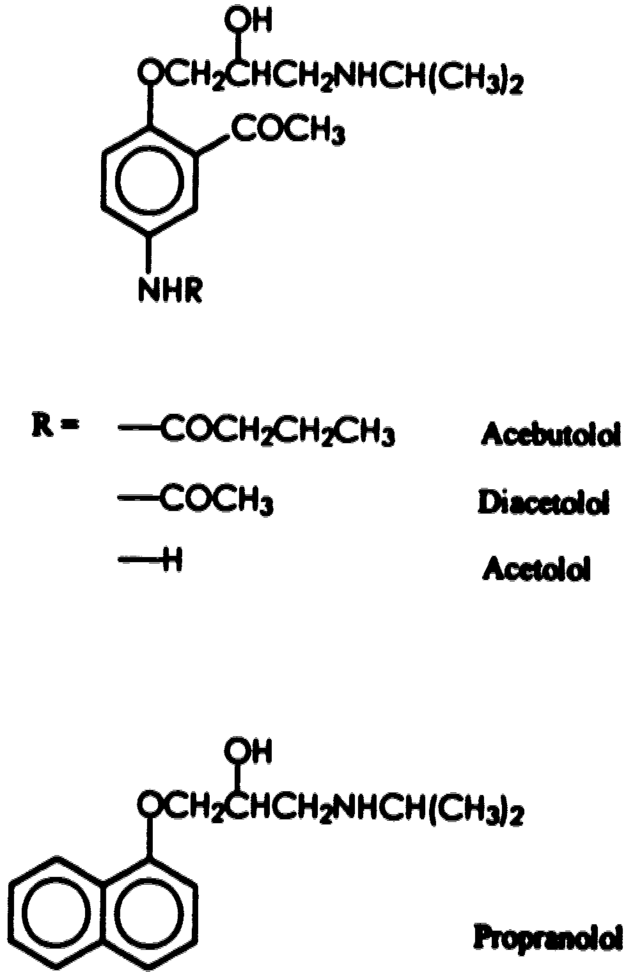

Figure 1-1 Structures of Acebutolol, diacetolol, acetolol, and propranolol

2. EXPERIMENTAL

2.1. Chemicals

Racemate and pure enantiomers of acebutolol, diacetolol, and internal standard (M&B 17764) were kindly provided by Rhone-Poulenc Ltd (Essex, England). Other β-blockers used in this study were racemates and obtained as gifts from the respective pharmaceutical firms: pindolol (Sandoz, Dorval, Que., Canada); atenolol (Imperial Chemical Industries, Cheshire, England); nadolol (Squibb, Montreal, Que., Canada); toliprolol (Boehringer Ingelheim, F.R.G.); alprenolol (Astra, Switzerland); bupranolol (Logeais, Issy-les-Molineaux, France); labetolol (Glaxo, Toronto, Ont. Canada); metoprolol (Ciba, Mississauga, Ont., Canada); (±)-, (+)- and (-)-sotalol (Bristol Myers, Candiac, Quebec, Canada); and tocainide (Astra, England). Acebutolol tablets (Sectral, Rhone-Poulenc, Montreal, Canada) were purchased commercially.

Racemate and pure enantiomers of propranolol, ketoprofen, all reagents required for microsomal incubations, Lowry kits for the determination of total protein and urethane were purchased from Sigma Chemical Co. (St. Louis, MO, USA). S-(+)-1-(1-Naphthyl)ethyl isocyanate (NEIC) was obtained from Aldrich (Milwaukee, WI, U.S.A.). Hexane, chloroform, ether, isopropyl alcohol, acetonitrile, acetic acid and methanol (BDH, Toronto, Ont., Canada) were all analytical grade. HPLC grade water, triethylamine, polyethylene glycol 400 and squalene were purchased from Fisher Scientific (Edmonton, Canada). Methoxyflurane (Metofane) was obtained from Pitman-

Moore Ltd (Mississauga, Canada), *Mycobacterium butyricum* from Difco Lab (Detroit, USA) and heparin (Hepalean) from Organon Teknika (Toronto, Canada).

2.2 Assays

2.2.1. Apparatus

Samples were mixed using a Vortex Genie 2 mixer (Fisher Scientific, Edmonton, Canada) and centrifuged with a Dynac II centrifuge (Becton Dickinson, Parsippany, NJ, U.S.A.). Evaporation of solvents utilized a Savant Speed Vac concentrator-evaporator (Emerston Instruments, Scarborough, Ont., Canada). The HPLC consisted of a Waters auto-injector WISP 712 and model 590-programmable pump (Waters, Mississauga,Ont, Canada). The detector was an Applied Biosystems model 980 fluorescence detector (Technical Marketing Associates, Edmonton, Canada). A Hewlett-Packard (Palo Alto, CA, U.S.A.) model 3390A integrator was used to record the peak areas. Diastereomers were separated on a Whatman Partisil 5 (25-cm) stainless steel silica column (Clifton, N.J., U.S.A.) using a mobile phase of hexane-chloroform-methanol pumped at a flow rate of 2 ml/min. Both sample preparation and chromatography were conducted at ambient temperature (22-25°C). Acebutolol diastereomers were also separated on a 8 mm x 10 cm Novapak C18 Radial-pak reversed-phase column (Waters, Mississauga,Ont) using a solution of acetate buffer pH 5.6: acetonitrile (60:40) pumped at a flow rate of 1 ml/min. Mass spectra (fast atom bombardment) of acebutolol diastereomers were obtained on a high resolution mass spectral system (AEI, MS9, Manchester, U.K.) via direct insertion probe, utilizing positive ion mode with glycerol as the medium.

2.2.2. Standard Solutions

Stock solutions of acebutolol, diacetolol, propranolol and internal standards (as hydrochloride salt) were prepared in water to a final concentration of 50 mg/l of the base (solution 1). Stock solutions of the same concentrations were prepared in 0.01% (v/v) triethylamine in chloroform (Solution 2) for determination of extraction and derivatization yields. These solutions were kept at 4 °C. Prior to sample preparation, the stock solutions were diluted with water.

A solution of 1% (v/v) S-(+)-1-(1-naphthyl)ethyl isocyanate was prepared in hexane and stored under nitrogen at -20°C. The NEIC solution was diluted to the appropriate concentration with hexane or chloroform immediately prior to use.

2.2.3. Sample Preparation & Analysis

2.2.3.1. Acebutolol Assay.

Drug free human plasma and urine samples were spiked with solutions of acebutolol and diacetolol to give enantiomer concentrations of 10, 25, 50, 100, 200, and 500 ng/ml for plasma and 0.5, 1, 2.5, 5, 10, 20, 40 and 100 mg/l for urine. To 1.0 ml plasma or 0.1 ml urine (diluted to 1 ml with water) was added 0.05 ml of a internal standard (IS) solution (10 μg/ml pindolol or 1 μg/ml M&B 17764) and 0.15 ml of 1 M sodium hydroxide. The mixture was then extracted with 5 ml of diethyl ether, mixed on a Vortex for 30 sec and centrifuged at 1800 g for 5 min. The organic layer was transferred to clean tubes and evaporated to dryness using a Savant Speed Vac concentrator-evaporator. The residues remaining after evaporation were derivatized with a 0.1% (v/v) solution of NEIC in chloroform (0.20 ml) and mixed for 60 sec.

Aliquots of 0.015 to 0.20 ml were injected into the HPLC and chromatographed using a mobile phase of hexane:chloroform:methanol (63:35:2). The fluorescence detector was set at 238 and 389 nm for excitation and emission, respectively.

When reverse-phase chromatography was utilized, the extracted samples were derivatized with 0.1% NEIC in dichloromethane (0.2 ml) at room temperature for 45 min before adding 0.2 ml of 0.1% ethanolamine in dichloromethane. After 15 min, samples were evaporated to dryness and reconstituted to 0.20 ml with mobile phase.

2.2.3.2. Propranolol Assay

Enantiomers of propranolol were quantitated using the HPLC assay described above with some modifications. Drug free rat plasma was spiked with propranolol to give enantiomer concentrations of 50, 500, 1000 and 2500 ng/ml. To 0.1 ml of plasma, 50 μl of internal standard (bupranolol 2.5 ug/ml) and 250 μl of 0.2 M NaOH was added. The mixture was then extracted with 5 ml of diethyl ether, vortex-mixed, and centrifuged. The organic layer was evaporated to dryness and derivatized with 185 μl of 0.02% S-NEIC in chloroform:hexane (50:50) and vortex-mixed for 60 sec. Aliquots of

100 to 150 μl were injected onto the HPLC. The formed diastereomeric derivatives were chromatographed using a mobile phase consisting of hexane:chloroform:methanol (75:25:0.4) and the fluorescence detector was set at 225 and 280 nm for excitation and emission, respectively.

2.2.3.3. Other Compounds

To assess the universal suitability of this assay, other compounds possessing a secondary amine with a hydroxy group in the β position were also examined. These included the racemates atenolol, alprenolol, bupranolol, labetolol, metoprolol, nadolol, pindolol, sotalol, toliprolol and tocainide. A small amount of drug powder (approximately 0.1 mg/ml) was dissolved in 0.01% (v/v) triethylamine in chloroform and derivatized with 1% S-NEIC in chloroform. Aliquots of 5 to 50 μl were injected onto the HPLC. The resulting diastereoisomer peaks were examined under various compositions of mobile phase and excitation wavelength of the florescence detector was altered between 220-320 nm. An emission filter of 280 nm was used for all measurements.

2.2.4. Extraction Yields

Aliquots (1 ml) of plasma and urine were spiked with acebutolol or diacetolol. After these samples (n=6) were extracted, derivatized and chromatographed the peak areas of the diasterisomers were compared with identical concentrations of the drugs in chloroform (Solution 2) which were directly derivatized with 0.1% NEIC (n=6). Loss of drug in extracted samples that occurs by taking 4 of 5 ml of the organic phase was accounted for. Percent recovery was calculated as the peak area of extracted drug divided by the peak area of the unextracted drug.

2.2.5. Derivatization Yields

Acebutolol or propranolol solutions were added to dry test tubes (n=8) to give concentrations of 50 and 500 ng/ml in 0.01% (v/v) triethylamine/chloroform. Half of the samples were evaporated to dryness and derivatized as described above. The amount of underivatized drug was measured. To determine the unreacted acebutolol, the mobile phase was modified to isopropyl alcohol-chloroform-triethylamine (80:20:0.01 v/v) and the fluorescence detector set to 238 and 389 nm for excitation and emission, respectively.

To determine the unreacted propranolol, the mobile phase was modified to 75:25 hexane:chloroform. The derivatization yields were calculated by comparing peak areas of underivatized drug to the amount of unreacted drug after derivatization. The reaction time was measured by comparing peak area at 5, 15, 30, and 60 min after derivatization of each drug with NEIC.

2.2.6. Mass Spectrometry

To determine the identity of the diastereoisomers of acebutolol, both peaks corresponding to the enantiomers of acebutolol were collected after HPLC separation and evaporated. The collected fractions were subjected to high resolution mass spectral analysis by the Mass Spectrometry Laboratory (Chemistry Services) at the University of Alberta.

2.2.7. Treatment of Data

Calibration curves were forced to linearity by least-square regression analysis of peak-area ratios of the enantiomers to internal standard against standard concentrations of each drug.

The % error was calculated as the difference between estimated and added concentrations of drug. The reproducibility (precision) was reported as an intra- and inter-day assay coefficient of variation of nine samples (three replicates of each concentration/day X three days). The lowest concentration used in the calibration curve was used as the limit of quantification. The limit of detection, however, was taken as a signal-to-noise ratio of 4:1.

Identification of peaks corresponding to the isomers of acebutolol, diacetolol and propranolol was accomplished by derivatization and chromatography of the racemate and pure R- and S- enantiomers of each drug. All results are reported as the mean ± standard deviation.

2.3. In Vivo Pharmacokinetic Studies

2.3.1. Human Subjects

All experimentation involving human subjects was performed at the Clinical Investigational Unit at the University of Alberta. A total of twelve healthy young (Table 2-I), nine healthy aged (Table 2-II), nine arthritic and nine healthy age-matched (Table

2-III) volunteers participated in the pharmacokinetic study after giving written informed consent. The protocol was approved prior to the study by the Human Ethics Review Committee of the University of Alberta Hospital, Edmonton. All volunteers were judged to be in good health as determined by physical examination and routine blood chemistry. None suffered from conditions in which beta-adrenergic antagonists were contraindicated and no other medications were taken for 24 h during the study.

2.3.2. Drug Administration and Sample Collection.

On the day of the experiment, a single 200 mg acebutolol tablet was given orally (at 0800 h) with approximately 240 ml water following an overnight fast (from 2400 h). Venous blood (5 ml) was drawn into heparinized tubes at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, and 24 h via an indwelling heparinized catheter inserted into a forearm vein. The subjects remained in a sitting position for 4 h, then ate a lunch of their choice. Blood samples were centrifuged and the plasma was separated. Urine (total output) was collected at time 0 and then at intervals of 0-3, 3-6, 6-12 and 12-24 h. All samples were stored at -20 °C prior to analysis. Blood pressure was monitored immediately before blood sample collection at times 0, 1, 2, 3, 4, 6, 8, 12, and 24 h. Unfortunately due to inaccurate and/or incomplete urine collection, we were not able to accurately measure acebutolol and diacetolol enantiomer urine concentrations in the first 12 healthy young subjects examined.

2.3.3. Rat Pharmacokinetic Studies

2.3.3.1. Induction of Arthritis.

To induce severe adjuvant arthritis (AA), female Sprague-Dawley (200-250 g) or Lewis rats (150-200 g) were intradermally inoculated into the tail base with 0.05 ml of heat-killed, freeze-dried *Mycobacterium butyricum* suspended in squalene (10 mg/ml). To induce mild AA, female Lewis rats weighing approximately 150 g were intraperitoneally inoculated with 35 mg of *Mycobacterium butyricum* in 1 ml normal saline (n=6). Rats were studied approximately 10-16 days after tail base injection or 21-28 days after ip injection of adjuvant upon the appearance of arthritis. Control rats were not pretreated. In order to minimize inter-rat variation, controls were matched with AA rats in terms of age, time period in cage and time of administration.

The degree of arthritis was assessed through visual and objective measures. Hindpaw swelling was quantified by measurement of diameter using a caliper and by water displacement. An arthritis index score (AI) was obtained by visually grading each hindpaw on a 0 to 4 basis (0, none; 1, involvement of single joint; 2, involvement of > 1 joint and/or ankle; 3, involvement of several joints and ankle with moderate swelling; or 4, involvement of several joints and ankle with severe swelling) and each forepaw on a 0 to 3 basis (0, none; 1, involvement of single joint; 2, involvement of > 1 joint and/or wrist; or 3, involvement of wrist and joints with moderate to severe swelling). A maximum score of 14 could thus be obtained. Erythrocyte sedimentation rates were measured with 200 μl blood using the Wintrobe micromethod (Laboratory Medicine &

Pathology Procedure Manual, University of Alberta Hospital, p52).

2.3.3.2. Surgical Methods and Sample Collection

The day before drug administration, silastic catheters (0.025 " i.d. X 0.047" o.d.; Dow-Corning, Midland MI) were implanted into the right jugular vein of female Sprague Dawley or Lewis rats and the animals were allowed to recover overnight. All surgical procedures were performed under anaesthesia, using diethyl ether for induction and methoxyflurane for maintenance. Drug doses (1 ml/kg) were given orally by gastric intubation or intravenously as bolus doses into the jugular vein cannula. Following iv doses, the cannulas were flushed with 0.5 ml of saline solution. Serial blood samples (0.20 ml) were then collected through the jugular vein cannula. After each sample the catheters were flushed with an equal volume of heparin in saline (100 units/ml). Each blood sample was immediately centrifuged and the plasma was separated and stored at -20 °C until assayed for drug enantiomers. We examined for contamination of cannulas from iv solutions of AC or PR by flushing a 4 inch section of catheter tubing with 0.5 ml of drug solution followed by 0.5 ml of normal saline (n=3), subsequent samples of drug-free buffer were obtained (n=3) using these cannulas. In each case, residual drug could not be detected in buffer samples.

Unless otherwise stated, rats were fasted for 12 h prior to and 4 h after drug administration. All animals were housed in plastic metabolic cages during experiments, with free access to water.

2.3.3.3. Drug Administration.

<u>2.3.3.3.1. Pharmacokinetics of acebutolol in healthy rats.</u> Oral (50 mg/kg, n=8), iv (10 mg/kg and 50 mg/kg, n=4) and ip (10 mg/kg, n=4) doses of racemic acebutolol in normal saline (1 ml/kg) were administered to unanesthetized jugular vein cannulated Sprague-Dawley rats. These animals were allowed free access to food and water. Fifty mg/kg oral doses were also administered to fasting rats (n=7). Blood samples (200μl) were collected at 0, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after dosing. After iv doses an additional blood sample at 0.25 h was collected. A single 24 h urine sample was collected from each animal.

For determination of biliary excretion, an additional six rats were anaesthetized with single 1.2 mg/kg ip doses of urethane and cannulated at the common bile duct using 0.01 inch internal diameter Tygon microbore tubing (Norton, Akron, OH) and at the right jugular vein as described above. Incisions were promptly sutured and animals were placed under a heat lamp to maintain body temperature. Immediately after surgery acebutolol was injected to the anaesthetized rats intraduodenally (50 mg/kg, n=3) or iv (10 mg/kg, n=3). Intraduodenal (id) rather than oral doses were administered to prevent aspiration and to minimize the effect of anaesthesia-induced alterations in gastrointestinal motility on drug absorption. Id doses were administered by injecting acebutolol solution (1 ml/kg) with a 26 gauge syringe directly into the duodenum, 0.5 cm distal to the pyloric sphincter. A 0.20 ml blood sample and total bile output were collected at 0, 5, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min after dosing. After each sample, 0.3

ml of normal saline was re-injected to replace fluid loss.

2.3.3.3.2. Pharmacokinetics of acebutolol in arthritic rats. Severe AA was induced in female Sprague-dawley rats. Racemic acebutolol (25 mg/kg) was administered to unanesthetized control (n=36) and AA (n=36) rats. Six rats were sacrificed at each predetermined time point (0.5, 1.0, 2.0, 4.0, 6.0, or 8.0 h). The rats were anaesthetized and blood was collected via cardiac puncture into heparinized blood collection tubes. Animals with an arthrogram score less than 2 were excluded from the pharmacokinetic study.

2.3.3.3.3. Pharmacokinetics of propranolol in arthritic rats. Mild AA was induced in Lewis rats (n=6). When arthritis was apparent in the AA rats (21-28 days after induction), all rats were jugular vein cannulated and allowed to recover overnight. Racemic propranolol in saline solution was administered orally (30 mg/kg) and intravenously (2 mg/kg) to AA (n=5) and healthy control (n=6) rats in a cross-over fashion with a 48 hour washout period between doses. Blood samples (175-200 μl) were collected through the jugular vein at 0, 15, 30, 45, 60, 120, 180, 240 and 360 min after oral and at 0, 5, 15, 30, 45, 60, 90, 120, 180 and 240 min after intravenous doses. For ethical reasons, due to the severity of arthritis inflicted in one rat, this rat could not be cannulated therefore only five of the six AA rats could be used for the pharmacokinetic study.

<u>2.3.3.3.4. Pharmacokinetics of propranolol in ketoprofen-treated arthritic rats.</u> Mild (n=15) and severe AA (n=15) were induced in female Lewis rats. One-half of each group received ketoprofen in polyethylene glycol 400 (2 mg/kg/day) orally by gastric intubation (1 ml/kg) for the entire duration of the study. When paw swelling was evident in >80% of control AA rats, both non-treated ($\text{MILD}_{\text{control}}$, $\text{SEVERE}_{\text{control}}$) and ketoprofen-treated (MILD_{KT}, $\text{SEVERE}_{\text{KT}}$) groups were jugular vein cannulated. Racemic propranolol (30mg/kg) in normal saline was administered orally and serial blood samples (175 µl) were collected at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6 and 8 h.

2.4. In Vitro

2.4.1. Everted intestinal preparations.

Six Sprague-Dawley rats were anaesthetized with ether and the intestines were exposed through a vertical midline incision. The duodenal and jejunum segments were removed 1 cm and 30 cm distal to the pyloric sphincter, respectively and the ileum was removed proximal to the caecum. The excised intestines were immediately placed in ice-cold Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 2 g/l of dextrose and everted on a glass rod. One end was tied off and the sac was filled with 37° C, drug-free, oxygenated buffer and placed in a jacketed glass organ bath containing 50 ml of either 1.0 µg/ml (n=3) or 250 µg/ml (n=3) of racemic acebutolol in Krebs-Henseleit bicarbonate buffer (pH 7.4) which was maintained at 37°C and continually oxygenated

(95% oxygen-5% carbon dioxide) for 2 h. The mucosal (0.10 ml) and serosal (0.05 ml) fluids were sampled at 0, 15, 30, 60, 90 and 120 min. Contents from gut segments were pooled and incubated with 250 μg/ml racemic acebutolol as described above.

2.4.2. Microsomal isolation and incubation.

Microsomal oxidation reactions were carried out using fresh liver microsomes from livers of control and arthritic Lewis rats. Briefly, freshly excised rat liver was immediately placed in ice-cold 100 mM phosphate-sucrose buffer (pH 7.4) and homogenized using a Potter Elvehjem tissue grinder (Talboys Engineering, Montrose, PA). Homogenates were centrifuged at 10,000 *g* for 20 min. The supernatant was then collected and centrifuged at 105,000 *g* for 60 min, resulting in a small protein pellet which was suspended in buffer and again centrifuged at 105,000 *g* for 60 min. The final pellet was resuspended in phosphate-sucrose buffer. Protein concentration was determined by the Lowry Method (Sigma kit, Sigma Chemical Co., St Louis, MO, USA). The incubation reaction mixture was similar to that used by Fujita *et al.* (1982) for microsomal oxidation of propranolol. Reaction mixtures (total volume 3 ml) containing 5 mM $MgCl_2$, 10 mM glucose 6-phosphate, 0.75 mM $NADP^+$, 2 U/ml glucose 6-phosphate dehydrogenase and 15 ug liver microsomes in 0.05 M Tris-HCL buffer (pH 7.4) were spiked with 2 mg/l racemic propranolol, and shaken in a water bath at 37°C. At 0, 0.5, 1 and 2 h after incubation, the reaction was stopped by transferring aliquots (0.5 ml) to tubes containing 0.5 ml 1 N NaOH. Samples were stored at -20°C until assayed for propranolol enantiomers. Microsomal metabolism of AA was compared to that of controls by measuring the % loss of propranolol versus time in the microsomal mixture.

2.4.3 Protein Binding Studies

2.4.3.1. Apparatus and technique

For all protein binding studies, binding was measured *in vitro* by equilibrium dialysis for 4 h at 37°C using a Spectrum Equilibrium Dialysis apparatus (Los Angeles, CA) with Teflon half cells separated by a Spectra/Por (Spectrum, Los Angeles, CA) cellulose membrane (molecular weight cut off = 12,000 daltons). Plasma (1 ml) was dialyzed against an equal volume of isotonic Sorensen's phosphate buffer (pH 7.4) spiked with either racemic acebutolol or propranolol. Samples from each side of the cell were then collected and stored at -20°C until analyzed for drug concentration. Before dialysis, plasma was adjusted to pH 7.4 by addition of 0.1 N HCL.

2.4.3.2. Binding studies in the Rat

2.4.3.2.1. Acebutolol The protein binding of acebutolol was compared between drug free plasma pooled from control and arthritic rats. Four replicates of blank plasma (1 ml) were dialyzed for 4 h against buffer spiked with either 0.20 or 1.0 mg/L of racemic acebutolol.

2.4.3.2.2. Propranolol. The protein binding of propranolol enantiomers was measured in triplicate in plasma pooled from healthy and arthritic rat at concentrations of 2.0 and 10 mg/L racemic propranolol. The protein binding of 10 mg/L racemic propranolol was also determined in duplicate in blank plasma obtained from individual ketoprofen treated and nontreated arthritic rats.

2.4.3.3. Binding Studies in human plasma

The protein binding of acebutolol was compared between drug free plasma pooled from healthy young and elderly human subjects. Four replicates of pooled plasma were dialysed against buffer containing 1.0 mg/L of racemic acebutolol.

2.5. Data Analysis

2.5.1. Pharmacokinetic indices

The terminal elimination half-life ($t_{1/2}$) for each enantiomer was determined by $0.693/\beta$, where β (elimination rate constant) was calculated using the regression slope of the log-linear terminal elimination phase. The area under the plasma concentration versus time curve (AUC_{0-t}) for each enantiomer was determined using the linear trapezoidal rule for 0 h to the time of the last measured plasma concentration (C_{last}). The extrapolated AUC ($AUC_{t-\infty}$) was calculated as C_{last}/β. The $AUC_{0-\infty}$ (AUC) was calculated by adding AUC_{0-t} and $AUC_{t-\infty}$. Renal clearances (CL_{renal}) of acebutolol and diacetolol enantiomers were estimated by dividing their cumulative urinary excretions (ΣXu_{0-t}) by the respective AUC_{0-t}. The fraction unbound (fu) and percent of protein binding was calculated as: fu = C_{buffer}/C_{plasma} and % bound = (1- fu) x 100, respectively.

2.5.2. Human Studies

In humans, oral clearance (CLo) of acebutolol was determined by CLo = Dose/AUC. Volume of distribution (Vd/F) was calculated using Vd/F = CLo/β. As the fraction of dose reaching the systemic circulation (F) was not known, both Vd and CL terms were expressed relative to F. Creatinine clearance (CL_{cr}) was estimated from serum creatinine concentration (Cockcroft and Gault, 1976).

To ascertain whether age or creatinine clearance had an effect on the pharmacokinetics of acebutolol and diacetolol enantiomers, plots of AUC, β, CL/F, CL_r and ΣXu were examined. Least-squares linear regression analysis was performed on these relationships.

2.5.3. Animal Studies

2.5.3.1. Pharmacokinetics of acebutolol in rats.

Systemic clearance (CL_{sys}) was calculated by dividing the administered iv dose by the corresponding $AUC_{0-\infty}$. The volume of distribution was calculated by $Vd = CL_{sys}/\beta$. The oral clearance (CL_{oral}) was determined by $CL_{oral} = Dose_{po}/AUC_{0-\infty,\,po}$. Absolute bioavailability (F) was calculated by dividing the mean AUC_{po} or AUC_{ip} by the mean AUC_{iv} after the same dose. Other bioavailability indices were calculated based upon equation $F_{oral} = F_{gut} * F_{liver} * F_{lung}$ (Gibaldi, 1982), therefore assuming the entire ip dose reaches the portal circulation:

$$F_{liver} = AUC_{ip} * DOSE_{iv} / AUC_{iv} * DOSE_{ip}$$

$$F_{gut} = AUC_{po} * DOSE_{ip} / AUC_{ip} * DOSE_{po}$$

Renal clearance (CL_{renal}) and biliary (CL_{bile}) clearance were estimated by dividing the cumulative urinary (ΣXu_{0-t}) or biliary (ΣXb_{0-t}) excretion, respectively, by the AUC_{0-t}. Nonrenal clearance of acebutolol was calculated by $CL_{nr} = CL_{sys} - CL_{renal}$. Clearance values and Vd were corrected for weight in all animals.

For the single point study of acebutolol, an area under the average plasma concentration versus time curve (AUC_{0-t}) was calculated from mean concentrations at each time point for AA and control rats using the linear trapezoidal rule. An average t½ was estimated by $0.693/\beta$, where β was calculated using the regression slope of the log-linear terminal elimination phase of the mean plasma concentrations versus time curve (4-8 h). The average oral clearance (CL_{oral}) for AA and control rats was calculated by dividing administered dose with the corresponding AUC.

To ascertain the effect of concentration on the enantiomer (S:R) ratio, plots of acebutolol concentrations versus S:R ratios were examined by linear least-squares regression analysis.

2.5.3.2. Pharmacokinetics of propranolol.

Systemic clearance (CL_{sys}) was determined by $CL_{sys} = Dose_{iv} / AUC_{0-\infty,iv}$. The volume of distribution (Vd) was calculated by CL_{sys}/β. The oral clearance (CL_{oral}) was calculated by $CL_{oral} = Dose_{po}/AUC_{0-\infty,po}$. The CL_{sys}, CL_{oral} and Vd were corrected for weight in all animals. Bioavailability (F) was calculated for each rat by $F = Dose_{iv} * AUC_{0-\infty,po} / Dose_{po} * AUC_{0-\infty,iv}$.

To examine the effect of severity of inflammation on the pharmacokinetics of propranolol, plots of AUC, CL_{oral}, $t_{½}$ and fraction unbound (fu) versus arthritic index were examined by linear least squares regression analysis.

2.5.4. Statistical Analysis.

Statistical significance was evaluated using Student's paired or unpaired t test and by one-way ANOVA, as deemed appropriate. Least-squares linear regression analysis (Epistat) 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 ± standard deviation.

Table 2-I- Patient Characteristics- Young Subjects

Patient	Age, years	Sex	Weight, kg	Creatinine Clearance mL/min
1	19	M	75.1	134
2	25	F	61.3	108
3	26	M	63.5	90
4	23	M	81.5	143
5	24	F	53.4	95
6	20	F	65.1	107
7	20	F	63.5	124
8	31	M	100.5	125
9	25	M	88.0	163
10	23	M	79	132
11	40	F	66.8	118
12	27	F	64.7	112
MEAN	25		71.9	121
SD	6		13.3	21

Table 2-II. Patient Characteristics- Aged Subjects

Patient	Age, years	Sex	Weight, kg	Creatinine Clearance mL/min
13	60	F	58.6	82.9
14	60	F	66.0	88.7
15	65	F	51.4	60.0
16	66	M	82.0	88.6
17	68	F	60.0	49.9
18	70	F	55.5	58.7
19	72	M	75.4	78.6
20	74	F	63.0	52.9
21	75	F	66.0	45.7
MEAN	67		64.2	67.3
SD	5.5		9.6	17.3

Table 2-III. Patient Characteristics- Arthritic & Healthy Subjects

Patient	Age, years	Sex	Weight, kg	Clcr, mL/min	Meds[*]	ESR[+], mm/hr
HEALTHY						
22	25	F	63.6	108		n.d.
23	27	F	52.0	86.4		7
24	43	F	63.0	87.3		n.d.
25	43	F	68.5	90.1		n.d.
26	52	M	84.5	n.d.		n.d.
27	60	F	66.0	89.0		n.d.
28	62	F	62.7	83.0		n.d.
29	65	F	52.0	60.0		n.d.
30	66	M	82.0	89.0		n.d.
MEAN	49		66.0	86.6		
(SD)	(16)		(11.3)	(13.1)		
ARTHRITIC						
31	25	F	55.9	n.d.	A,B	10
32	26	F	69.6	127	C	25
33	35	F	75.8	n.d.	B	27
34	51	M	93.9	113	A,B	8
35	60	F	63.0	71.1	A	18
36	60	F	80.2	n.d.	A,B	n.d.
37	62	F	57.1	73.7	A,B	39
38	63	M	99.2	n.d.	A,B	55
39	63	F	59.7	46.6	A,B,C	58
MEAN	49		72.7	86.3		30
(SD)	(16)		(15.9)	(32.9)		(19)

[*], medications; [+], erthrocyte sedimentation rate
A, NSAIDs; B, Disease modifying agents; C, analgesics
n.d., not determined

3. RESULTS

3.1 Assays

3.1.1. Acebutolol Assay

Our attempts to separate enantiomers of acebutolol using reversed-phase HPLC, although successful, resulted in an additional peak eluting as late as 90 min after injection (Fig. 3-1). This limited the convenience of the assay under isocratic conditions. As separation of the enantiomers using normal-phase was found to be more convenient, we therefore chose to use normal-phase HPLC.

With normal-phase, virtual baseline separation of the peaks corresponding to acebutolol and diacetolol was attained (resolution, AC: R_s=1.45; DC: R_s=1.50). The chromatograms were free from any interfering peaks. Analysis of the pure enantiomers of acebutolol and diacetolol did not reveal racemization. Under these conditions, peaks corresponding to R-,S- acebutolol enantiomers eluted at approximately 12 and 13 minutes; internal standard (pindolol) enantiomers eluted at 6 and 7 minutes, respectively (Fig. 3-2). The retention times for R- and S-diacetolol were 23 and 27 minutes, respectively (Fig. 3-3).

In all cases, excellent linearity was observed between the peak area ratios (drug:internal standard) in plasma and urine ($r > 0.995$) over the concentration range examined. Typical calibration curves where Y is the peak area ratio (R- or S-drug/internal standard) and X is the enantiomer concentration could be described for R and S-acebutolol by $Y = 0.075 + 0.071(X)$ and $Y = 0.067 + 0.066(X)$ in plasma and $Y = -0.092 + 0.119(X)$ and $Y = -0.078 + 0.111(X)$ in urine, respectively. Typical calibration curves for diacetolol in plasma can be described by $Y = 0.0085(X) +$

0.0496 and $Y = 0.0085(X) + 0.0427$ for the R- and S-enantiomers, respectively.

The observed assays interday and intraday coefficient of variation for acebutolol (Table 3-I) and diacetolol (Table 3-II) were always less than 10% over the examined concentration range in plasma. Error of measurement was also determined to be less than 10% (Table 3-I, 3-II). Quality control samples were analyzed daily and accuracy of of these measurements were found to be within the accepted limits of error (<10%). Minimum assay detection limit, defined as signal to noise ratio of at least 4 to 1, was determined to be 1 ng/ml for the enantiomers of both acebutolol and diacetolol.

The extraction yields of acebutolol in plasma were 91.1±7.8 and 80.4±7.0 for 25 and 500 ng/ml, respectively. In urine, yields were 80.8±3.85 and 87.4±7.19 for 2 and 100 mg/l, respectively. Extraction of R-and S-diacetolol from spiked samples of plasma were 26.0±3.0 % at 50 ng/ml and 28.4±3.4 % at 500 ng/ml. Recovery of diacetolol from urine was similar and was determined to be 30.1±2.8 % at 0.5 mg/L and 29.0±3.8% at 5.0 mg/L.

The derivatization yields of acebutolol and diacetolol were found to be 100% within 10 min, as underivatized drug could not be detected. Peak areas of derivatized diacetolol and acebutolol diastereoisomers were also found to be maximal within 5 minutes. Therefore longer reaction times were not necessary. Furthermore, the diastereoisomers were found to be stable at room temperature in chloroform for at least 72 hours.

Identification of the two peaks corresponding to acebutolol enantiomers indicated that the urea, and not the carbamate, derivative was formed. The molecular ion (MH^+) was observed at m/z 534. Another ion at m/z 516 suggests the loss of H_2O. These results indicate that the derivative is mono-substituted. Furthermore, substitution must be on the nitrogen atom, as the loss of H_2O suggests a free hydroxyl group. Increasing the reaction time of derivatization did not result in formation of the carbamate derivative.

3.1.2. Propranolol Assay.

HPLC peaks corresponding to R- and S-propranolol were free from any interference and eluted with a resolution factor = 3.5 at approximately 12 and 19 minutes, respectively (Fig. 3-4). Internal standard peaks eluted at approximately 6 and 10 minutes (Fig. 3-4).

Calibration curves were linear over the concentration range of 50-10000 ng/ml of propranolol enantiomers (correlation coefficient $r > 0.99$). Coefficients of variation were less than 10% over the examined concentration range (Table 3-III). The limit of detection is 10 ng/ml, if one uses a signal-to-noise ratio of 4:1.

The derivatization yield of propranolol was found to be 100% within 30 min, as underivatized drug could not be detected. Peak areas of derivatized propranolol enantiomers were also found to be maximal within 30 minutes. Furthermore, the diastereoisomers were found to be stable at room temperature in chloroform for at least 72 hours. However, samples were injected within 48 hours of derivatization, as longer times resulted in appearance of an additional peak which interfered with the internal standard.

3.1.3. Other Racemic Compounds

Derivatization with S-NEIC appears to have wide applicability as acebutolol, atenolol, metoprolol, nadolol, pindolol, propranolol, sotalol, toliprolol and tocainide were successfully separated and detected. Only minor modifications to the mobile phase and to the excitation and emission wavelengths of the acebutolol assay were required for detection of these compounds.

3.2. Human Pharmacokinetic Studies

3.2.1 Young subjects

In the young subjects, we observed a slight but significant stereoselective disposition for acebutolol (Fig. 3-5, Table 3-IV). In these subjects, S-(-)-enantiomer AUC values were significantly greater than those of the antipode (S:R AUC = 1.20 ±0.05). Although peak plasma concentrations were reached at the same time for each enantiomer (T_{max}), concentrations (C_{max}) were consistently and significantly higher for the S-enantiomer. Oral clearance of R-acebutolol was significantly greater than that of S-acebutolol. Terminal $t_{1/2}$ of the enantiomers, however, were not significantly different.

Diacetolol disposition was also stereoselective (Fig. 3-6, Table 3-V). Peak plasma concentrations (C_{max}), although occurring at the same time for each enantiomer (T_{max}), were significantly higher for R-diacetolol (R/S 1.4±0.2). The AUC_{0-24} of R-diacetolol was also significantly greater than that of the S-enantiomer although their

$AUC_{0-\infty}$ were not statistically different. The enantiomeric ratios of S:R-diacetolol in plasma steadily increased from 0.7±0.1 at Tmax to 1.5±0.4 at 24 h. Values of $t_{½}$ were significantly longer for S-diacetolol than R-diacetolol. Furthermore, the $t_{½}$ of S-diacetolol but not R-diacetolol was significantly longer than that of the parent compound. The pharmacokinetics of acebutolol and diacetolol were not significantly different between male and female subjects.

3.2.2 Elderly Subjects

3.2.2.1. Pharmacokinetic differences between enantiomers.

Similar to young subjects, acebutolol and diacetolol disposition in the elderly subjects was stereoselective (Fig. 3-7, Tables 3-VI,3-VII). The AUC and ΣXu of the S-enantiomer of acebutolol were 16.5% and 18.5% greater than its antipode, respectively. The oral clearance of R-acebutolol was subsequently 17.9% greater than S-acebutolol. Although there was a trend of longer $t_{½}$ for S-acebutolol (7 of 9 subjects), the difference did not reach significance. Renal clearance of R- and S-acebutolol were not significantly different (Table 3-VII).

The AUC_{0-24} and C_{max} of R-diacetolol were significantly greater than for S-diacetolol (Table 3-VIII). Although plasma levels of R-diacetolol were greater, the $t_{½}$ of S-diacetolol was significantly longer, thus resulting in prolonged plasma levels, hence the $AUC_{0-\infty}$ of the enantiomers were not significantly different. The CL_{renal} and ΣXu of R-diacetolol were 14% and 46% greater than S-diacetolol, respectively.

3.2.2.2. Correlations between acebutolol and age.

For R-acebutolol there was a significant correlation between age and CL_{renal} (r=0.679), whereby an increase in age was associated with a decline in CL_{renal} (Fig. 3-9a). Correlations of age versus CL/F (r=0.473), AUC (r=0.525), ΣXu (r=0.568) or β (r=0.599) were not significant (Table 3-VI). For S-acebutolol, a significantly declining β (r=0.681), CL_{renal} (r=0.736) and ΣXu (r=0.670) with increasing age was observed. Correlations with CL/F (r=0.343), and AUC (r=0.411) were not significant.

3.2.2.3. Correlations between acebutolol and creatinine clearance.

Declining CL_{cr} was associated with a significant reduction in CL_{renal} (r=0.745) (Fig. 3-9c) and ΣXu (r=0.761) of R-acebutolol. However, no relationship was observed with AUC (r=0.429), CL/F (r=0.444), or β (r=0.429). Similarly, for S-acebutolol, CL_{renal} (r=0.780) and ΣXu (r=0.853) were highly correlated to CL_{cr}; whereas associations with AUC (r=0.279), CL/F (r=0.235) and β (r=0.569) were not significant.

3.2.2.4. Correlations between diacetolol and age.

For R-diacetolol, age was highly correlated to β (r=0.900), whereby β decreased with increasing age (Fig. 3-9b). ΣXu was also negatively associated with age (r=0.750). Correlations of age with AUC (r=0.345) or CL_{renal} (r=0.615) were not significant. For S-diacetolol, age-related reductions in β (r=0.734), CL_{renal} (r=0.760) and ΣXu (r=0.738) were significant. The correlation between age and AUC of S-diacetolol was not significant (r=0.382).

3.2.2.5. Correlations between diacetolol and creatinine clearance.

Renal clearance (r=0.917) and ΣXu (r=0.710) of R-diacetolol were observed to significantly decline with decreasing CL_{cr} (Fig. 3-9d). Associations of CL_{cr} with β (r=0.606) and AUC (r=0.642) were not significant. Similarly for S-diacetolol, positive correlations of CL_{renal} (r=0.847) and ΣXu (r=0.723) versus CL_{cr} were seen (Fig. 3-9), whereas no significant associations were observed with AUC (r=0.567), and β (r=0.300).

3.2.2.6. Correlations between age and enantiomer disposition.

Graphs of the S:R ratios of acebutolol and diacetolol enantiomers in plasma and urine versus age are shown in Fig. 3-10. For acebutolol, an age-related decline in the S:R ratio of acebutolol enantiomers in urine (ΣXu) was observed (r=0.677). However, age was related to but not significantly associated to the AUC S:R ratio of acebutolol (r=0.628). For diacetolol, neither AUC (r=0.395) nor ΣXu (r=0.565) S:R ratios were significantly related to age.

3.2.2.7. Correlations between CL_{cr} and enantiomer disposition.

The enantiomeric ratios of acebutolol in plasma (AUC S:R; r=0.807) and urine (ΣXu S:R; r=0.807) was highly correlated to CL_{cr}. As CL_{cr} values declined, a subsequent decline in the S:R ratio of acebutolol towards unity was observed (Fig. 3-10).

Similarly, a decline in the S/R ratio of diacetolol in urine was evident (Fig. 3-10). The AUC S:R ratio of diacetolol was however, not significantly associated with CL_{cr} ($r=0.060$)

3.2.2.8. Protein binding.

Plasma protein binding of acebutolol was neither stereoselective nor age-related. The % binding was calculated to be 7.3±1.2% and 6.6±2.0% for R- and S-acebutolol in these subjects; and 16±9.1% and 14±9.2% for R- and S-acebutolol in younger subjects. These differences were not significant.

3.2.3. Arthritic Subjects

3.2.3.1. Pharmacokinetic differences between enantiomers.

Similar to healthy subjects, acebutolol disposition in arthritic subjects was stereoselective (Fig. 3-11, Tables 3-IX, 3-X). Plasma concentrations (AUC) and ΣXu of the S- enantiomer of acebutolol were 25% and 17% greater than its antipode, respectively. Subsequently, the oral clearance of R-acebutolol was significantly greater than S-acebutolol. Terminal $t_{½}$ of the enantiomers, however, were not significantly different. The renal pathway only constituted a minor elimination route for acebutolol (generally less than 20% of an oral dose was recovered unchanged in urine within 24 h). Furthermore, in healthy subjects renal clearance of the two enantiomers were not significantly different (Table 3-IX). Hence, the enantiomeric ratio of acebutolol excreted in the urine of these subjects (ΣXu S:R = 1.21±0.11) reflected the ratio found in plasma (AUC S:R, 1.24±0.08). Renal clearance of acebutolol in arthritic subjects, however, was stereoselective in favour of the R enantiomer.

The AUC_{0-24} and C_{max} of R-diacetolol were significantly greater than S-diacetolol (Table 3-X). Although plasma levels of R-diacetolol were greater, the $t_{½}$ of S-diacetolol was significantly longer, thus resulting in prolonged plasma levels. Hence in arthritic subjects, the $AUC_{0-\infty}$ of the enantiomers were not significantly different whereas differences were significant in healthy volunteers. In all subjects, CL_{renal} of diacetolol was stereoselective in favour of the R enantiomer. Urine collection of diacetolol generally accounted for approximately 15% of the acebutolol oral dose with 50% greater amounts of R as compared to S-diacetolol collected (ΣXu S:R arthritic, 0.63±0.04; healthy, 0.65±0.06).

3.2.3.2. Effect of arthritis on pharmacokinetics of acebutolol.

Although a trend existed in which arthritis was associated with higher plasma concentrations of the acebutolol enantiomers, such that the CL_{oral} of R- and S-acebutolol were 22 and 25% lower, respectively, this was only significant for the S enantiomer (Table 3-IX). However, the terminal $t_{½}$ values of S-acebutolol as well as those of R and S-diacetolol were significantly prolonged in arthritis (Table 3-IX, 3-X). Plasma concentrations of the diacetolol metabolite, however, were similar in arthritic and healthy subjects (Table 3-X). Lower CL_{renal} values of acebutolol and diacetolol enantiomers in arthritic subjects as compared to controls were not significant. In addition, arthritis was not found to alter enantiomer S:R ratios of acebutolol and diacetolol in plasma or urine.

3.3. Animal Studies

3.3.2. Pharmacokinetics of Acebutolol in Healthy Rats

3.3.2.1. Intravenous doses.

Following iv administration, plasma levels of acebutolol enantiomers declined in similar patterns (Fig. 3-12a,b), with no significant difference in either Vd or $t_{1/2}$ (Table 3-XI). After 8 h, following the 10 mg/kg dose, plasma levels were below the limit of detection. Consequently, $t_{1/2}$ was determined from the slope of the 6-8 h elimination phase. Nevertheless, the $t_{1/2}$ values were not significantly different between the various iv, ip and oral doses. After the 10 mg/kg dose, CL_{nr} and CL_{sys} were significantly greater for the R-enantiomer, consequently plasma concentrations of the S-enantiomer were slightly greater. A significant decrease in the CL_{sys} of R-acebutolol was seen when the iv dose was increased from 10 mg/kg to 50 mg/kg (Table 3-XI). A similar trend was also seen with S-acebutolol, but failed to reach a significant level. Hence, increasing dose resulted in a small but significant decrease in the AUC S:R ratio from 1.06±0.025 to 0.96±0.018. After both doses, CL_{renal} of S-acebutolol was stereoselective (Table 3-XIII).

With regard to the metabolite, plasma concentrations of R-diacetolol were significantly greater than S-diacetolol after the 50 mg/kg dose (Fig. 3-12b). Changing dose did not significantly alter the AUC (Table 3-XI) or S:R ratio (10 mg/kg, 0.91±0.1; 50 mg/kg, 0.86±0.1) of diacetolol. CL_{renal} of diacetolol was stereoselective in favour of the R enantiomer (Table 3-XIII).

3.3.2.2. Intraperitoneal doses.

After ip doses (Fig. 3-12c), enantiomer concentrations of acebutolol were not significantly different from one another (AUC S:R, 1.03±0.033). Assuming the entire ip dose reaches the portal circulation, bioavailability reflects F_{liver}, and was found to be 0.86 and 0.84 for R- and S-acebutolol, respectively. Unfortunately, due to an interfering peak resulting from analytical problems, diacetolol levels were not measured after ip doses.

3.3.2.3. Oral doses.

We observed fluctuations in plasma concentrations of acebutolol in most rats after oral doses, in the absence (Fig. 3-12d) or presence (Fig. 3-12e) of food. However, food was found to significantly decrease the AUC of the enantiomers of both acebutolol and diacetolol (Table 3-XII) by the same extent. The greater absolute F in fasting rats (R, 0.59; S, 0.63), as compared to the fed rats (R, 0.31; S, 0.33) reflects a 48% food-related decrease in bioavailability. In fed rats, the F_{gut} was calculated to be 48% for R and S-acebutolol. The AUC S:R ratio of acebutolol was not significantly different from unity in both fasting (1.02±0.03) and non-fasting (1.04±.04) rats. However, CL_{iv} was significantly higher for the R enantiomer. T_{max} and $t_{½}$ values were not altered by the presence of food. An insignificant trend of higher C_{max} was seen in fasting rats.

The AUC of R- and S-diacetolol after the 50 mg/kg oral doses were not significantly different from those of iv doses, however when oral bioavailability was considered and the values were corrected for the corresponding AUC of acebutolol, twice the amount of diacetolol was present after oral doses (Table 3-XII). Plasma concentrations of diacetolol enantiomers were not significantly different from one another (Fig. 3-12d,3-12e). The S:R ratio after oral administration (0.92±0.1) was not significantly different than that seen after iv doses (0.89±0.1)

The CL_{renal} of acebutolol was stereoselective in favour of the S enantiomer (Table 3-XIII) in both fasting and fed rats. Greater amounts of acebutolol enantiomers were collected in the urine of fasting rats (Table 3-XIII) which was consistent with the food-induced lower F. Nevertheless, ΣXu S:R ratios were not affected by food (Fasting, 1.12±0.03; Non-fasting, 1.16±0.07) nor were they significantly different from those observed after iv (1.14±0.06) or ip (1.12±0.03) doses. With diacetolol, CL_{renal} was stereoselective in favour of the R-enantiomer (Table 3-XIII) and similar S:R ΣXu ratios were observed between oral (0.68±0.04) and iv (0.69±0.04) doses.

3.3.2.4. Bile Duct Cannulation.

The CL_{bile} of acebutolol and diacetolol after iv administration was not significantly different from that observed after intraduodenal (id) doses (Table 3-XIV). With each dose, CL_{bile} was stereoselective in favour of the S-enantiomer for both acebutolol and diacetolol. A smaller % of drug and metabolite was collected in bile after the id as compared with the iv dose, nevertheless, when corrected for F, these differences were not significant. Likewise, the ΣXb S:R ratio of acebutolol (iv, 1.19±0.065; id, 1.17±0.029) and diacetolol (iv, 0.90±0.05; id 0.81±0.08) was not affected. While the AUC enantiomer S:R ratio of acebutolol was not significantly different from unity (AUC S:R iv 1.01±0.011; id 1.00±0.034), stereoselectivity in diacetolol plasma concentrations was observed after both iv (AUC S:R 0.77±0.069) and id (AUC S:R 0.77±0.049) doses.

3.3.2.5. In Vitro Gut.

The percent of acebutolol transferred through the intestinal wall to the serosal side was independent of mucosal concentration in all segments (Fig. 3-13). Significant differences between intestinal segments were not observed. Hence, for the sake of clarity, the means of data from all gut segments were depicted in figure 3-13. In all cases, mucosal and serosal enantiomer concentrations were not significantly different from unity. Intestinal metabolism of acebutolol enantiomers was not observed as mucosal concentrations of acebutolol were not altered after incubation with gut segments and their contents.

3.3.3. Pharmacokinetics of Acebutolol in Arthritic Rats

3.3.3.1. Induction of Arthritis.

Approximately 10-16 days after injection, skin nodules on the ears and tail and swelling of the hind and fore paws were observed in the arthritic rats (arthrogram, 9.4±3.9), while physical changes were not observed in the control rats. Weight gain was less in AA (-6.65±16.2 g) than in controls (27.1±14.3 g), therefore the mean weight of the AA rats (239±24.1 g) was slightly but significantly less than that of controls (266±22.1 g) at the time of pharmacokinetic study.

3.3.3.2. Acebutolol in control rats.

In non-cannulated control rats, plasma concentrations of the active enantiomer, S-acebutolol, were slightly but significantly greater than R-acebutolol at all measured times except for 8 h. Both enantiomers attained their maximum concentration at 2 h (T_{max}). The estimated average $t_{1/2}$ of the enantiomers were similar (R-acebutolol, 2.9 h; S-acebutolol, 2.8 h). Interestingly, the S:R ratio at earlier sampling times (0.5-2 h) appeared to be concentration dependent. A decline in the S:R ratio from 1.5 towards unity was observed with increasing plasma concentration (Fig. 3-14.) during this absorption and distribution phase. Linear regression slopes of concentration ($r=0.647$) and log concentration ($r=0.787$) versus S:R ratios were found to be significant ($p<0.001$). The enantiomer ratios during the elimination phase, at 4-8 h, were not concentration dependent.

Concentrations of S-diacetolol were 18% greater than R-diacetolol at 0.5 h in controls. At other time points, differences were not significant. However, the plasma concentrations of diacetolol were very low and undetectable in a few samples, exhibiting large variability in both AA and control rats.

3.3.3.3. Acebutolol in arthritic rats.

Plasma concentration vs time curves for R- and S-acebutolol in AA as compared to control rats are shown in Fig. 3-15. Plasma concentrations of both enantiomers were significantly raised in AA as approximately 5, 10 and 3 fold greater concentrations were seen at 0.5, 1 and 2 h, respectively. Subsequent plasma levels were not significantly different from controls. The AUC values calculated from mean plasma concentrations was approximately 150% greater in AA rats for both R- (AA: 1719, control: 689 ug.h/L) and S-acebutolol (AA: 1843; control: 750 μg.h/L). AA did not affect T_{max} (2 h in both groups). The terminal $t_{1/2}$ of the enantiomers were not prolonged by AA (control: S, 2.8 h, R, 2.9 h; AA: S, 1.8 h, R, 2.0 h). Plasma concentrations of S-acebutolol were significantly greater than R-acebutolol only at 0.5 and 1 h in AA as compared to 0.5-6 h in controls. The S:R ratio of acebutolol, however, was not altered by AA (Table 3-XV). Linear regression slopes of concentration ($r=0.715$) and log concentration ($r=0.838$) versus S:R ratios of acebutolol at 0.5-2 h were also found to be significant in AA ($p<0.001$). This concentration-dependency appeared to be slightly greater than in controls but this was not significant (Fig. 3-14).

Plasma concentrations of both R- and S-diacetolol were generally greater in AA rats (Fig. 3-16). However, this difference was only significant at 2 h (Table 3-XV). The calculated AUC value for R and S-diacetolol was approximately 150% greater in AA (AA: R, 403; S, 394 ug.h/L vs control: R, 159; S, 158 ug.h/L). T_{max} was also attained faster in AA rats (AA: 2.0 h, control: 4.0 h). There was no significant effect of AA on the ratio of metabolite to parent (diacetolol:acebutolol) drug concentrations, although variability of this ratio was high in both control and AA rats. Interestingly, the S:R ratio of diacetolol enantiomers was significantly lower in AA as compared to the control rats at the early sampling times (Table 3-XV).

3.3.3.4. Protein binding.

At 100 ng/ml, the extent of binding to plasma proteins was 8.0±0.8 and 9.0±4.9 for R-acebutolol, and 7.0±1.2% and 7.0±3.0% for S-acebutolol in control and arthritic rats, respectively. At 500 ng/ml protein binding was 10±4.6 and 11±4.2% for R-acebutolol, and 12±5.0 and 12.5±5.5% for S-acebutolol in control and arthritic rats, respectively. Protein binding was neither concentration dependent nor stereoselective and was not significantly altered in adjuvant induced arthritis.

3.3.4. Effect of Arthritis on the Pharmacokinetics of Propranolol in Rats.

3.3.4.1. Induction of arthritis.

Approximately 21 days after ip injection of adjuvant, mild to moderate swelling of the hind paws was evident in the AA rats (AI= 2.3±1.9). In AA all erythrocyte sedimentation rates (ESR) were greater than 3 mm in the first hour. Physical changes were not observed in the control rats. On the day of propranolol administration, the mean weight of the AA rats (220±28 g) was slightly but not significantly less than that of controls (256±36 g).

3.3.4.2. Propranolol disposition in healthy control rats.

In order to account for first-pass metabolism, we chose larger doses of propranolol for oral administration as compared with iv doses. Accordingly, in the control rats, plasma levels after an oral dose of 30 mg/kg were comparable to those after a 2 mg/kg iv dose (Fig. 3-17). A pronounced stereoselectivity in the plasma concentrations was observed in all rats; with concentrations of R-propranolol being consistently greater than those of the active *S*-enantiomer.

After iv administration, the R:S concentration ratio was observed to decline with time from 6.3±1.4 at 5 minutes to 0.65±0.86 at 180 minutes. Overall, the AUC of the R enantiomer was significantly greater (AUC R:S, 3.0±1.1) and its CL_{sys} was less than half of *S*-propranolol. The Vd of *S*-propranolol was over 6 times greater than R-propranolol. Similarly, $t_{½}$s of *S*-propranolol were longer than R-propranolol (Table 3-XVI).

After oral administration of propranolol to control rats an AUC R:S ratio of 4.3±1.2 was observed. Similarly the peak plasma level (Cmax) of R-propranolol was significantly greater than that of *S*-propranolol in all animals (Cmax R:S, 4.9±1.3) while the time to reach C_{max} (T_{max}) was not significantly different between the enantiomers (Table 3-XVII). Similar t½s for R- and *S*- enantiomers were observed. These $t_{½}$ values, however, were longer than those observed after iv administration.

In all rats, CL_{oral} of *S*-propranolol was significantly greater than its antipode. Hence, bioavailability was stereoselective and was calculated to be 21±10% for R-propranolol and 12±9% for *S*-propranolol in controls.

3.3.4.3. Effect of adjuvant arthritis in vivo.

3.3.4.3.1. I.V. dose. As depicted in Table 3-XVI and Fig. 3-18, the disease did not affect the disposition of propranolol or the S:R ratio of propranolol in plasma (AUC S:R AA: 0.39±0.23; Control: 0.37±0.11).

3.3.4.3.2. Oral dose. After oral administration, CL_{oral} of both enantiomers were significantly decreased, resulting in a significantly greater AUC and bioavailability of both R- and S-propranolol (Fig. 3-19, Table 3-XVII). Although significant for both enantiomers, this effect was more pronounced on the less active R- enantiomer: 13 and 4 fold increases in the AUC of R- and S-propranolol were seen, respectively. This resulted in a significant change in the AUC R:S ratio of propranolol from 4.3±1.2 in controls to 14±3.0 in arthritic rats.

The T_{max} was not significantly different from controls, although AA resulted in a 4 and 10 fold greater C_{max} for S and R-propranolol, respectively, (C_{max} R:S AA: 11±0.81, control: 4.9±1.3). The effect of AA on $t_{1/2}$ was insignificant.

3.3.4.4. In Vitro metabolism.

Microsomal oxidative activity was apparent in livers from both AA and control rats. The time-course of *in vitro* metabolism of R- and S-propranolol are shown in Fig. 3-20. Loss of propranolol was significantly slower in AA as compared to controls. This effect appeared to be stereoselective; in AA, there was a significantly greater percent of the S- enantiomer remaining at each sampling time than R-propranolol. In control microsomes, however, differences between enantiomers was not observed.

3.3.5. Effect of Disease Severity and NSAID Treatment on the Pharmacokinetics of Propranolol in Arthritic Rats.

3.3.5.1. Induction of arthritis.

Approximately 10-16 days or 21-28 days after induction of severe or mild AA, skin nodules on the ears and tail and swelling of the hind and fore paws were observed in the arthritic rats. The observed inflammation in severe AA, as measured by changes in AI, paw diameter and water displacement was significantly greater than that seen in mild AA (Table 3-XVIII). The mean ESR values were not significantly different between the groups.

3.3.5.2. Stereoselective disposition of propranolol.

A pronounced stereoselectivity in the plasma concentrations was observed in all rats; with concentrations of R-propranolol being consistently greater than those of the active S-enantiomer (Fig. 3-21). Hence the CL_{oral} of S-propranolol was significantly greater than its antipode. Similarly the peak plasma level (C_{max}) of R-propranolol was significantly greater than that of S-propranolol in all animals. while the time to reach

C_{max} (T_{max}) was not significantly different between the enantiomers. Similar t½s for R- and S- enantiomers were observed.

3.3.5.3. Effect of ketoprofen treatment.

Treatment with ketoprofen significantly reduced the AI only in the severe model of AA. In mild AA, the AUC_{0-8} of R- and S-propranolol was not significantly different in ketoprofen rats (Table 3-XVIII). On the other hand, in severe AA the AUC_{0-8} of both enantiomers were significantly lower in ketoprofen as compared to control AA (Table 3-XIX). Neither the $t_{½}$s nor the enantiomer S:R ratios of propranolol were significantly altered by ketoprofen treatment.

3.3.5.4. Effect of disease severity.

In nontreated AA controls, greater AUCs and hence lower CL_{tot} of propranolol were observed in rats displaying severe AA. Although more pronounced with the R-enantiomer, this was significant for both enantiomers. Hence, the AUC R:S ratio was also significantly altered (Table 3-XVIII). A high correlation between the AI and the AUC_{0-8} of R- ($r=0.79$) and S-propranolol ($r=0.80$) was found (Fig. 3-22). The AUCs of R- and S-propranolol were also significantly related to other measures of disease severity such as changes in paw diameter and volume, although higher correlation coefficients were obtained with AI.

Similarly, in ketoprofen-treated rats, greater AUCs and lower CL_{tot} were seen in the severe model of AA (Table 3-XVIII). As this was more pronounced with the R-enantiomer, the AUC R:S ratio was significantly higher in the $SEVERE_{KT}$ group. The relationship between disease severity (AI) and AUC_{0-8} of R- (r=0.74) and S-propranolol (r=0.59) was also significant in these rats (Fig. 3-22). Disease severity had no significant effect on the t½ values of propranolol in either the ketoprofen-treated or control groups.

3.3.6. Protein Binding of Propranolol.

3.3.6.1. Healthy versus arthritic rats.

Protein binding was stereoselective in favour of the R enantiomer in plasma obtained from arthritic and healthy rats. The unbound fraction of both propranolol enantiomers were significantly reduced in arthritis at both 2 and 10 mg/L (Table 3-XIX). At 10 mg/L this increased binding was more pronounced with the R enantiomer resulting in a significant increase in the fu S:R ratio from 1.44 in controls to 8.18 in arthritis. Concentration dependency, in that protein binding is reduced with increasing concentration, was observed with both enantiomers in plasma obtained from controls but not arthritic rats.

3.3.6.2. Ketoprofen and nontreated arthritic rats.

The plasma protein binding of propranolol was stereoselective in favour of the R-enantiomer in both control and ketoprofen-treated rats. Unfortunately, due to the inadvertent loss of sample, we were unable to collect sufficient plasma from rats with mild AA. Therefore plasma protein binding was only determined in $\text{SEVERE}_{\text{control}}$ and $\text{SEVERE}_{\text{KT}}$ AA rats. Treatment with ketoprofen was found to significantly increase the fu of both R- and S-propranolol (Table 3-XIX). The fu S:R ratio decreased from 8.1±1.7 in controls to 4.4±1.2 in ketoprofen. When protein binding of all rats were combined, a significant relationship between the AI and fu of R- ($r=0.62$) and S-propranolol ($r=0.69$) was observed (Fig. 3-23). In addition, the relationships between AUC and fu of R-propranolol ($r=0.63$) and the AUC and fu of S-propranolol ($r=0.62$) were significant (Fig. 3-24).

Table 3-I. Accuracy and Precision- Acebutolol

Mean (standard deviation), n=9

Concentration (ng/mL)			Error %		Precision *%	
Added	Measured					
	R	S	R	S	R	S
10	10.4 (0.46)	10.4 (0.42)	3.5	4.4	4.5	4.0
25	25.3 (1.3)	25.0 (1.0)	1.1	0.1	4.9	4.0
50	51.9 (4.2)	51.4 (3.5)	3.8	2.8	8.0	6.9
100	101 (7.3)	100 (6.1)	1.7	0.3	7.1	6.0
200	204 (16)	202 (16)	2.1	1.4	7.8	7.9
500	499 (3.0)	499 (3.4)	-0.1	-0.1	0.70	0.67

Table 3-II. Accuracy and Precision- Diacetolol

Mean (standard deviation), n=9

Concentration (ng/mL)			Error %		Precision * %	
Added	Measured					
	R	S	R	S	R	S
10	10.9 (0.9)	10.9 (0.3)	9.2	9.7	8.2	2.6
25	24.8 (1.8)	25.2 (1.6)	-0.9	0.8	7.1	6.3
50	53.5 (3.9)	52.7 (5.0)	7.0	5.4	7.3	9.5
100	101 (6.7)	101 (6.7)	0.6	1.0	6.7	6.6
250	260 (11)	261 (13)	3.9	4.5	4.4	4.8
500	498 (3.6)	497 (4.4)	-0.5	-0.5	0.70	0.9

*, Coefficient of Variation % .

Table 3-III. Accuracy and Precision- Propranolol

Mean (standard deviation), n=9

Concentration (ng/mL)			Error %		Precision* %	
Added	Measured					
	R	S	R	S	R	S
50	51.8 (4.0)	52.8 (4.3)	3.63	5.63	7.74	8.14
500	504 (26.9)	512 (13.4)	0.85	2.34	5.33	2.61
1000	990 (36.7)	993 (32.7)	-1.01	-0.77	3.71	3.29
2500	2432 (83.7)	2448 (52.4)	-2.73	-2.08	3.44	2.14

*, Coefficient of Variation %

Table 3-IV. Pharmacokinetic Parameters of Acebutolol- Young Healthy Subjects

Subject	AUC (μg.h/L)		C_{max} (μg/L)		T_{max},h	$t_{1/2}$ (h)		Clo (L/h)		Vd/F (L/kg)	
	R	S	R	S	R,S^{+}	R	S	R	S	R	S
1	972	1200	151	157	2.0	6.1	6.9	103	83	12.2	11.1
2	1077	1279	222	239	2.0	2.5	2.6	93	78	5.4	4.8
3	1666	1957	494	560	2.0	4.7	5.1	60	51	6.3	5.9
4	792	1052	94	120	4.0	5.4	5.8	126	95	11.9	9.7
5	1698	1935	584	643	2.0	8.3	5.8	59	52	13.2	8.2
6	1130	1391	225	260	2.0	5.2	5.1	89	72	10.2	8.1
7	1034	1218	174	189	1.5	3.9	3.9	97	82	8.6	7.4
8	620	788	112	120	2.0	4.4	4.2	161	125	10.3	7.6
9	795	956	160	176	2.0	6.1	6.3	126	105	12.6	10.9
10	957	1105	170	175	2.0	7.9	7.9	105	91	11.2	10.2
11	818	918	137	163	4.0	8.0	5.3	122	109	17.4	12.2
12	774	946	128	150	4.0	6.2	6.3	129	106	16.2	13.9
Mean	**1028***	**1229**	**221***	**246**	**2.5**	**5.7**	**5.4**	**106***	**87**	**11.3***	**9.2**
SD	339	375	155	172	0.9	1.8	1.4	30	22	3.5	2.6

*, significantly different from the S-enantiomer

$^{+}$, T_{max} of R and S-AC not different from one another

Table 3-V. Pharmacokinetic Parameters of Diacetolol- Young Healthy Subjects

Subject	AUC (μg.h/L)		AUC_{24} (μg.h/L)		Tmax (h)	Cmax (μg/L)		$t_{1/2}$ (h)	
	R	S	R	S	R,S	R	S	R	S
1	2118	2427	1932	2192	4.0	215	152	6.4	6.9
2	1658	1449	1346	961	4.0	208	131	3.9	6.0
3	3067	2341	2773	1985	4.0	325	188	7.0	8.6
4	2168	2213	1965	1734	2.0	214	155	6.5	10.0
5	2025	1900	1374	1789	6.0	366	210	10.4	15.1
6	3620	4747	3368	4031	4.0	341	263	6.1	8.3
7	2622	2490	2569	2357	4.0	347	288	4.1	5.5
8	2294	2127	2143	1878	4.0	268	218	5.7	7.5
9	1443	1303	1361	1179	2.0	187	133	7.4	8.4
10	991	869	874	733	2.0	73	52	7.3	8.3
11	2930	3164	2767	2515	4.0	390	310	5.5	10.3
12	1886	2051	1718	1603	4.0	248	203	6.3	10.3
Mean	2235	2257	2016	1913*	3.7	265*	192	6.4*	8.8
SD	735	990	705	822	1.1	93	73	1.7	2.5

*, significantly different from the S-enantiomer

Table 3-VI. Effect of Age and Creatinine Clearance on Pharmacokinetic Parameters (r = Pearson correlation coefficient)

Acebutolol		Age	CLcr
		r	r
AUC	R	+ 0.525	- 0.429
	S	+ 0.411	- 0.279
β	R	- 0.599	+ 0.429
	S	- 0.681*	+ 0.569
CL_{renal}	R	- 0.679*	+ 0.745*
	S	- 0.736*	+ 0.780*
ΣXu	R	- 0.568	+ 0.761*
	S	- 0.670*	+ 0.853*
AUC	S:R	- 0.628	+ 0.807*
ΣXu	S:R	- 0.677*	+ 0.807*

Diacetolol		Age	CLcr
		r	r
AUC	R	+ 0.282	- 0.659
	S	+ 0.362	- 0.594
β	R	- 0.900*	+ 0.606
	S	- 0.734*	+ 0.300
CL_{renal}	R	- 0.615	+ 0.915*
	S	- 0.760*	+ 0.847*
ΣXu	R	- 0.750*	+ 0.710*
	S	- 0.738*	+ 0.723*
AUC	S:R	- 0.395	+ 0.060
ΣXu	S:R	- 0.565	+ 0.689*

+, positive or, -, negative relationship

*, statistically significant ($p < 0.05$)

Table 3-VII. Pharmacokinetic Parameters of Acebutolol- Elderly Subjects

Subject	AUC (μg.h/L)		Cmax (μg/L)		T_{max}	$t_{1/2}$ (h)		Clo (L/h)		Vd/F (L/kg)		ΣXu (mg)		CL_{renal} mL/min	
	R	S	R	S	a	R	S	R	S	R	S	R	S	R	S
13	1215	1470	233	270	2.0	2.9	3.1	82	68	5.8	5.1	7.3	9.0	107	110
14	938	1227	375	418	1.0	5.4	4.5	106	81	12	8.1	7.1	10	129	142
15	816	900	139	124	0.8	8.5	9.8	123	111	29	31	5.8	5.9	132	128
16	723	941	105	132	4.0	5.1	5.3	138	106	13	11	5.0	6.2	120	116
17	1207	1448	306	323	1.0	4.3	5.0	83	69	8.6	8.2	3.7	4.2	52	49
18	1436	1527	190	173	4.0	12	16	70	66	21	27	3.9	4.5	53	63
19	1308	1554	243	271	4.0	6.2	6.3	76	64	9.1	7.8	7.6	8.8	104	101
20	1912	2151	189	181	1.0	12	12	52	46	15	12	3.9	4.3	42	41
21	1077	1165	106	102	4.0	5.9	6.8	93	86	12	13	4.3	4.4	71	69
Mean	1181*	1376	209	221	2.4	6.9	7.6	92*	78	14	14	5.4*	6.4	90	91
SD	359	380	91	106	1.5	3.3	4.0	27	21	7.2	8.9	1.6	2.4	36	36

a, Tmax values for both R- and S-Acebutolol

*, significantly different from the S-enantiomer

Table 3-VIII. Pharmacokinetic Parameters of Diacetolol- Elderly Subjects

Subject	AUC_∞ (µg.h/L)		AUC_{24} (µg.h/L)		T_{max}	Cmax (µg/L)		t1/2 (h)		ΣXu (mg)		Clr (mL/min)	
	R	S	R	S	a	R	S	R	S	R	S	R	S
13	2806	1924	2338	1564	4.0	355	223	4.0	4.3	14	9.8	103	105
14	3970	3428	3801	2923	1.5	939	618	5.5	9.3	24	18	107	101
15	2731	1509	2527	1408	4.0	276	97.6	6.2	5.6	13	7.4	84	88
16	2462	2231	2175	1849	4.0	252	191	7.2	8.8	13	9.2	104	83
17	4600	5540	4095	3759	1.5	444	299	7.6	7.8	8.2	5.3	33	23
18	3581	3339	3160	2470	4.0	368	253	6.9	11	9.6	6.0	51	41
19	1964	1897	1687	1413	4.0	177	123	8.1	12	11	6.9	110	81
20	5300	5409	3992	3528	4.0	294	215	12	14	8.7	5.5	36	26
21	4352	3404	3328	2453	2.0	229	130	11	12	9.8	6.3	49	42
Mean	3530	3187	3147*	2464	3.2	370*	239	7.6*	9.4	12*	8.2	75*	66
SD	1113	1483	959	916	1.2	228	156	2.4	3.2	4.9	3.9	32	32

a, T_{max} values for both R- and S-Acebutolol
*, significantly different from the S-enantiomer

Table 3-IX. Pharmacokinetic Parameters of Acebutolol- Arthritic & Healthy Subjects

Subject	AUC (mg.h/l)		C_{max} (mg/l)		T_{max} (h)	$t_{1/2}$ (h)		Clo (l/h)		ΣXu (mg)		Clr (ml/min)	
	R	S	R	S	a	R	S	R	S	R	S	R	S
Healthy													
22	951	1104	86	94	6.0	5.9	6.4	105	90.6	11	12	193	181
23	1144	1382	286	341	3.5	2.3	2.4	87	72	14	17	203	199
24	610	828	153	258	3.5	3.3	3.2	164	121	5.2	6.6	141	132
25	1009	1233	339	378	2.5	2.9	3.0	99	81	12	14	201	191
26	981	1202	191	236	4.0	2.9	3.3	102	83	12	15	209	211
27	938	1227	375	418	1.0	5.4	4.5	106	81	7.1	10	129	142
28	1782	2264	489	572	2.0	4.6	4.6	56	44	16	20	150	143
29	820	900	140	125	0.75	8.5	9.8	123	111	5.8	5.9	132	128
30	725	940	105	130	4.0	5.1	5.3	138	106	5.0	6.2	120	115
Mean	995	1231*	240	284*	3.0	4.6	4.7	109	88*	9.8	11.8*	164	160
SD	336	428	140	158	1.6	1.9	2.3	30.7	23.1	4.1	4.9	36.5	35.1
Arthritic													
31	1485	1690	393	413	2.0	5.9	5.6	67.3	59.2	7.9	8.7	88.3	86.2
32	825	1255	167	192	3.0	3.5	9.1	121	79.7	13	16	258	214
33	1709	2132	382	401	1.0	4.9	[illegible]	[illegible]	46.0	16	19	153	145
34	971	1243	250	354	2.0	6.2	[illegible]	[illegible]	[illegible]	[illegible]	12	172	159
35	857	1319	160	200	3.0	5.8	[illegible]	117	[illegible]	[illegible]	[illegible]	150	116
36	1292	1569	205	216	3.0	[illegible]	4.2	77.4	[illegible]	[illegible]	[illegible]	155	150
37	1458	1846	359	408	2.0	6.8	7.4	[illegible]	[illegible]	[illegible]	[illegible]	91.2	89.6
38	1294	1514	117	129	6.0	7.5	7.3	77.3	[illegible]	[illegible]	[illegible]	144	139
39	1313	1467	201	197	2.5	8.9	10	76.2	[illegible]	[illegible]	[illegible]	97.9	89.1
Mean	1245	1559*	248	279*	2.7	5.9	7.2#	85.1	[illegible]	[illegible]	[illegible]	146	132*
SD	301	293	104	113	1.4	1.7	2.2	22.7	11.4	2.8	3.6	52.5	41.7

a, values given for both R- and S-Acebutolol

*, significantly different from the S-enantiomer

#, significantly different from healthy subjects

Table 3-X. Pharmacokinetic Parameters of Diacetolol- Arthritic & Healthy Subjects

Subject	AUC (mg.h/l)		AUC$_{ss}$ (mg.h/l)		T_{max} (h)	Cmax (mg/l)		t1/2 (h)		ΣXu (mg)		Clr (ml/min)	
	R	S	R	S	a	R	S	R	S		S	R	S
Healthy													
22	1252	1080	1169	919	6.0	107	70	5.4	7.8	15	8.7	201	134
23	--	--	--	--	--	--	--	--	--	25	16	--	--
24	1594	1478	1388	1146	3.0	219	151	3.4	4.7	17	11	177	127
25	2296	1944	1765	1257	3.0	350	207	5.1	7.2	25	15	180	131
26	2594	2538	1784	1258	4.0	256	170	5.9	10	28	18	182	121
27	3970	3428	3801	2923	4.0	939	618	5.5	9.3	24	18	107	101
28	3152	2677	3022	2406	2.5	398	280	5.1	7.1	19	12	98	74
29	2731	1509	2527	1408	4.0	276	97.6	6.2	5.6	13	7.4	84	88
30	2462	2231	2175	1849	1.5	252	191	7.2	8.8	13	9.2	104	83
Mean	2506	2111*	2204	1646*	3.5	345	207*	5.2	7.3*	20	13*	142	107*
SD	851	766	881	696	1.2	237	169	1.3	2.0	5.8	4.2	47.4	23.8
Arthritic													
31	2051	2074	1854	1447	2.5	232	142	6.9	14	14	8.2	116	65.7
32	2545	2418	2404	1985	3.5	329	236	5.6	9.8	23	16	148	109
33	2849	2220	2646	1986	3.0	354	218	6.4	7.6	25	15	148	112
34	2185	1817	1839	1585	3.0	249	239	9.3	8.4	14	9.4	110	86.4
35	2056	1698	1983	1573	4.0	239	164	4.7	6.1	19	12	156	117
36	2942	3104	2702	2277	4.0	243	168	5.7	11	18	12	104	65.1
37	3260	2952	3043	2631	2.5	414	275	6.2	7.4	27	18	140	101
38	1648	1948	1395	1171	3.5	108	71	7.8	16	18	11	186	96.3
39	1890	1972	1704	1299	4.0	141	97	6.5	14	13	7.4	111	62.3
Mean	2181	2245	2174	1773*	3.3	257	179*	6.6#	10.4*#	19.2	12.1*	135	90.5*
SD	896	493	546	480	0.6	97.5	68.6	1.3	3.4	5.1	3.5	27.2	21.6

a, values given for both R- and S-Acebutolol *, significantly different from the S-enantiomer #, significantly different from healthy subjects

Table 3-XI. Pharmacokinetic Indices of Acebutolol Enantiomers in Healthy Rats after IV and IP Administration of Acebutolol (n=4).

	Acebutolol									
	AUC_{0-12} µg·hr/litre		Cl_s ml/min/kg		$t_{1/2}$ hr		Vd litre/kg		AUC_{DC}/AUC_{AC}	
	R	S	R	S	R	S	R	S	R	S
10 mg/kg iv										
Mean	1340	1420^{a}	61.9	58.5^{a}	1.8	1.8	9.6	8.9	0.14	0.12
SD	77.8	64.5	3.5	2.5	0.25	0.36	1.5	1.9	0.03	0.04
50 mg/kg iv										
Mean	9040^{b}	$8710^{a,b}$	46.5^{b}	48.3^{a}	2.3	2.5	9.5	10.5	0.11	0.10
SD	1190	1220	7.3	8.2	0.61	0.64	3.8	4.4	0.02	0.03
10 mg/kg ip										
Mean	1150	1190	71.7	69.5	2.5	2.4	nd	nd	nd	nd
SD	146	153	12.5	11.6	0.30	0.29				

a) Significantly different from the R enantiomer.
b) Significantly different from 10 mg/kg iv dose.
nd) Not determined.

Table 3-XII. Pharmacokinetic Indices of Acebutolol Enantiomers in Healthy Rats after Oral Administration of 50 mg/kg Racemate Acebutolol.

	Acebutolol										
	AUC_{0-12} µg·hr/litre		Cl_O ml/min/kg		$t_{1/2}$ hr		C_{max} µg/L		T_{max} hr	AUC_{DC}/AUC_{AC}	
	R	S	R	S	R	S	R	S		R	S
Fasted											
Mean (n=7)	5410[a]	5510[a]	78.4	77.1	2.8	2.7	1074	1133	2.0	0.22[a]	0.20[a]
SD	1190	1200	21.2	21.0	0.95	0.93	457	476	1.0	0.05	0.03
Fed											
Mean (n=8)	3360[a,b]	3510[a,b]	149[b]	145[b]	2.3	2.2	723	732	2.7	0.24[a]	0.21[a]
SD	1110	1190	77.7	75.1	0.96	1.1	431	407	1.9	0.06	0.063

a) Significantly different from iv doses.
b) Significantly different from fasted rats.
No significant differences were observed between enantiomers.

Table 3-XIII. Urinary Data in Healthy Rats after Acebutolol Administration [Mean ± (SD)]

Dose and Route of Administration	ΣX_u as % of dose				CL_{renal}, ml/min/kg			
	Acebutolol		Diacetolol		Acebutolol		Diacetolol	
	R	S	R	S	R	S	R	S
10 mg/kg iv	23.5 (3.1)	26.9^{a} (3.7)	4.9 (1.2)	3.4^{a} (0.8)	14.6 (2.2)	15.7^{a} (2.2)	17.0 (3.9)	13.1 (4.4)
50 mg/kg iv	26.6 (8.5)	30.2^{a} (9.6)	4.1 (1.6)	2.9^{a} (1.3)	12.0 (3.2)	14.2^{a} (3.7)	14.5 (5.0)	12.1^{a} (5.2)
10 mg/kg ip	18.7 (5.4)	21.1^{a} (6.2)	3.6 (0.8)	2.0^{a} (0.4)	13.1 (3.7)	14.3^{a} (4.3)	nd	nd
50 mg/kg po Fasted	23.0 (8.0)	25.7^{a} (8.9)	6.2 (2.0)	4.1^{a} (1.3)	16.9 (6.6)	18.8^{a} (7.4)	15.9 (5.8)	13.0^{a} (5.4)
50 mg/kg po Fed	$11.5^{b,c}$ (8.2)	$13.2^{a,b,c}$ (9.3)	4.6 (3.1)	3.1^{a} (2.1)	14.6 (2.2)	15.7^{a} (2.2)	17.0 (4.0)	13.1^{a} (4.4)

a) Significantly different from the R enantiomer.
b) Significantly different from 50 mg/kg iv dose.
c) significantly different from fasted rats.

Table 3-XIV. Biliary Data in Bile-Duct Cannulated Rats after Acebutolol Administration
[Mean ± (SD), n=3]

Dose and Route of Administration	ΣX_{bile}, as % of dose				CL_{bile}, ml/min/kg			
	Acebutolol		Diacetolol		Acebutolol		Diacetolol	
	R	S	R	S	R	S	R	S
10 mg/kg iv	10.1 (2.6)	12.2[a] (3.7)	1.22 (0.08)	1.10[a] (0.8)	1.93 (0.41)	2.27[a] (0.36)	3.15 (1.03)	3.65[a] (1.06)
50 mg/kg iv	4.3[b] (0.66)	5.0[a,b] (0.78)	0.87 (0.23)	0.67[a,b] (0.15)	1.53 (0.60)	1.83 (0.82)	1.74 (0.97)	1.82[a] (1.0)

a) Significantly different from the R enantiomer.
b) Significantly different from 50 mg/kg iv dose.

Table 3-XV. S:R Concentration Ratios in Plasma of Arthritic (AA) and Control Rats. Mean (± SD), n=36

Hours	Acebutolol AA	Control	Diacetolol AA	Control
0.5	1.27 ± 0.14	1.22 ± 0.05	0.92 ± 0.24[a]	1.22 ± 0.11
1.0	1.21 ± 0.15	1.31 ± 0.12	0.92 ± 0.16[a]	1.37 ± 0.19
2.0	1.03 ± 0.07	1.06 ± 0.05	0.95 ± 0.19	0.84 ± 0.19
4.0	1.14 ± 0.09	1.16 ± 0.05	1.11 ± 0.38	0.89 ± 0.11
6.0	1.09 ± 0.03	1.11 ± 0.06	1.53 ± 0.43	1.00 ± 0.07
8.0	1.02 ± 0.10	1.07 ± 0.08	1.23 ± 0.31	0.96 ± 0.16

[a] significantly different from controls.

Table 3-XVI Pharmacokinetic Parameters of Propranolol Enantiomers in Arthritic and Control Rats after IV Administration of Racemate (n=6).

	$AUC_{0-\infty}$ (μg.h/L)		CL_{sys} (L/h/kg)		$t_{1/2}$ (h)		Vd (L/kg)	
	R	**S**	**R**	**S**	**R**	**S**	**R**	**S**
<u>Control</u>								
Mean	1185	432$^+$	0.93	2.7$^+$	0.62	1.32	0.74	4.62$^+$
SD	374	175	0.29	0.86	0.36	0.59	0.23	1.17
<u>AA#</u>								
Mean	1518	525$^+$	0.80	2.3$^+$	0.40	1.22	0.48	3.56$^+$
SD	689	259	0.34	1.0	0.14	0.89	0.22	1.71

+, significantly different from R-PR.
#, not significantly different from control.

Table 3-XVII. Pharmacokinetic Parameters of Propranolol Enantiomers in Arthritic and Control Rats after Oral Administration of Racemate (n=6).

	AUC (µg.h/L)		Cmax (µg/L)		Tmax (min)	Clo (L/h/kg)		t1/2 (h)		F	
	R	S	R	S		R	S	R	S	R	S
Control											
Mean	3200	722+	1068	229+	38	6.31	22.4	1.94	2.27	0.21	0.12
SD	1540	181	196	59	14	4.24	6.20	1.12	0.73	0.10	0.04
AA											
Mean	43330 *	3180 *,+	11040 *	1030 *,+	30	0.36 *	4.82 *,+	2.74	2.76	1.56 *	0.46 *,+
SD	7860	386	2560	235	19	0.08	0.52	0.94	1.14	0.59	0.21

+, significantly different from R-PR.
*, significantly different from controls.

Table 3-XVIII. Disease Severity and Pharmacokinetics of Propranolol in KT-Treated and Control Arthritic Rats.

Parameters	*MILD* $_{CON}$	*MILD* $_{KT}$	*SEVERE* $_{CON}$	*SEVERE* $_{KT}$
Arthritic Index ^{a}	2.14 (2.5)	0.38 (0.52)	7.25 (2.5) ^{c}	3.57 (1.5) ^{b,c}
% Increase Paw Diameter ^{a}	0.24 (0.31)	0.04 (0.1)	2.78 (1.9) ^{c}	0.83 (.54) ^{b,c}
% Increase Paw Volume ^{a}	1.21 (0.73)	0.97 (0.53)	3.53 (2.2) ^{c}	0.93 (.37) ^{b}
AUC R-PR ^{a}	16200 (12600)	15800 (10100)	79900 ^{c} (18500)	39200 ^{b,c} (14300)
AUC S-PR ^{a}	1760 (1290)	1720 (970)	6880 ^{c} (2250)	2920 ^{c} (1270)
AUC R:S ^{a}	9.09 (2.09)	9.02 (2.67)	11.9 (1.37) ^{c}	14.2 (2.79) ^{c}
CLo R-PR ^{a}	1.35 (0.89)	1.61 (1.60)	0.164 ^{c} (0.89)	0.406 ^{b,c} (0.17)
CLo S-PR ^{a}	8.80 (4.46)	10.6 (9.39)	2.26 ^{c} (0.85)	6.05 ^{b,c} (3.33)
$t_{1/2}$ R-PR	2.4 (1.2)	3.2 (2.5)	2.1 (1.0)	1.7 (0.72)
$t_{1/2}$ S-PR	2.4 (1.0)	4.1 (3.1)	1.5 (0.55)	1.5 (0.44)

mean (standard deviation)
a), significantly different amoung means
b), significantly different from nontreated control group
c), significantly different from mild treated or control group

Table 3-XIX Fraction Unbound % of Propranolol Enantiomers in Plasma obtained from AA and Control Rats(n=3)

	Control		Arthritic	
	R	S	R	S
2 mg/L				
Mean	7.4	$33.2^{\#}$	2.7^{a}	$8.7^{\#a}$
(sd)	(1.5)	(4.5)	(0.7)	(0.7)
10 mg/L				
Mean	28.9^{*}	$41.6^{\#*a}$	1.8^{a}	$13.2^{\#a}$
(sd)	(0.1)	(0.1)	(0.7)	(3.8)

#, significantly different from R-enantiomer
*, significantly different from 2 mg/L
a, significantly different from control

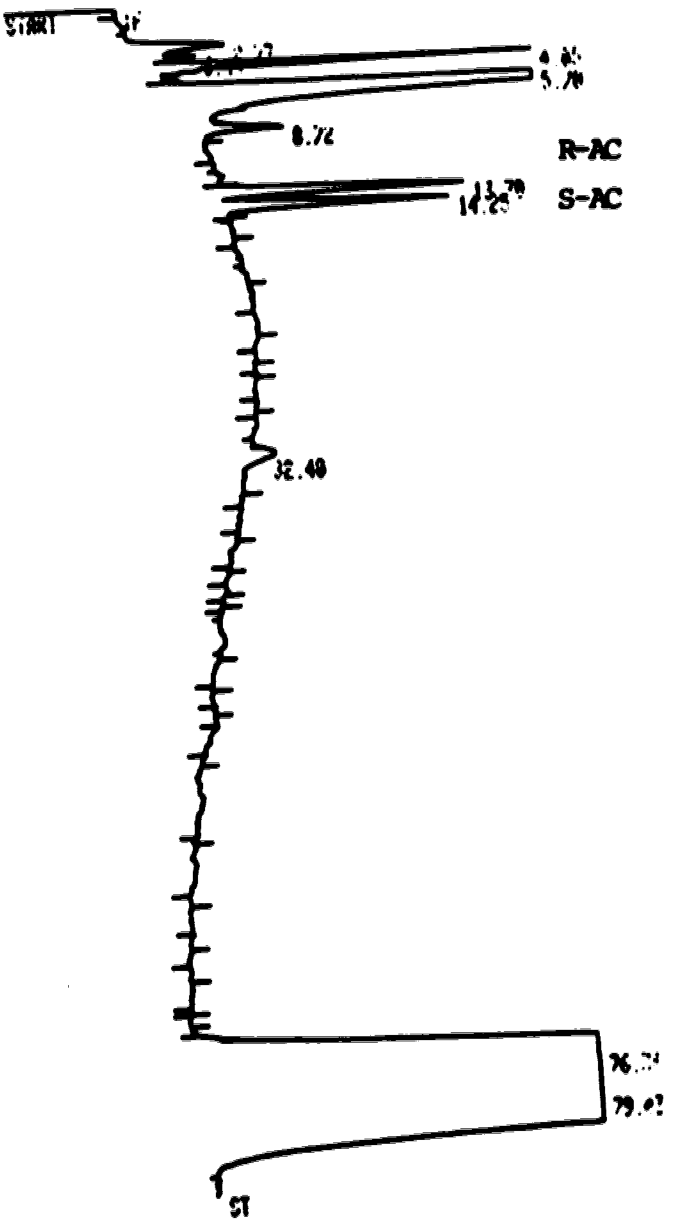

Figure 3-1 Chromatogram of plasma spiked with 50 ng/mL of ± AC using reversed-phase HPLC separation.

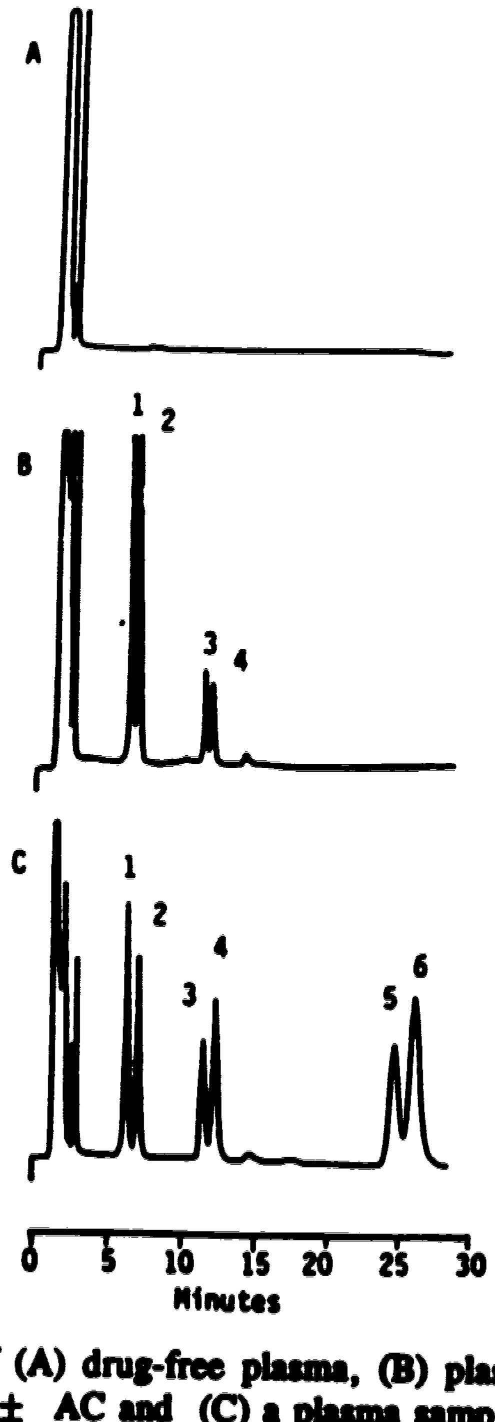

Figure 3-2 Chromatograms of (A) drug-free plasma, (B) plasma spiked with 20 ng/mL of ± AC and (C) a plasma sample taken 12 h following oral administration of a single 200 mg racemic dose of AC to a healthy subject. Peaks: 1 and 2 = internal standard; 3 and 4 = R and S-AC; 5 and 6 = R- and S-diacetolol (DC) diastereoisomers, respectively.

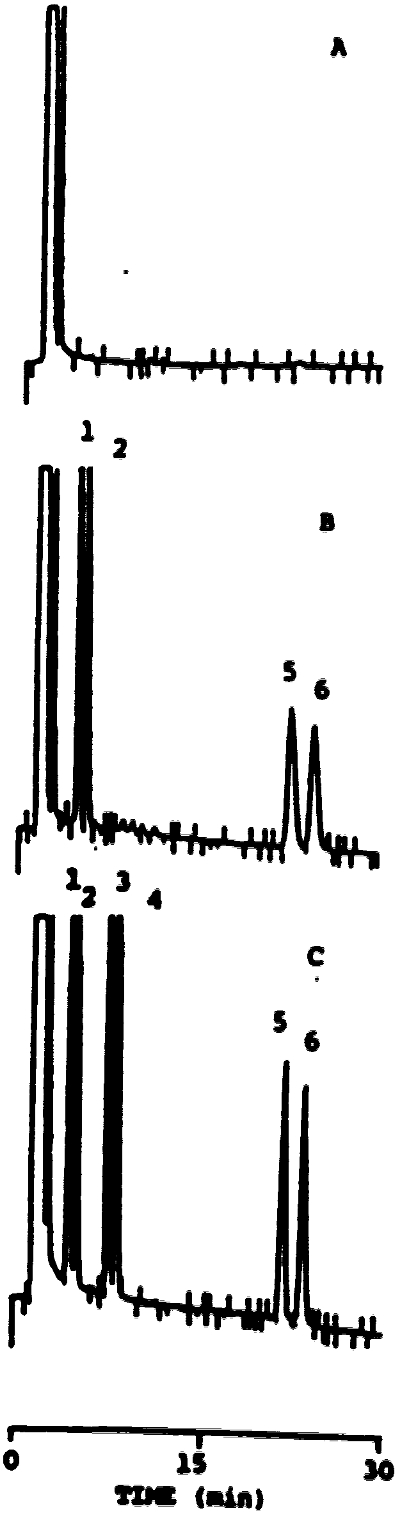

Figure 3-3 Chromatograms of (A) blank plasma, (B) plasma spiked with 100 ng/mL of ± DC and (C) plasma spiked with 100 ng/mL of ± AC and ± DC. Peaks: 1 and 2 = internal standard; 3 and 4 = R and S-AC; 5 and 6 = R- and S-DC diastereomers.

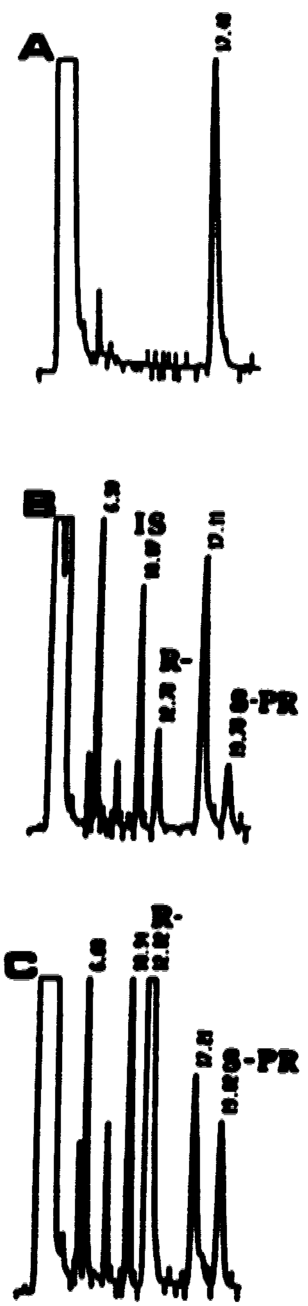

Figure 3-4 HPLC chromatograms of (A) drug-free plasma, (B) plasma spiked with 500 ng/mL of ± PR, and (C) a plasma sample taken 4h following oral administration of (±) PR (30 mg/kg) in an AA rat. Peaks at times 6.6 and 10.9 min, internal standard; at 12.7 min, R-PR; and at 19.8 min, S-PR diastereomers.

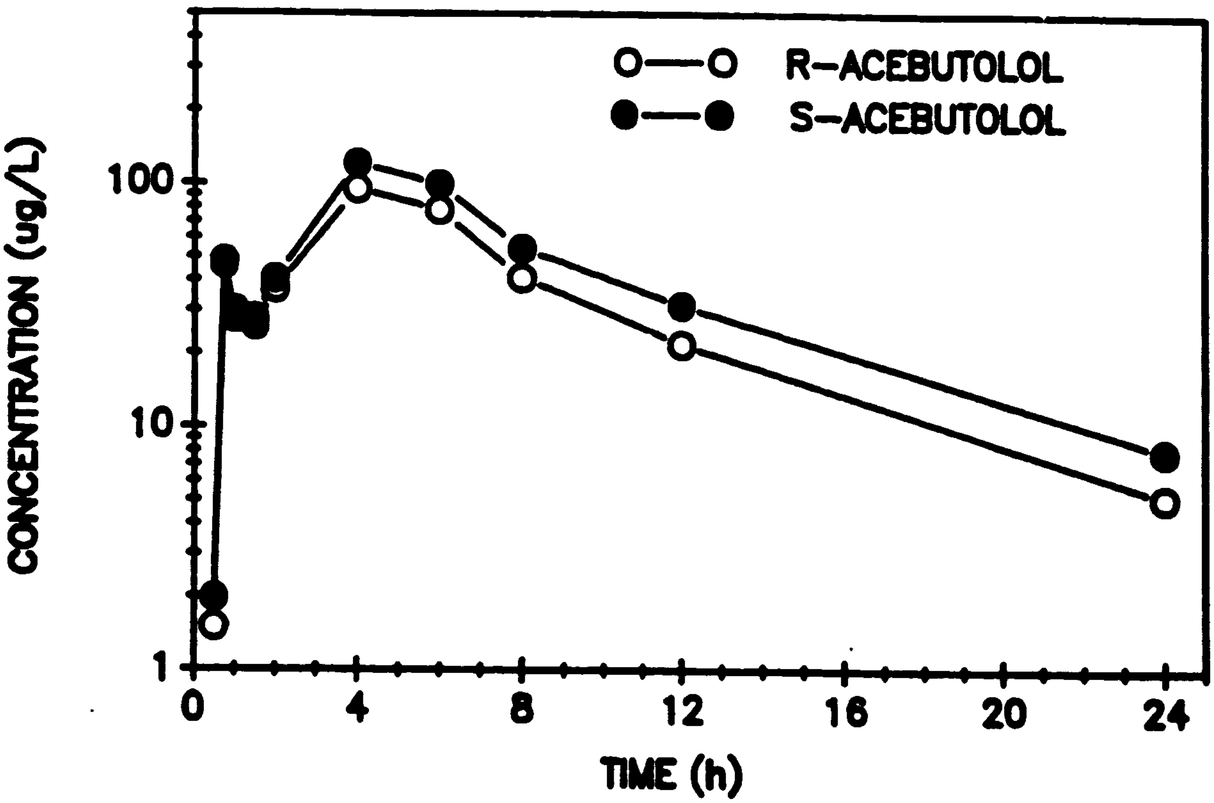

Figure 3-5 Plasma concentration versus time profiles for R and S-AC in a representative young healthy subject after a single 200 mg oral dose of AC (O) R-AC; (●) S-AC.

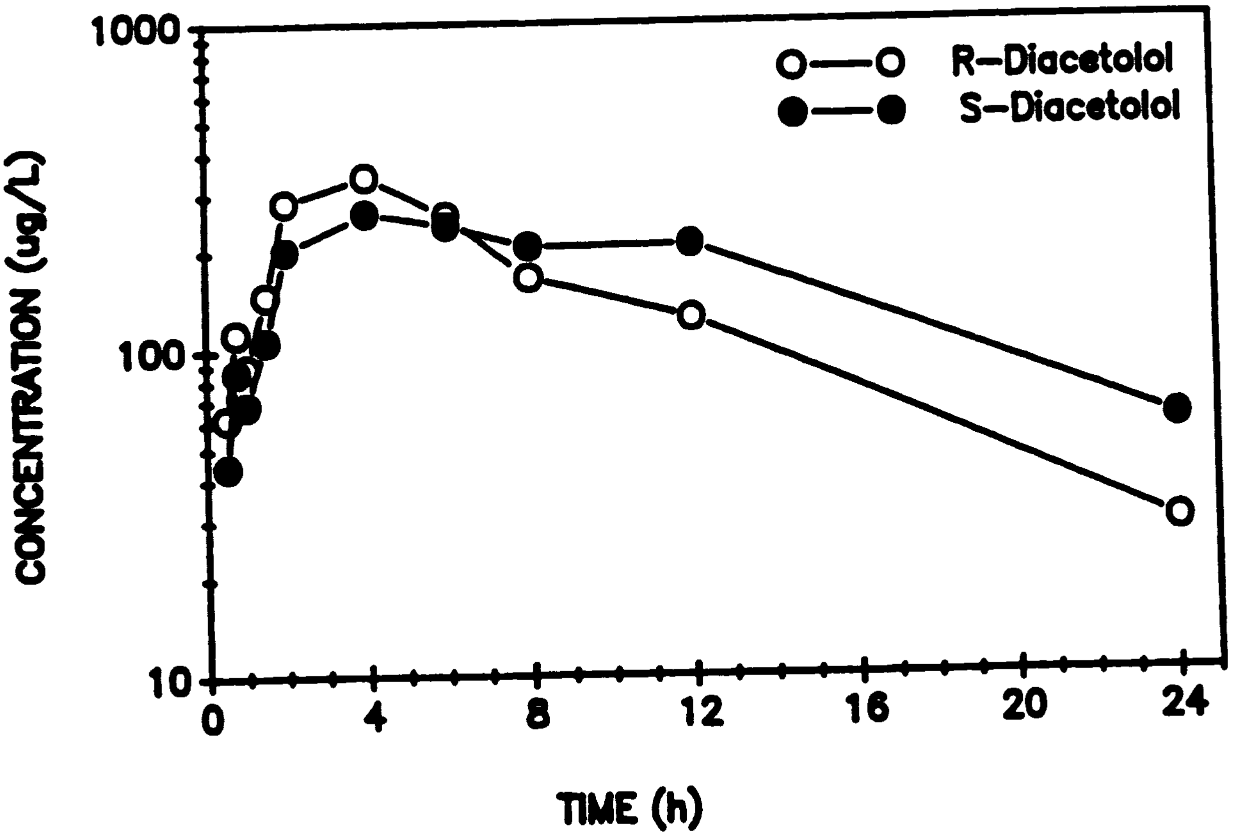

Figure 3-6 Plasma concentration versus time profiles for R and S-DC in a representative young healthy subject after a single 200 mg oral dose of AC. (○) R-DC; (●) S-DC.

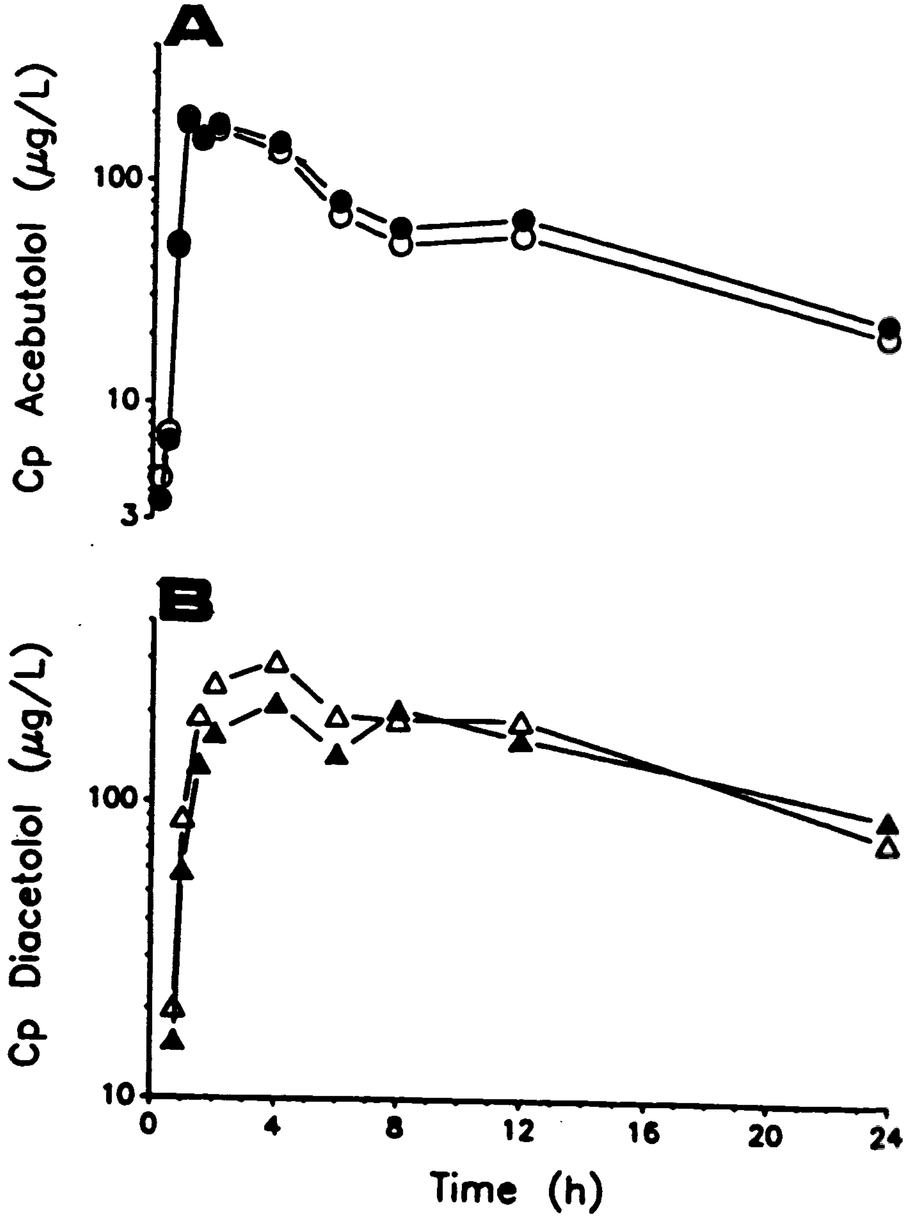

Figure 3-7 Plasma concentration versus time profiles for (A) R- and S-AC and (B) R-and S-DC in a representative aged healthy subject after a single 200 mg oral dose of AC.
(○) R-AC; (●) S-AC; (△) R- DC; (▲) S-DC.

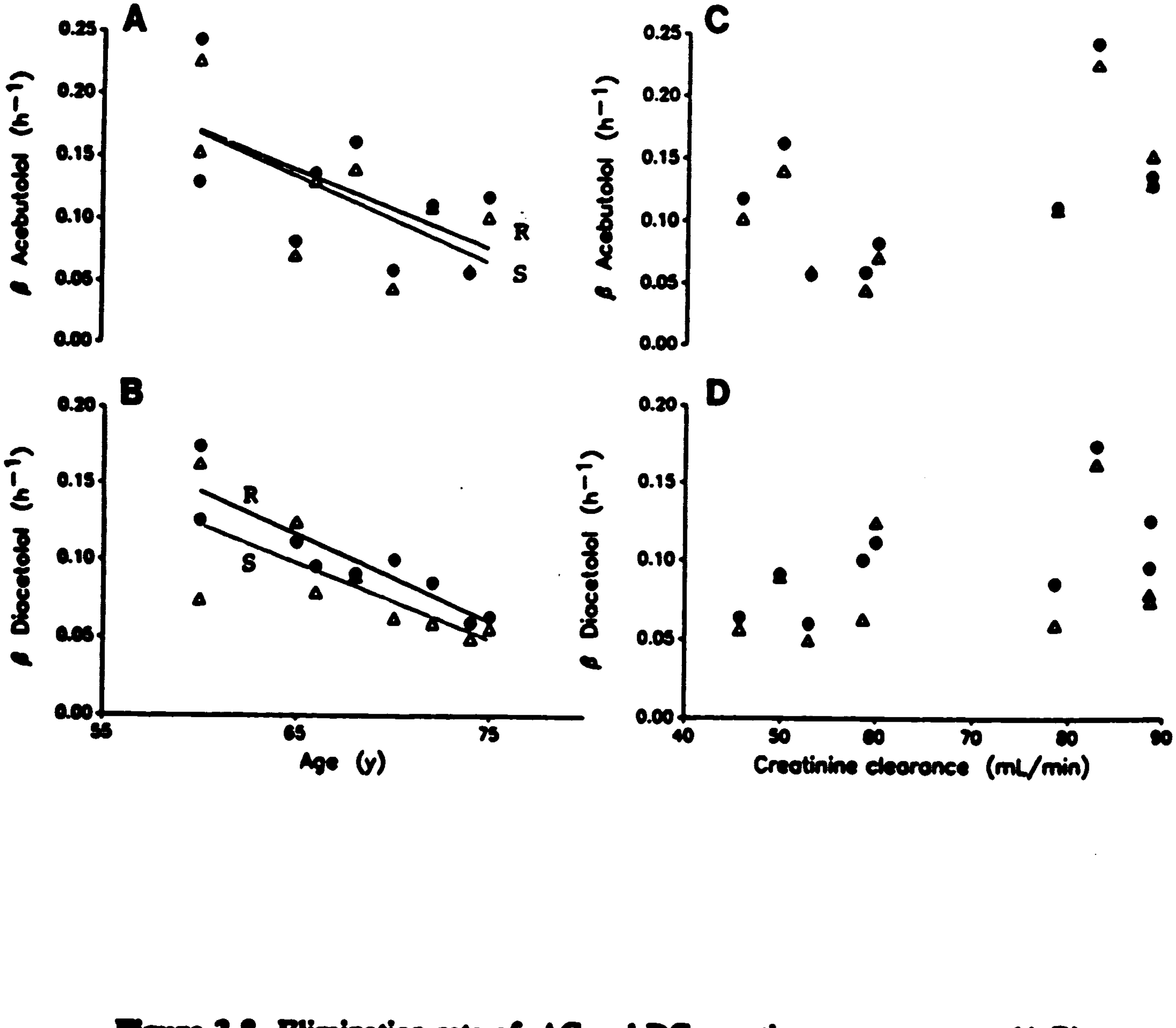

Figure 3-8 Elimination rate of AC and DC enantiomers versus age (A,B) or creatinine clearance (C,D). (R= ●- ; S = ▲-) .

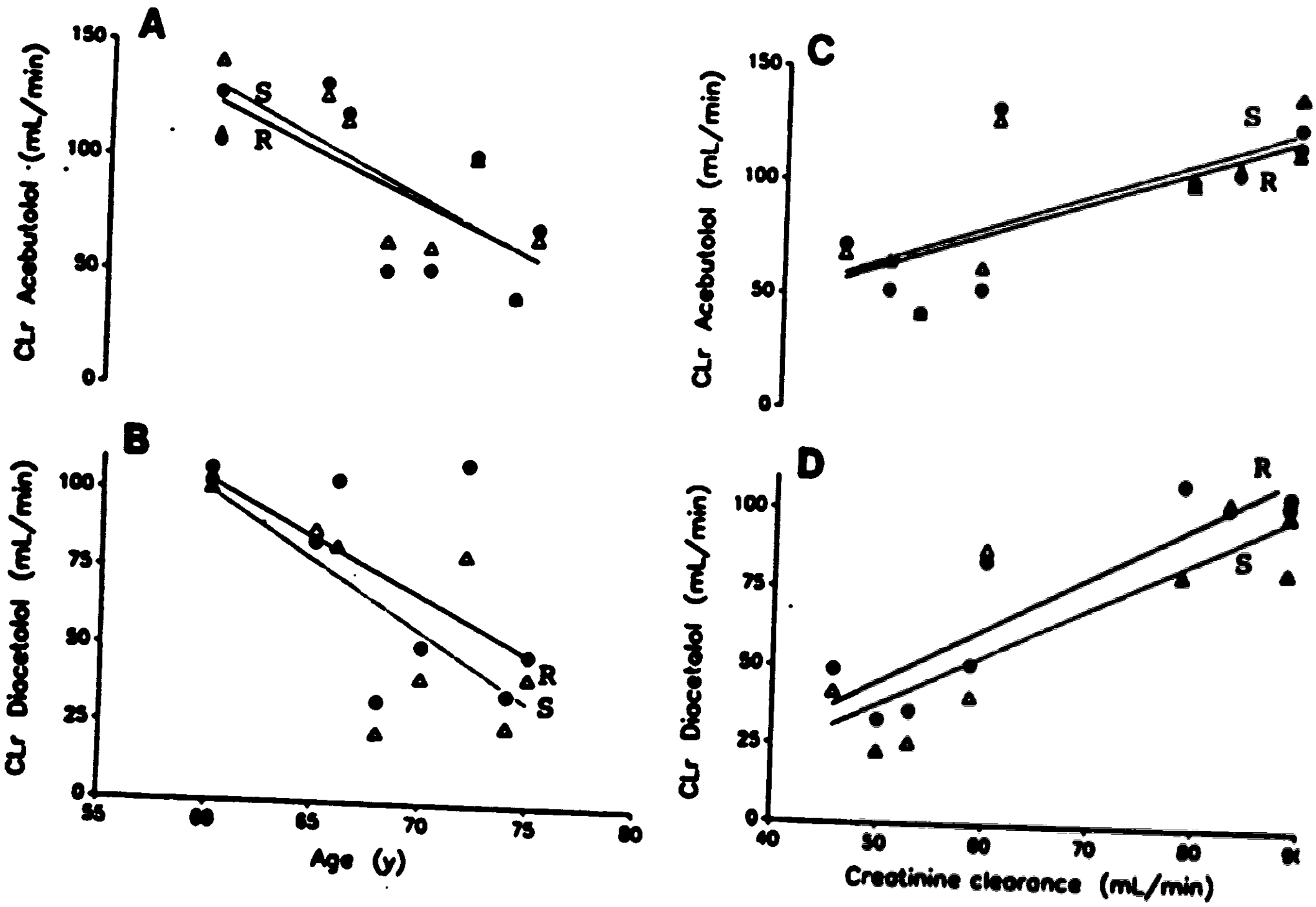

Figure 3-9 Renal clearance (CLr) of AC and DC enantimers versus age (A,B) or creatinine clearance. (R= ●- ; S = ▲-).

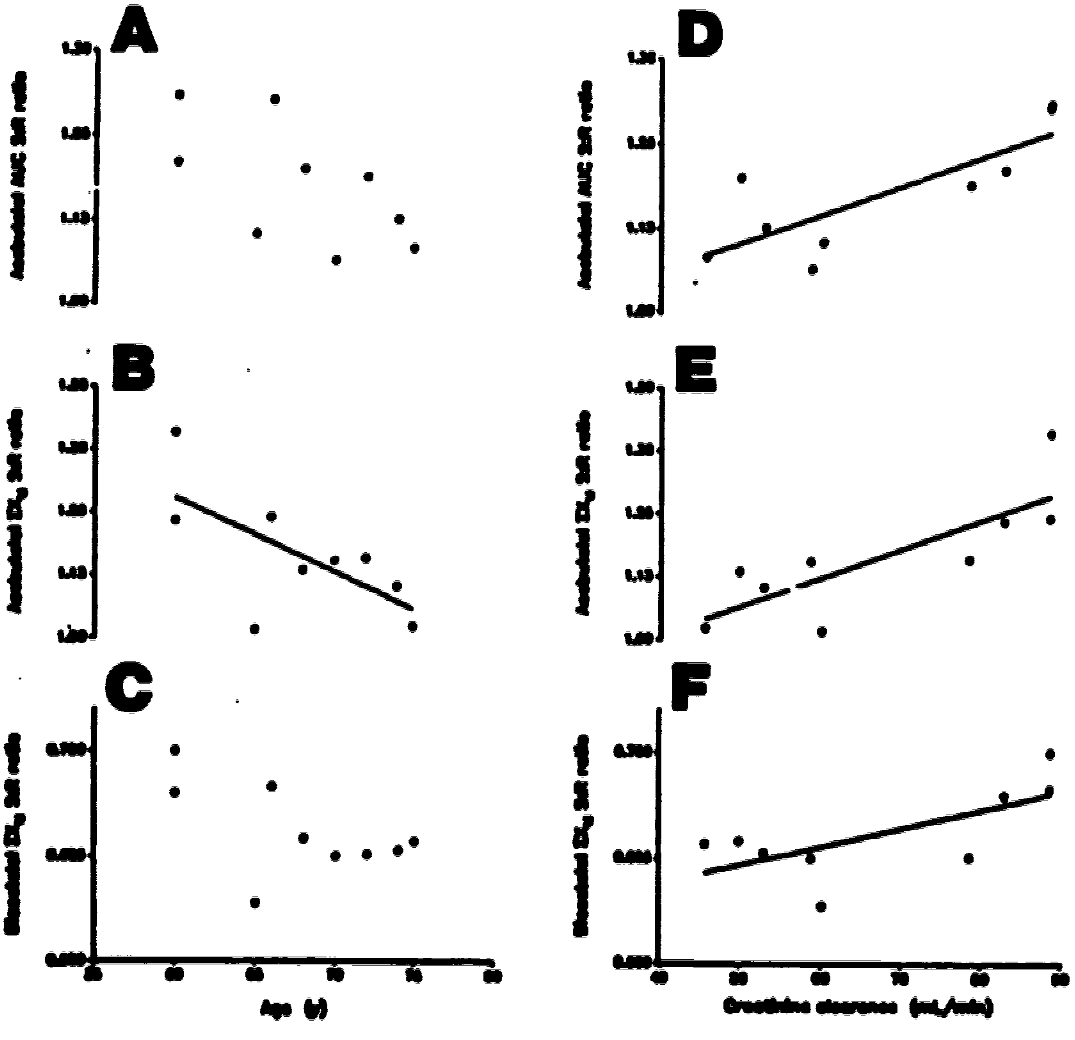

Figure 3-10 Plasma AUC S:R ratio of AC enantiomers versus age (A) or creatinine clearance (D). ΣXu S:R ratio of AC and DC enantiomers versus age (B,C) or creatinine clearance (E,F).

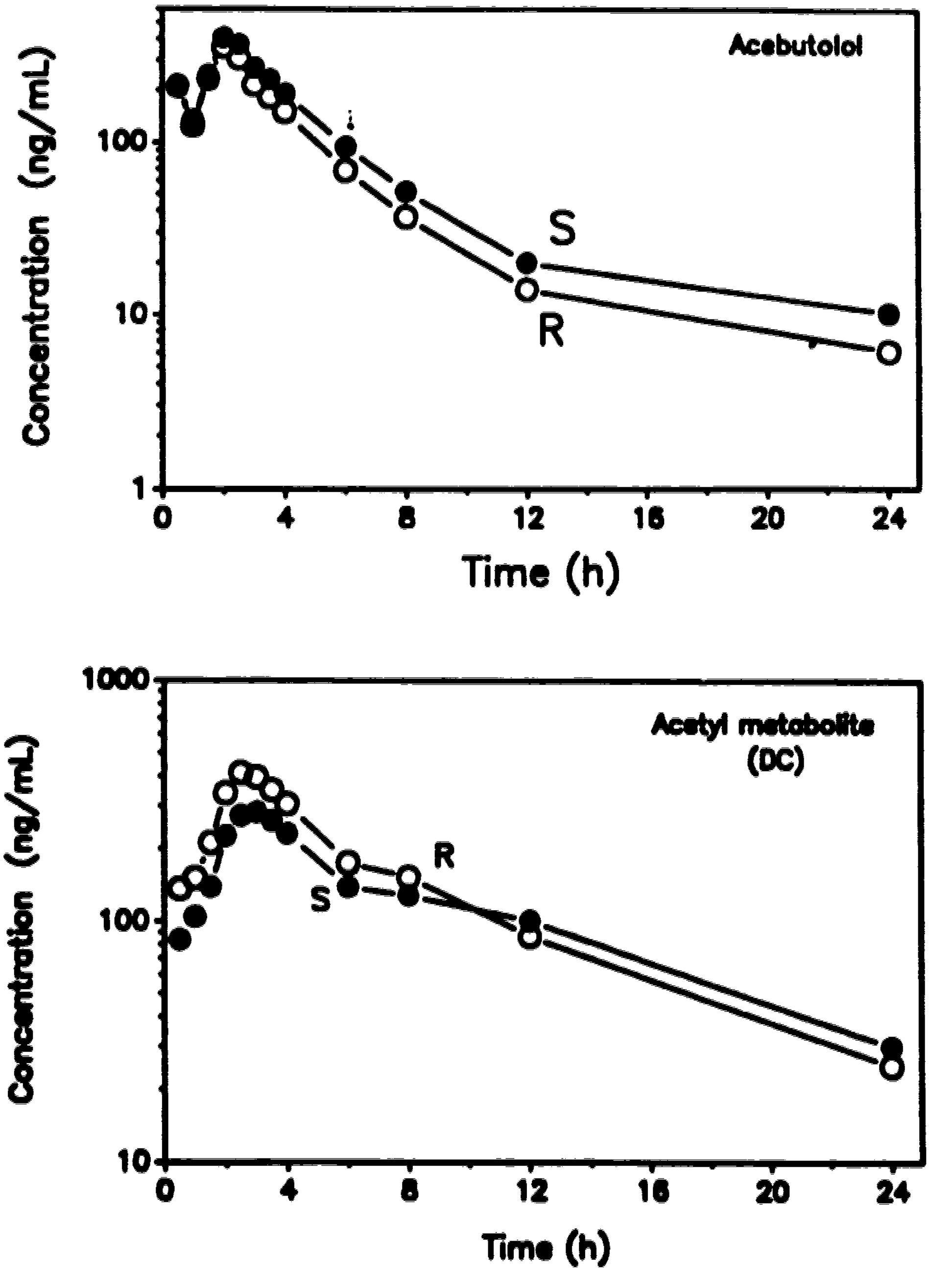

Figure 3-11 Plasma concentration versus time profiles for (A) R- and S-AC and (B) R-and S-DC in a representative arthritic subject after a single 200 mg oral dose of AC. (○) R-AC; (●) S-AC; (○) R- DC; (●) S-DC.

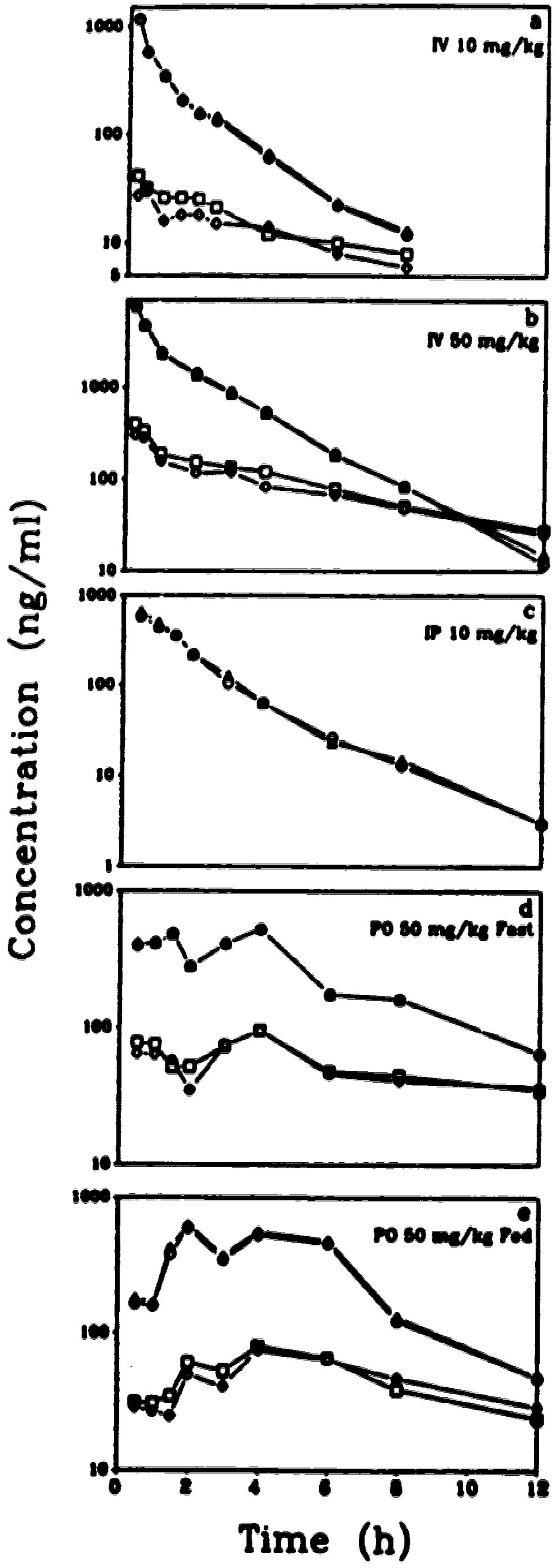

Figure 3-12 Plasma concentrations of R- AC (○) and S- AC (●) and acetylated metabolite (R-DC, □; S-DC, ◇) in representative rats after administration of ± AC

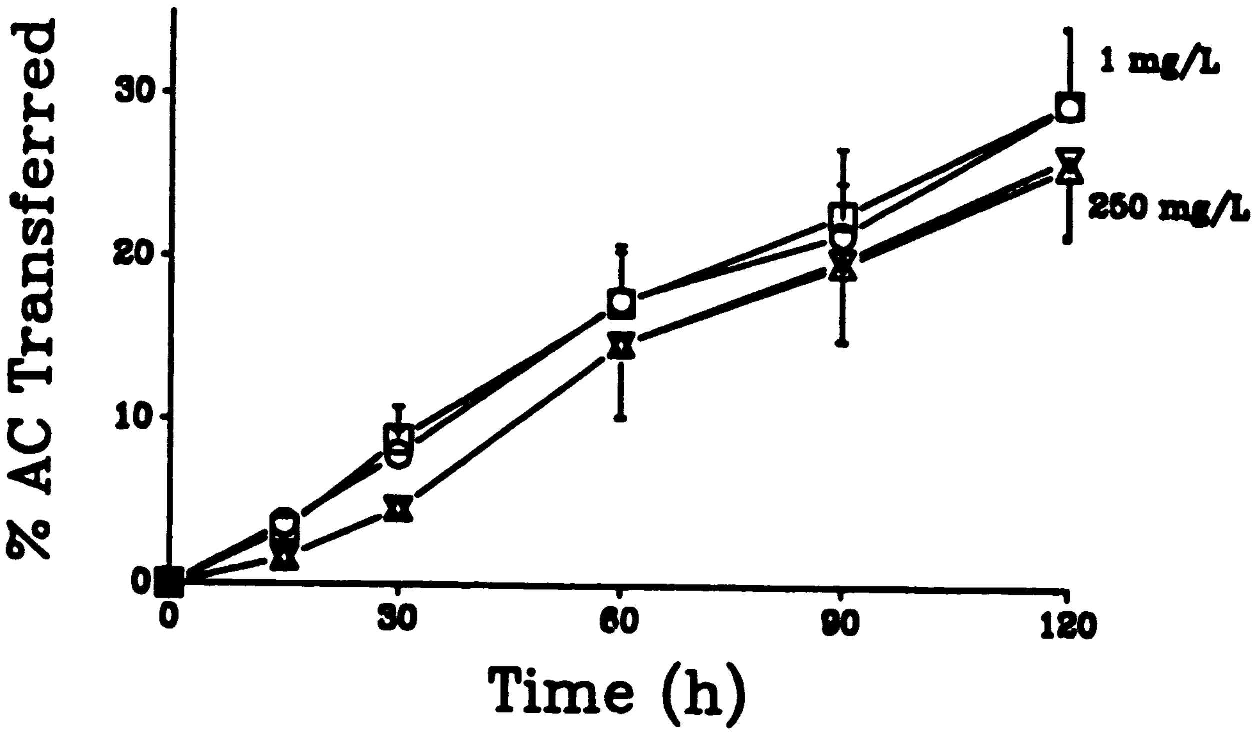

Figure 3-13 Percent of AC transferred into the intestinal sac (% serosal/mucosal concentration) after incubation with 1 mg/L (R, ○; S,□) or 250 mg/L (R, ▽; S, ▲) racemic AC (mean±SD, n=10).

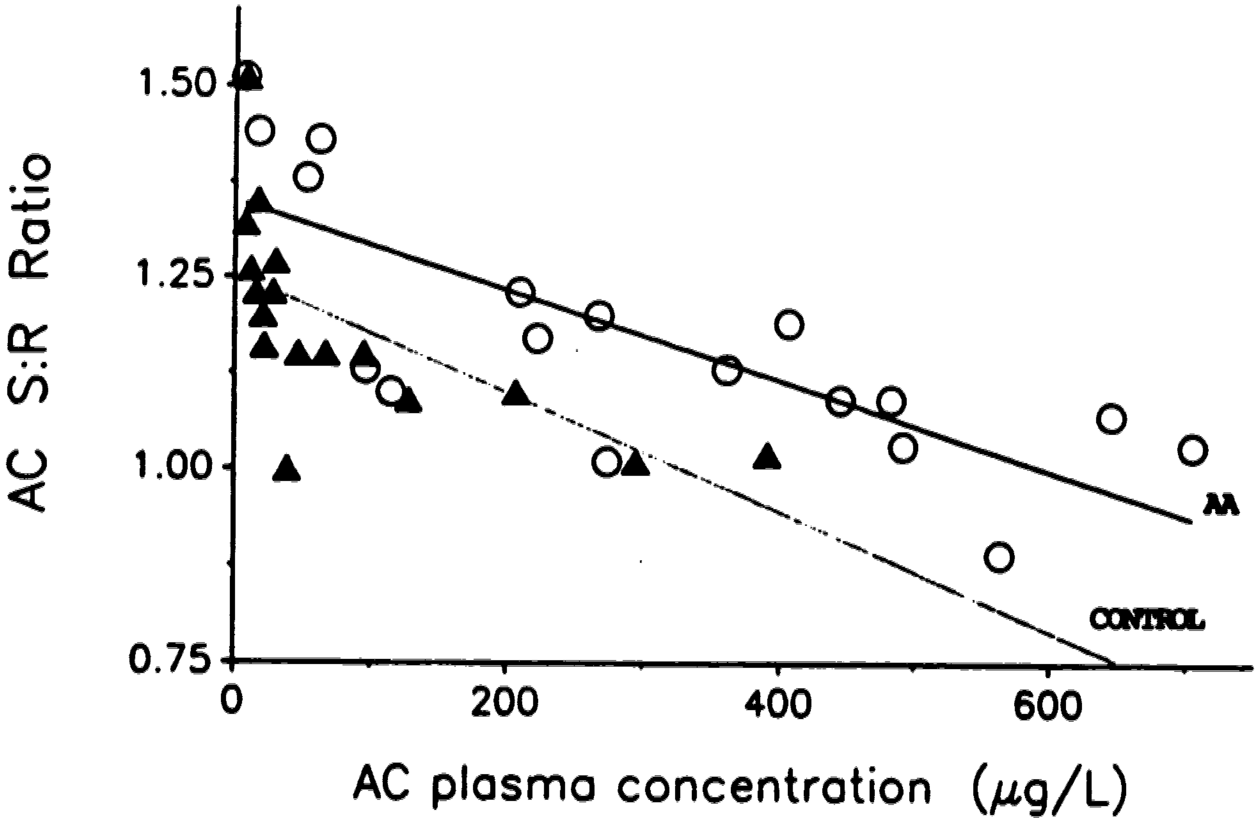

Figure 3-14 **S:R concentration ratio versus concentration of R-AC. AA (○); control (▲) during 0.5-2 h period.**

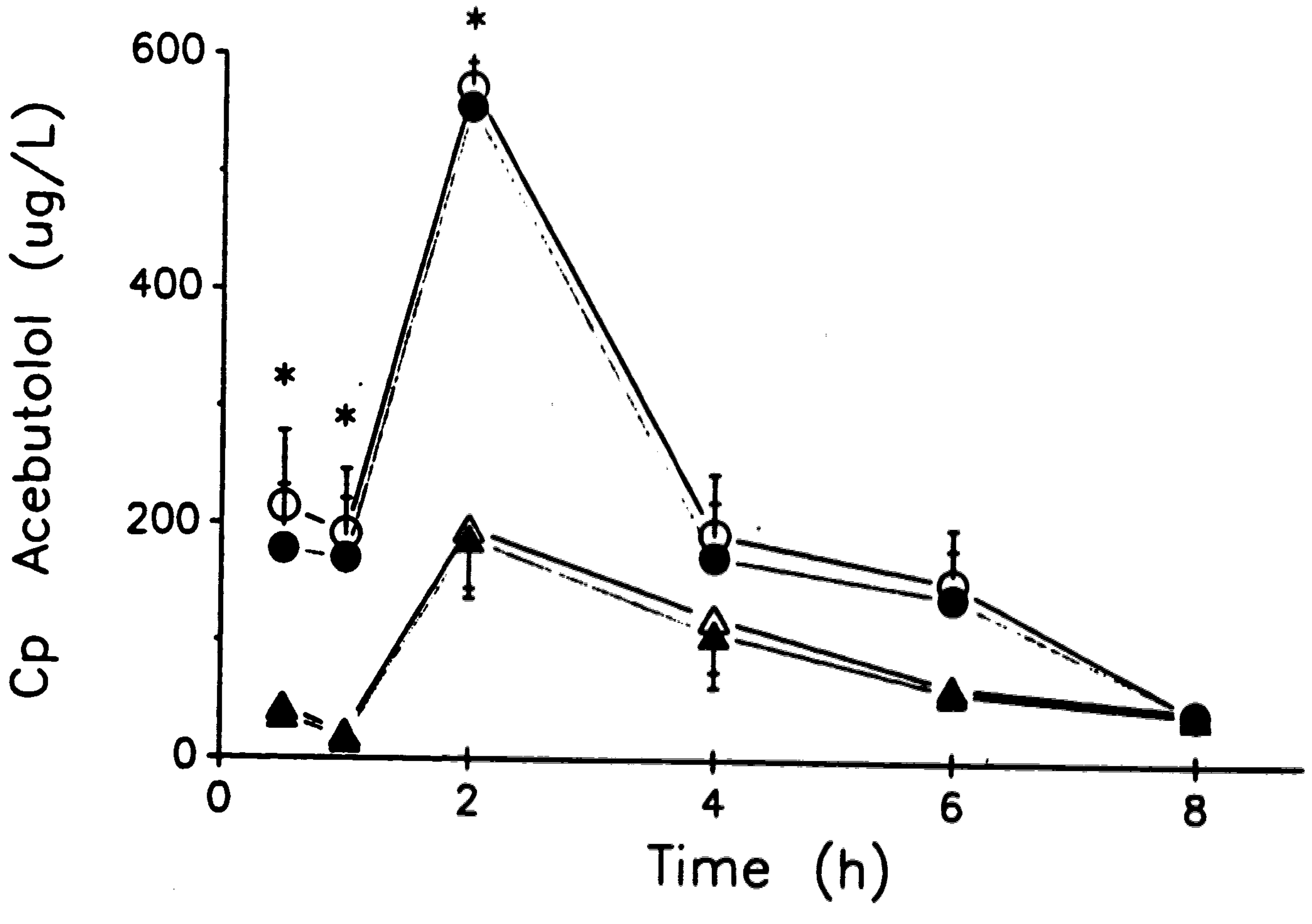

Figure 3-15 **Mean plasma concentration versus time curves for AC enantiomers in AA and controls. AA: R-AC (●); S-AC (O), CONTROL: R-AC (▲); S-AC (▲). Significantly different from controls (*).**

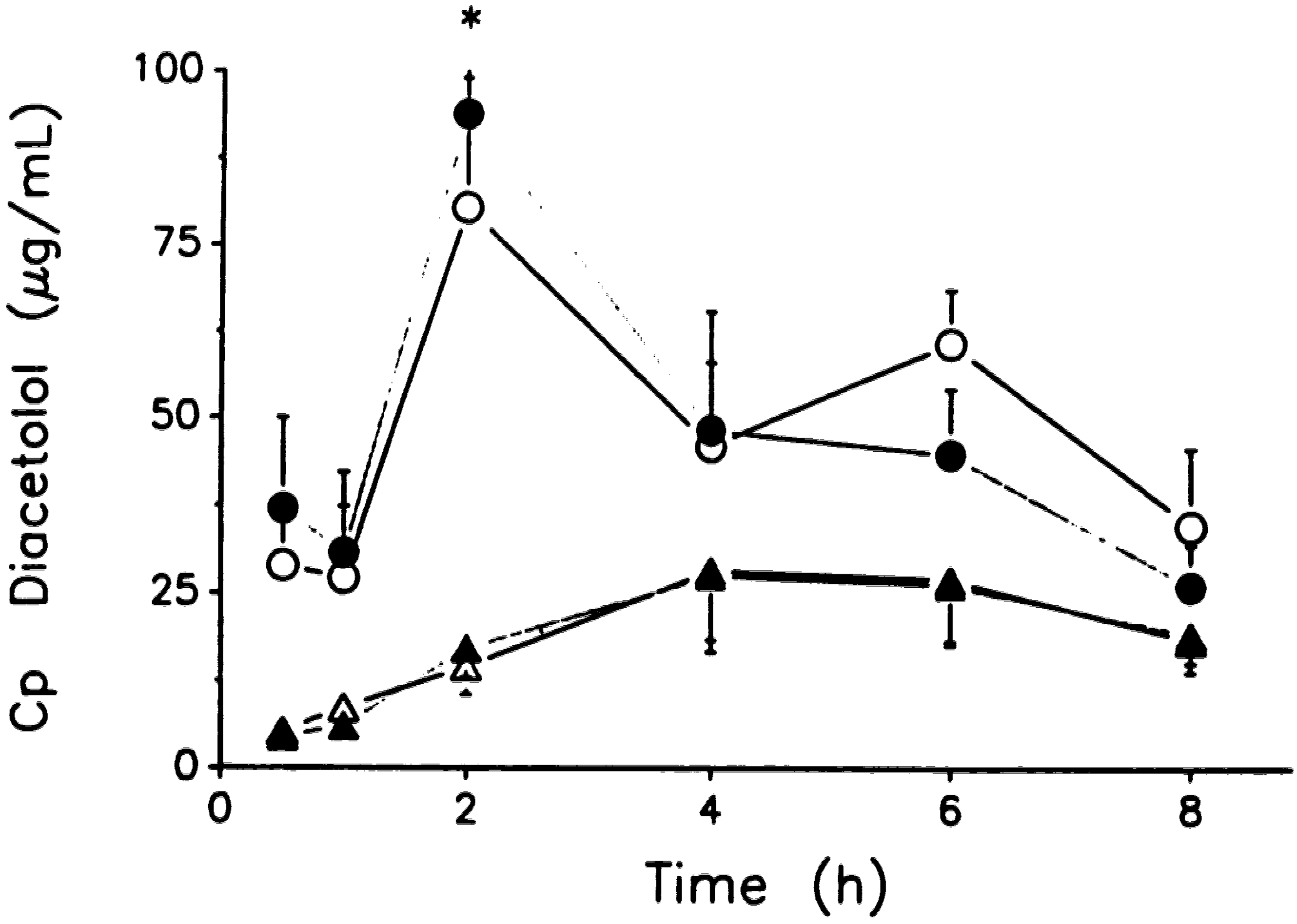

Figure 3-16 Mean plasma concentration versus time curves for DC enantiomers in AA and controls. AA: R-DC (●); S-DC (○), CONTROL: R-DC (▲); S-DC (△). Significantly different from controls (*).

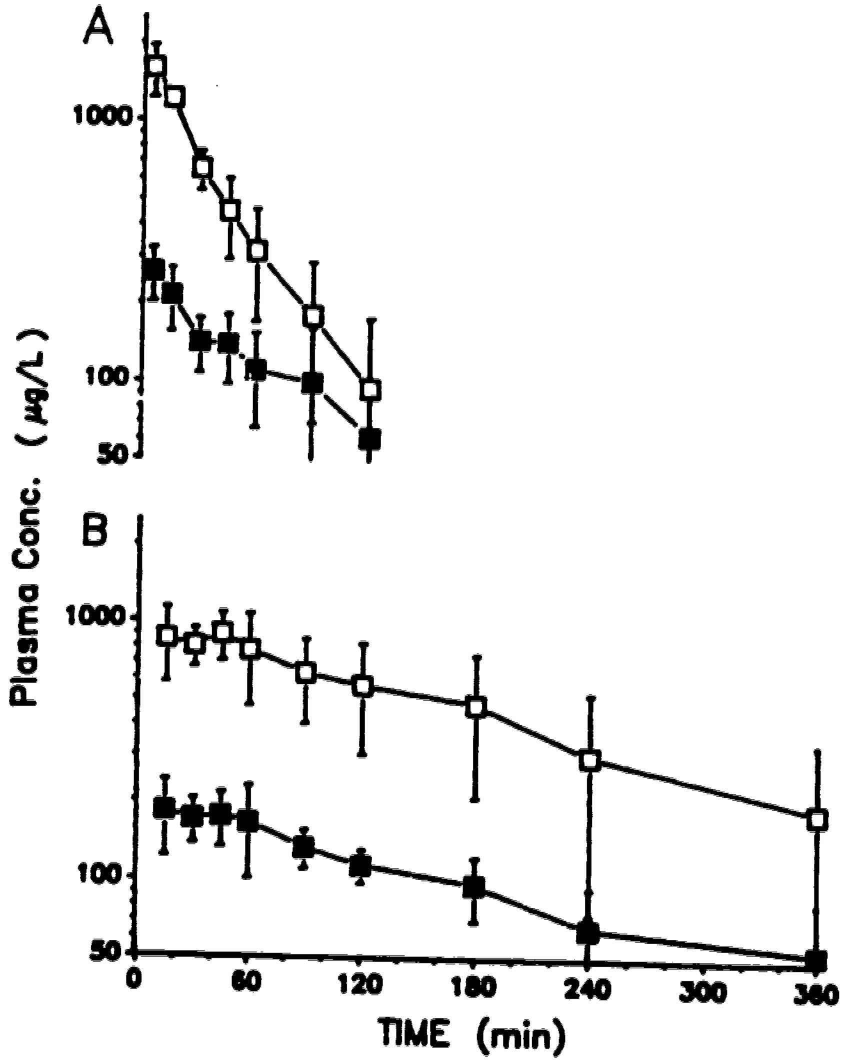

Figure 3-17 Mean plasma concentration-versus-time curves in control rats (n=6) after (A) iv and (B) oral doses of ± propranolol (PR) (R-PR □; S-PR ■).

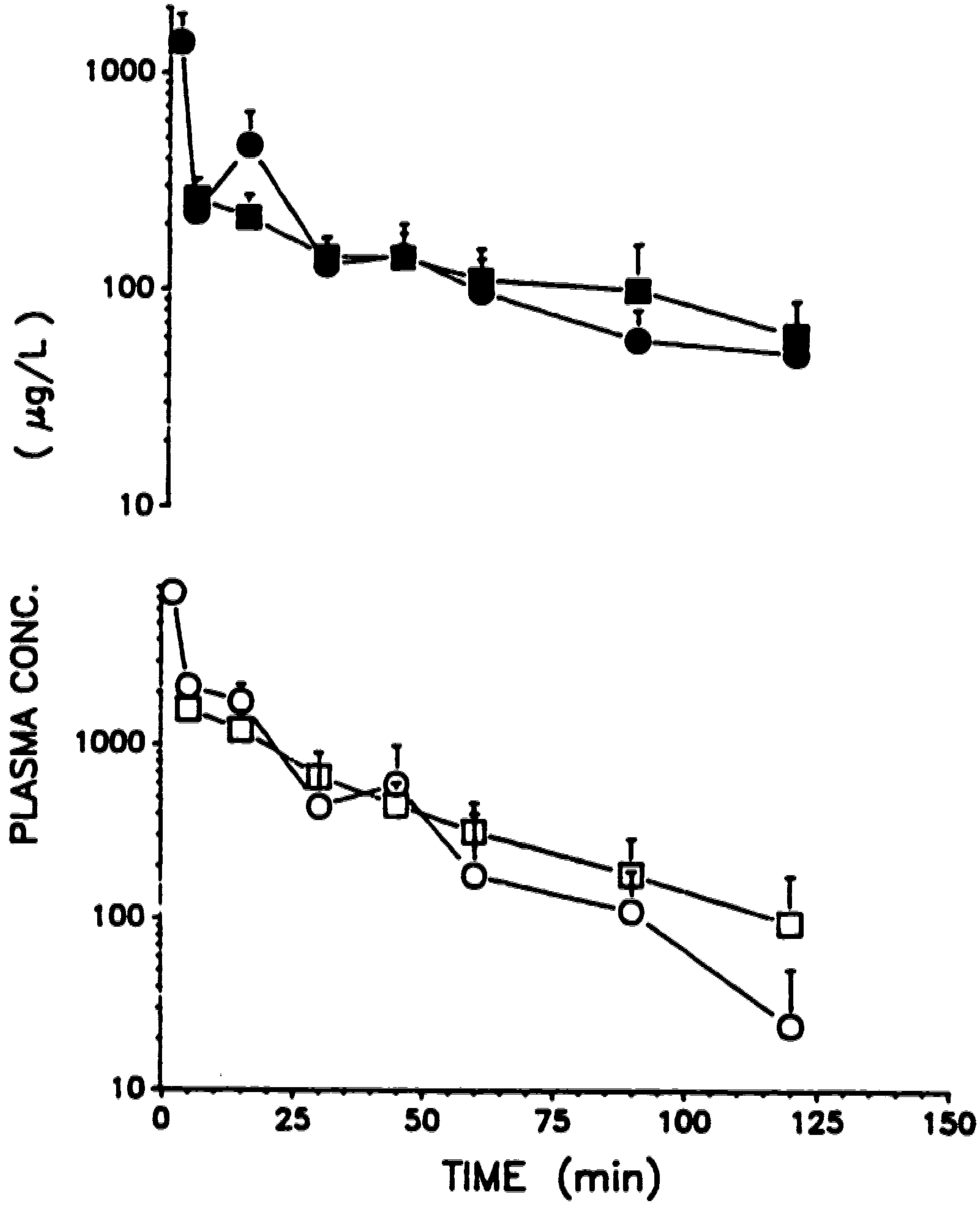

Figure 3-18 Mean plasma concentration-versus-time curves of (A) S-PR and (B) R-PR in arthritic (n=5) and control (n=6) rats after iv administration. AA: (R-PR, ○; S-PR, ●). Controls: (R-PR □; S-PR ■).

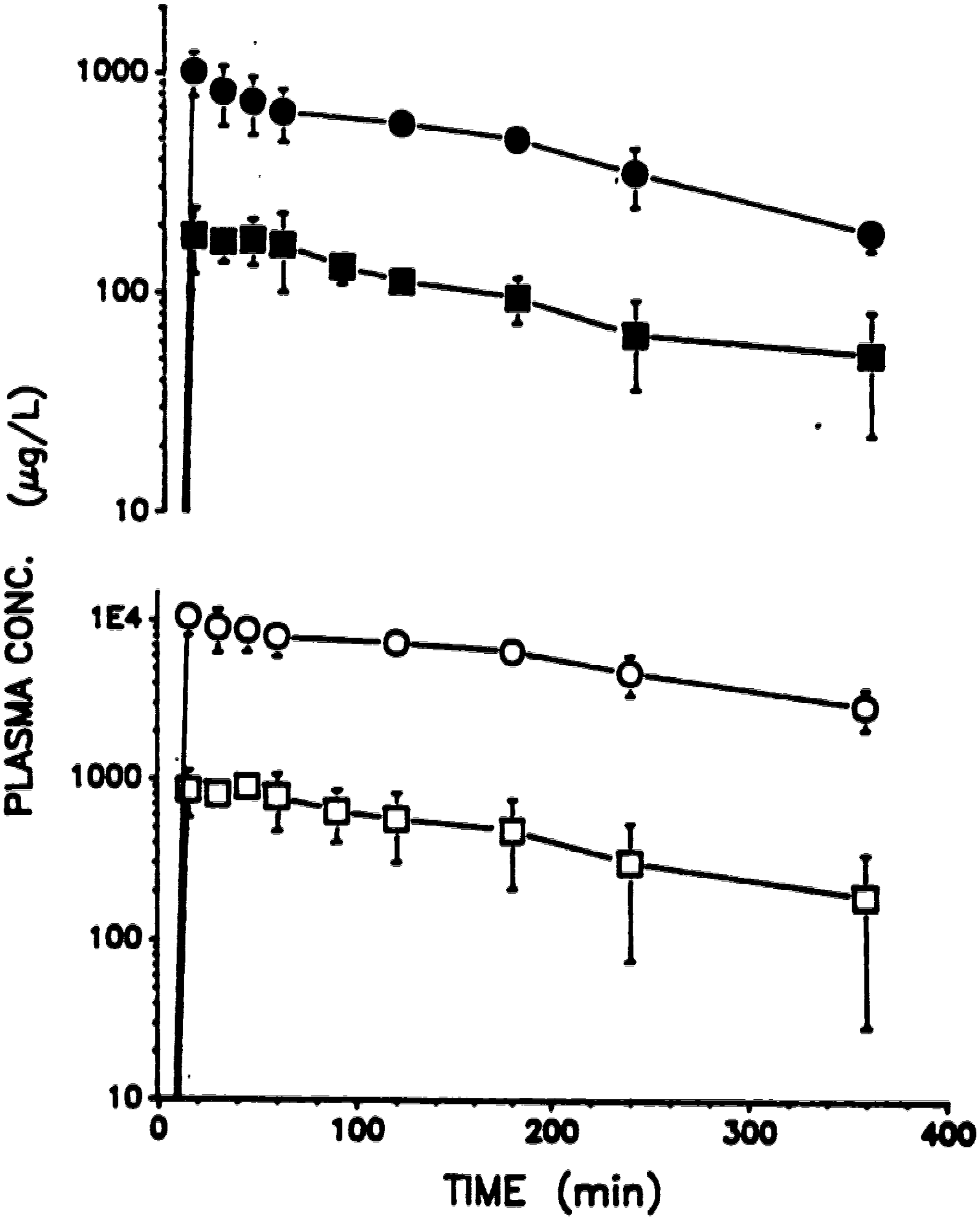

Figure 3-19 Mean plasma concentration-versus-time curves of (A) S-PR and (B) R-PR in arthritic (n=5) and control (n=6) rats after oral administration. AA: (R-PR, ○; S-PR, ●). Controls: (R-PR □; S-PR ■).

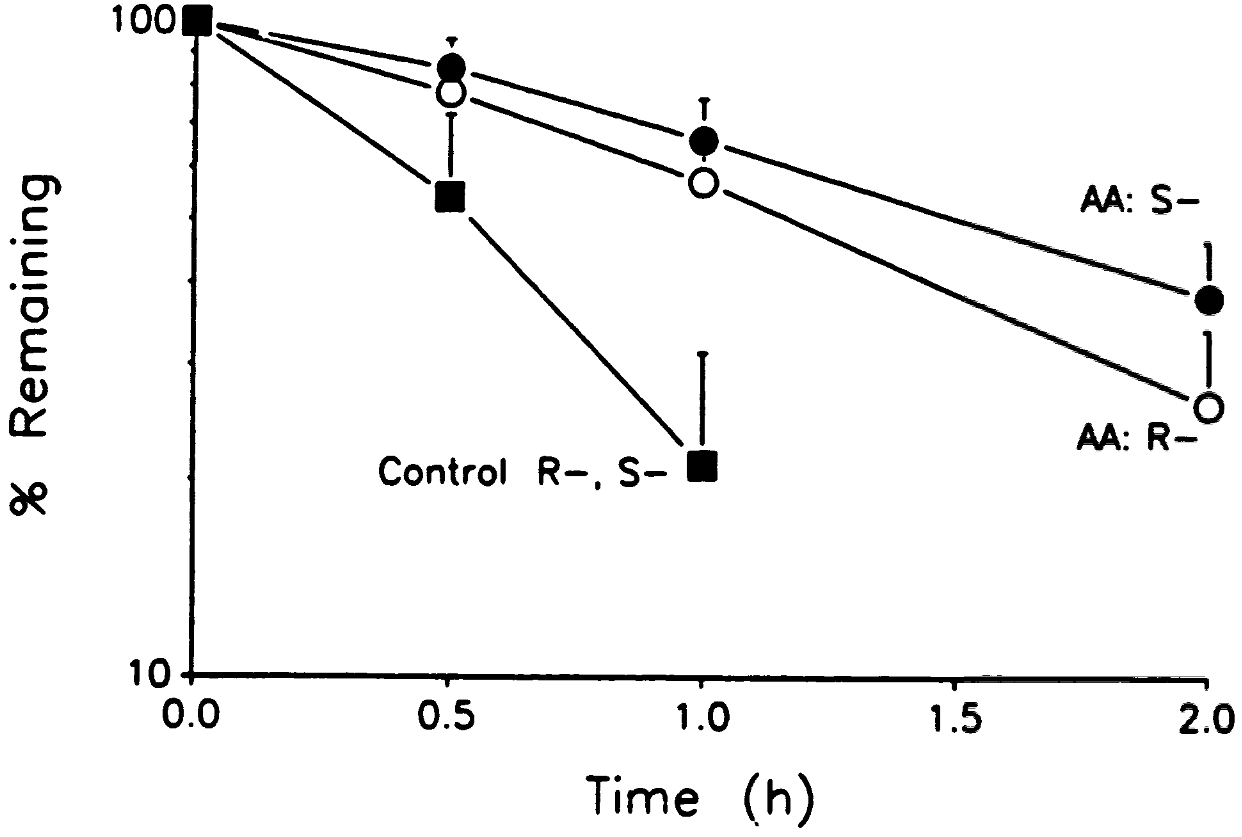

Figure 3-20 Mean percentage PR remaining versus time in microsomes from AA and controls (n=4) AA: (R-PR, ○; S-PR, ●). Controls: (■ R & S-PR).

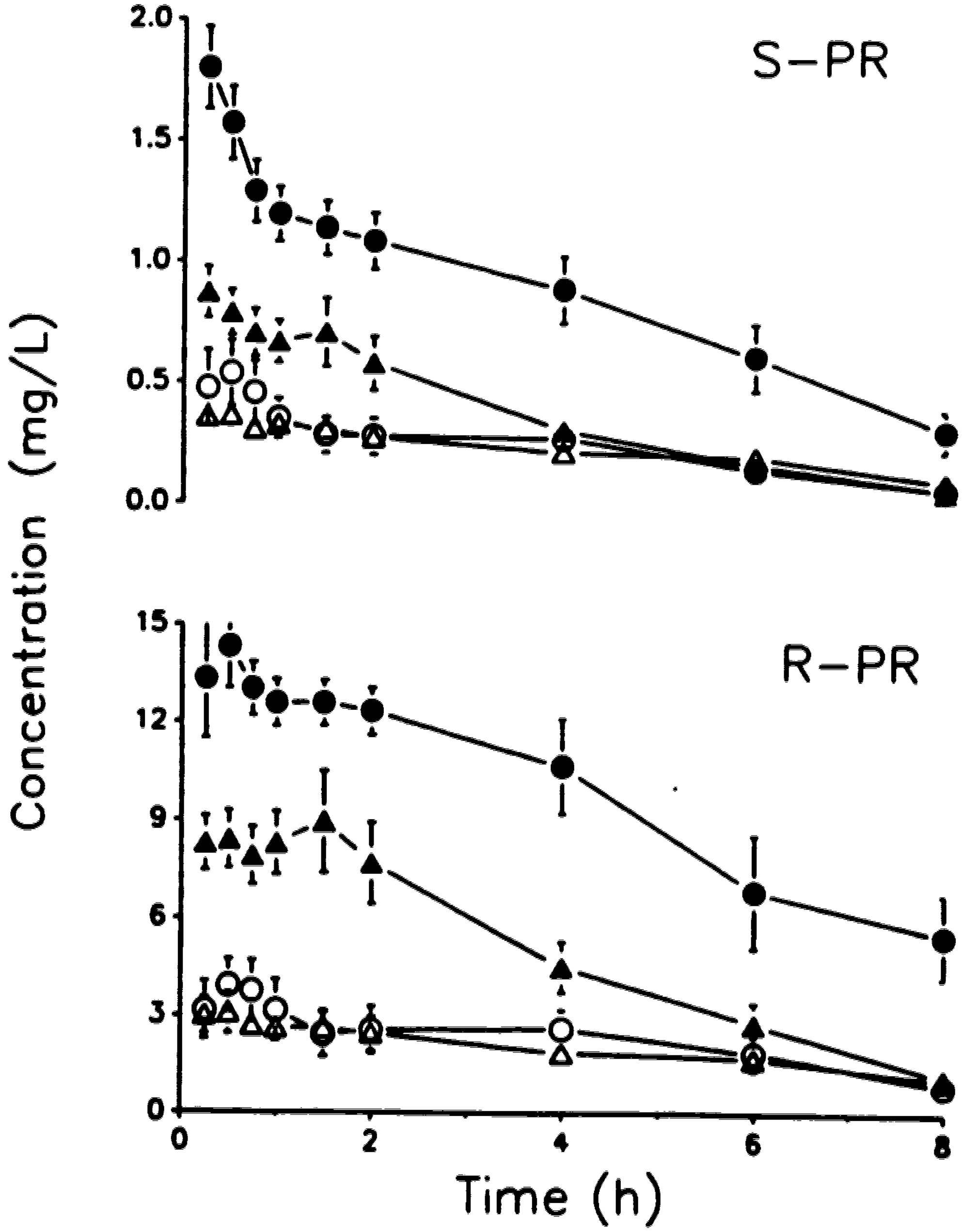

Figure 3-21 **Mean plasma concentration-versus-time curves of (A) S-PR and (B) R-PR in arthritic rats after oral administration. ($\text{SEVERE}_{\text{CONTROL}}$,● ; $\text{MILD}_{\text{CONTROL}}$, ○; $\text{SEVERE}_{\text{KT}}$, ▲; MILD_{KT}, △).**

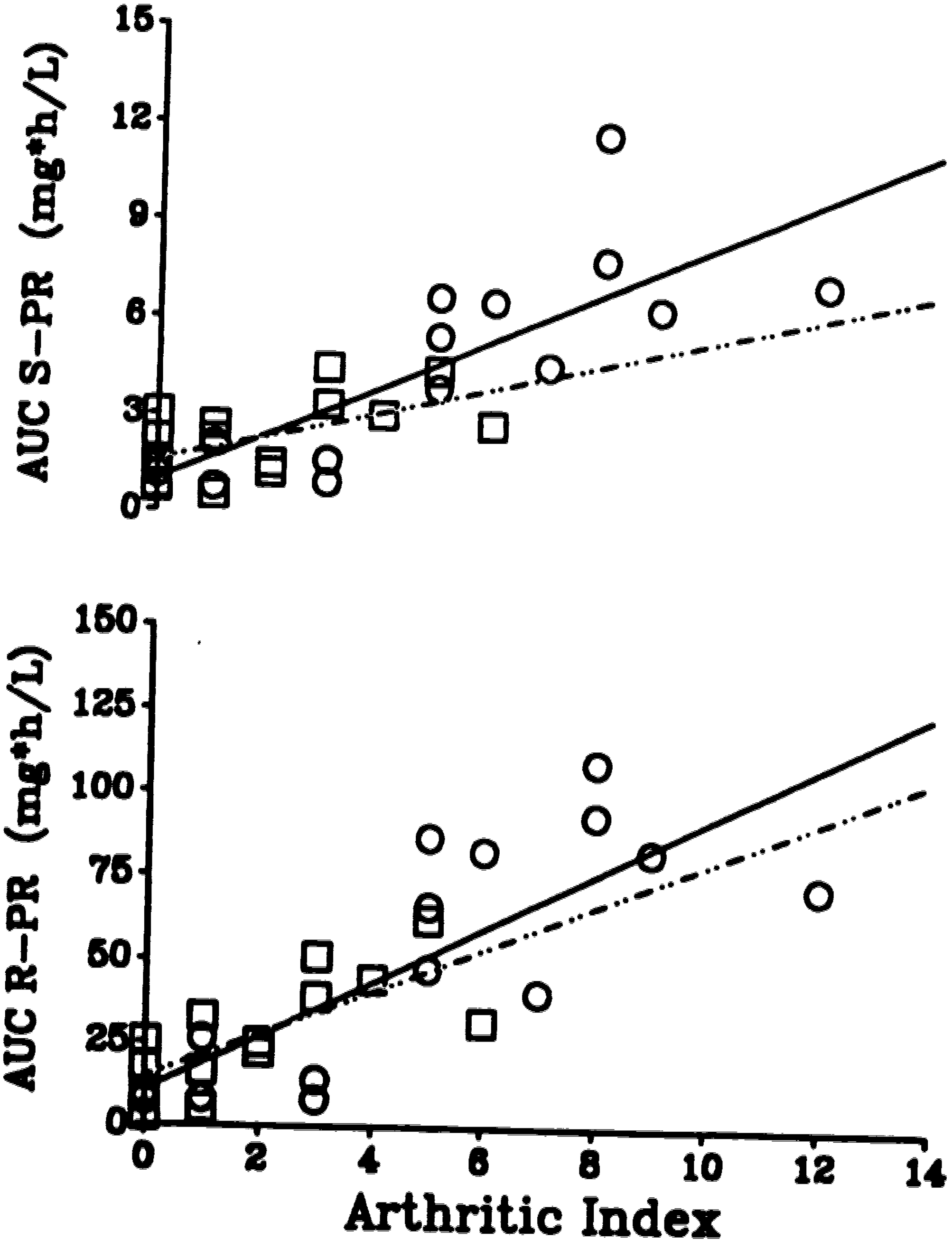

Figure 3-22 AUC of (A) S-PR and (B) R-PR versus arthritic index in ketoprofen treated (□) and control (○) arthritic rats.

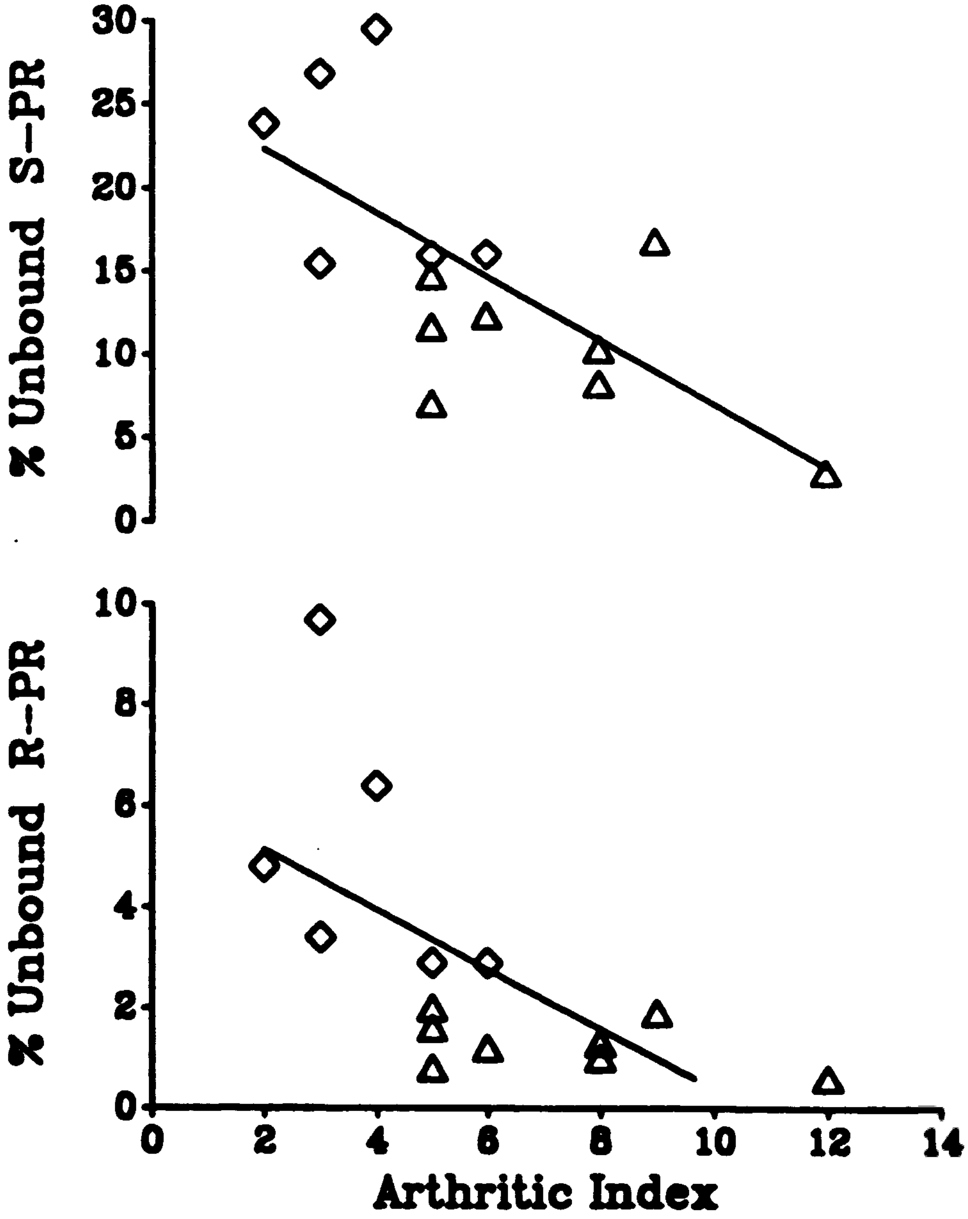

Figure 3-23 Fraction unbound (%) (A) S-PR and (B) R-PR versus arthritic index in ketoprofen treated (◇) and control (△) arthritic rats.

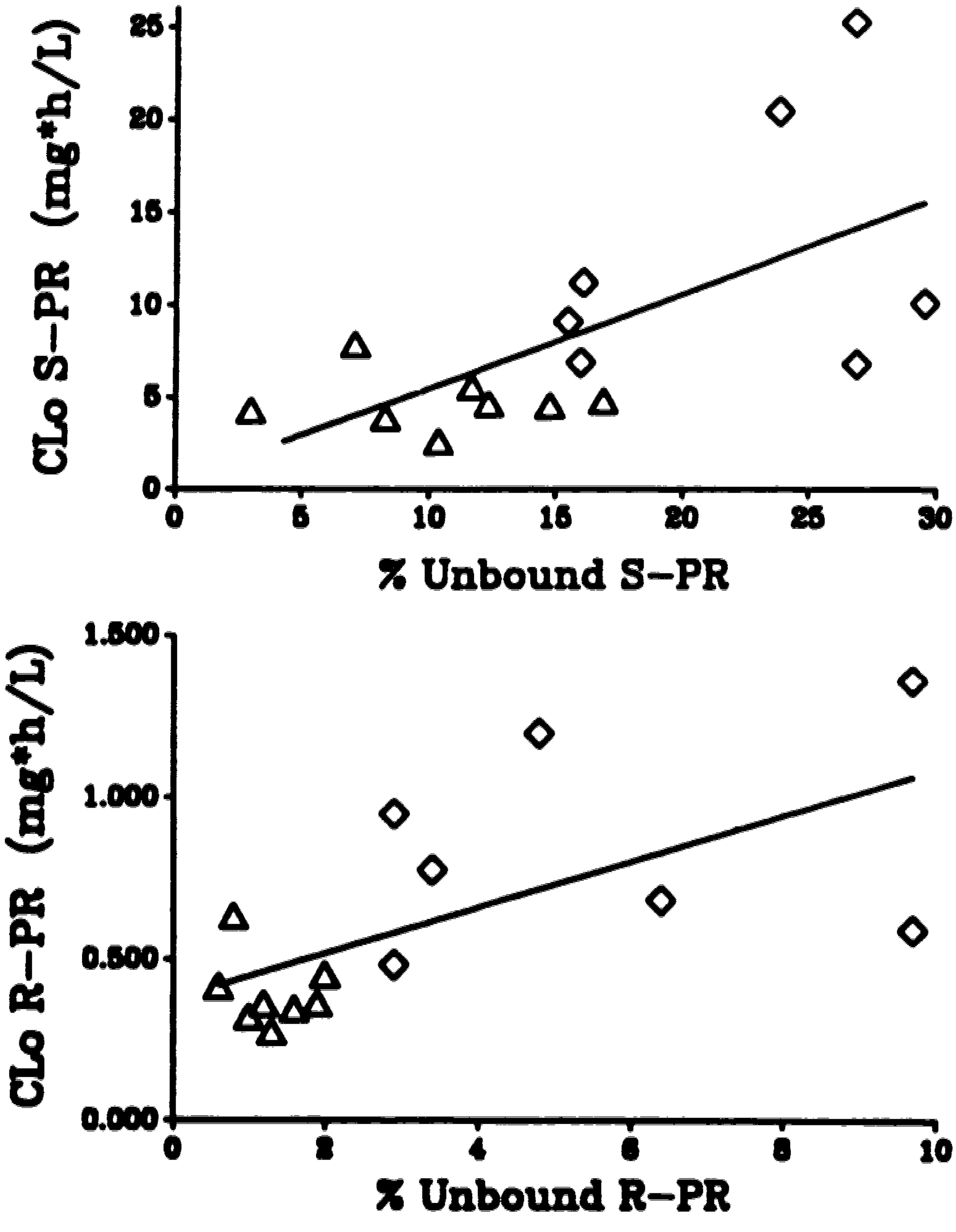

Figure 3-24 Oral clearance of (A) S-PR and (B) R-PR versus fraction unbound (%) in ketoprofen treated (◇) and control (△) arthritic rats.

4. DISCUSSION

4.1 HPLC Assays

4.1.1 Acebutolol

Chiral derivatization with subsequent separation of diastereoisomers has recently been reported for a number of β-adrenoceptor blocking drugs (Thompson *et al.*, 1982; Pflugmann *et al.*, 1987; Caccia *et al.*, 1978; Hsyu & Giacomini, 1986). Either enantiopure S-(-)- or R-(+)-1-phenylethyl isocyanate have been used for separation and determination of some of these compounds. Without exception, the resultant diastereoisomers have been the urea, as opposed to the carbamate derivatives.

To date, the two reports of separation of acebutolol and its active metabolite, diacetolol, utilize either optically pure S-(-)-N-trifluoroacetylprolyl chloride (Sankey *et al.*, 1984) or R-(+)-phenylethyl isocyanate (Gulaid, 1985) as derivatizing reagents. The shortcomings of S-(-)-N-trifluoroacetylprolyl chloride, such as racemization, as well as contamination (up to 15%) with the (+)-enantiomer has been well documented (Thompson *et al.*, 1982; Gulaid *et al.*, 1985). Previous studies with S-(-)-phenylethyl isocyanate suggest that a somewhat lengthy reaction time is required (Pflugmann *et al.*, 1987). Moreover, these previously reported methods lacked sensitivity for measurement of therapeutic concentrations of acebutolol, despite the use of fluorescence detection (Gulaid *et al.*, 1985). We decided to use optically pure NEIC as an alternative to the phenylethyl isocyanates as fluorescence and hence, sensitivity, of the derivative might be augmented. An increased sensitivity has been reported by other investigators using R-(+)- or S-(-)-napthylethyl isocyanate (NEIC) for derivatization of enantiomers (Gubitz

& Mihellyes, 1984; Darmon & Thenot, 1986; Spahn *et al.*, 1988).

Derivatization of acebutolol and diacetolol with NEIC was found to be virtually complete within 10 min. Studies with other isocyanates have shown that derivatization of beta-blockers is rapid (Gourmel *et al.*, 1980; Schieffer, 1980; Gabriel *et al.*, 1980). Contrary to these findings, a previous study (Darmon & Thenot, 1986), which utilized NEIC for separation and determination of betaxolol enantiomers, found that incubation of samples at 37° C for 1.5 h was necessary.

Formation of both the urea and carbamate derivatives of a racemic drug results in multiple diastereomer derivatives, thus obscuring analysis of the enantiomer concentrations. However, identification of the two peaks corresponding to acebutolol enantiomers indicated that the urea, and not the carbamate, derivative was formed. Consistent with other reports (Thompson *et al.*, 1982; Gulaid *et al.*, 1985), it was noted that NEIC in large excess did not result in formation of the carbamate derivative. Similarly, increasing the reaction time of derivatization did not result in formation of the carbamate derivative. Based on this finding, it may be reasonable to suggest that similar compounds possessing the secondary amino group, β to the hydroxyl functional group, form urea derivatives.

Although high extraction yields of R and S-acebutolol were obtained, the diethyl ether extractions of diacetolol enantiomers from plasma and urine were only about 30%. It is unclear why such a low recovery of diacetolol was seen since an extraction of 65-75% with diethyl ether has been previously reported (Gulaid *et al.*, 1985). However, we did not feel that modification of the extraction method was necessary since 1) linear and reproducible responses were attained within the therapeutic concentration range; 2) extraction of acebutolol was greater than 80 % and 3) sensitivity of diacetolol enantiomers lower than 10 ng/ml was not required for pharmacokinetic analysis.

Our attempts to separate enantiomers of acebutolol using reversed-phase HPLC, although successful, resulted in an additional peak eluting as late as 90 min after injection (Fig. 3-1). The identity of this peak, however, is unknown but may be a dimer or trimer of NEIC. This limited the convenience of the assay under isocratic conditions. Moreover, if a large excess of NEIC was used, or if derivatization was conducted under aqueous conditions, this peak increased in size. The peak, however, could be diminished in size by 1) the addition of ethanolamine, as suggested by Pflugmann *et al.* (1987) for metoprolol and phenylethyl isocyanate to stop the reaction, or 2) by utilizing normal-phase HPLC. The first alternative was not feasible, as it did not entirely eliminate the peak. We, therefore, chose normal-phase HPLC as the better alternative, as the method was more convenient, run times were shorter and late eluting peaks were absent under these conditions. Furthermore, with normal-phase, an improved baseline separation of the diastereoisomer peaks corresponding to acebutolol and diacetolol was attained (Fig. 3-2, 3-3). Sensitivity and accuracy (Table 3-I, 3-II) were adequate for our pharmacokinetic studies. Therefore, this normal phase HPLC assay was found to be convenient for the simultaneous determination, of the enantiomers of acebutolol and its active metabolite, diacetolol.

Perhaps more importantly, this method appears to be a 'general' method for the determination of a number of compounds which possess a secondary amino moiety β to the hydroxy group. Derivatization with NEIC appears to have wide applicability as atenolol, nadolol, pindolol, propranolol, sotalol, toliprolol and tocainide enantiomers were successfully separated and detected. Only minor modifications to the mobile phase, excitation and emission wavelengths were required for detection of these compounds.

4.1.2 Propranolol

Numerous methods involving chiral derivatization have been reported for the separation of propranolol enantiomers. Unfortunately, the applicability of these methods for our pharmacokinetic studies was limited as many lacked sensitivity (Sedman & Gal, 1983; Schmitthenner *et al.*, 1989) or convenience such that lengthy preparation times were required (Linder *et al.*, 1989), interfering peaks were present or they did not have an internal standard (Schaefer *et al.*, 1990). Furthermore, in our laboratory, the methods we reproduced were found to lack sufficient resolution to quantitate samples possessing large differences in enantiomer concentrations (Langaniere *et al.*, 1989). We thus utilized chiral derivatization with NEIC and developed a normal phase HPLC assay suitable for stereoselective pharmacokinetic studies of propranolol in rats. Separation of propranolol enantiomers with this assay was found to be more convenient than previously reported stereospecific methods (Langaniere *et al.*, 1989; Schaefer *et al.*, 1990; Linder *et al.*, 1989) as sample preparation was rapid (approximately 20-30 min) and only a 25 minute run time was required. Sensitivity and accuracy were adequate for pharmacokinetic studies (Table 3-III). In addition, excellent resolution of diastereomer peaks (Fig. 3-4) allowed accurate enantiomer quantification in plasma samples possessing high S:R ratios. The extraction yield was not determined as diethyl ether extraction was found to be suitable for the extraction and detection of relatively low concentrations (ng/ml) of propranolol enantiomers in small sample sizes. Furthermore, previous analytical methods using diethyl ether as an extraction solvent have reported extraction efficiencies of between 78-90% for the propranolol enantiomers (Langaniere *et al.*, 1989; Guttendorf *et al.*, 1989).

4.2 Disposition Studies of Acebutolol Enantiomers in Humans

4.2.1. Pharmacokinetics of acebutolol enantiomers in healthy subjects

The antihypertensive agent, acebutolol, introduced in France by May & Baker in 1974, is extensively used worldwide. Several articles which review the nonstereoselective physicochemical, pharmacology and pharmacokinetics properties of racemic acebutolol are available in the literature (Maxwell & Collins, 1974; De Bono *et al.*, 1985; Singh *et al.*, 1986; Foster & Carr, 1990). Acebutolol is a relatively hydrophilic cardioselective ß-adrenoceptor blocking agent which also possesses partial agonist activity. Pharmacological activity of its major metabolite, diacetolol, is similar to that of acebutolol.

In all subjects, we observed a stereoselective disposition for acebutolol, with slightly higher plasma concentrations of the active, S-(-)-enantiomer (Fig. 3-5). This is contrary to a previous report of one subject (Sankey *et al.*, 1984), in which plasma concentrations of acebutolol were superimposable. This discrepancy may be due to the fact that the previous study only analyzed plasma samples from one subject. Furthermore the previous study utilized S-(-)-N-trifluoroacetylprolyl chloride as a derivatizing reagent which is known to undergo racemization (Thompson *et al.*, 1982; Gulaid *et al.*, 1985), which may partially mask differences in enantiomer concentrations. Nevertheless, total (R + S) values we obtained in these subjects were similar to those previously reported for racemic acebutolol and diacetolol (Gulaid *et al.*, 1981; Zamon *et al.*, 1984; Roux *et al.*, 1980a; Roux *et al.*, 1983a).

After oral administration, acebutolol reportedly undergoes extensive first-pass metabolism to diacetolol, such that only 35% of a dose is systemically available in man (Roux *et al.*, 1983a). This pathway may be saturable as the pharmacokinetics of acebutolol have been shown to be dose-dependent after oral administration (Meffin *et al.*, 1978), but linear after iv administration (Meffin *et al.*, 1977a). However, based on previously reported values (non-stereospecific) for CL_{sys} which range from 528 to 615 mL/min after an intravenous dose and CL_{renal} values of approximately 150 ml/min (Roux *et al.*, 1983a; Meffin *et al.*, 1977a; Roux *et al.*, 1980a) and assuming a liver plasma flow of 0.8 L/min (Gibaldi & Perrier, 1982; Colburn, 1988), extraction ratios between 0.47 and 0.58 are calculated for acebutolol. This indicates that acebutolol undergoes a moderate rather than extensive degree of metabolism upon first pass through the liver. The 32% and 36% oral bioavailabilities we estimate for R- and S-acebutolol from our data are consistent with the reported values. Nevertheless, although acebutolol may only undergo a moderate degree of first-pass extraction, it may still be extensively metabolized.

Metabolism of acebutolol occurs primarily via hydrolysis of the butyramide group to form acetolol, followed by N-acetylation to diacetolol (Fig 1-1). After oral doses in man, the intermediate acetolol accounts for less than 10% and diacetolol more than 60% of recovered drug in urine (Andresen & Davis, 1979). Similar to others (Winkle *et al.*, 1977), an extensive formation of the diacetolol metabolite was seen. We observed both greater oral clearances for R-acebutolol and higher peak plasma concentrations for R-diacetolol. Furthermore the metabolite/drug AUC ratio was significantly greater for the R as compared to the S enantiomer. This suggests that the metabolism of acebutolol to diacetolol is stereoselective for the R enantiomer. Thus the formation rate and/or extent of biotransformation of R-acebutolol to R-diacetolol is greater than metabolism of S-acebutolol to S-diacetolol, resulting in higher concentrations of the R enantiomer of diacetolol. Thus a stereoselective metabolic conversion of acebutolol to diacetolol, in favor of the R enantiomer, could, in part, account for the observed stereoselectivity.

The presence of site specific transport proteins could, perhaps, result in stereoselective absorption and hence stereoselective differences in bioavailabilty. Boyd *et al.* (1989) suggested the existence of a stereoselective absorption process for atenolol. More recently, however, it was found that differences observed in the disposition of atenolol enantiomers could be explained solely by differences in renal clearance (Mehvar *et al.*, 1990). However, the absorption of acebutolol is virtually complete in man as only 11% of an oral dose is reportedly unabsorbed (Meffin *et al.*, 1978; Gabriel *et al.*, 1981; Roux *et al.*, 1983a). Furthermore, drug absorption is generally a passive process and there is no evidence for acebutolol to suggest involvement of an active process (ie. saturable absorption); thus stereoselective absorption of acebutolol seems unlikely. In fact, stereoselective absorption is more often observed for endogenous compounds requiring an active transport system. The few exceptions (eg. L-dopa; Wade *et al.*, 1973) include compounds which are similar to endogenous substances.

Administration of an acebutolol product which does not consist of equal proportions of the two enantiomers may also explain differences between enantiomer bioavailability. We tested this possibility by assaying both enantiomers in acebutolol tablets. In all cases, we consistently found that, indeed, the ratio of the two enantiomers was unity. Thus differences in the bioavailability of acebutolol enantiomers likely stems from stereoselectivity in its first pass metabolism rather than absorption.

As metabolism of acebutolol proceeds through hydrolysis with subsequent acetylation (Andresen & Davis, 1979), stereoselective metabolism could occur with either process. To date, existence of stereoselective acetylation has not been reported. Stereoselective oxidative metabolism of the beta-blockers has been frequently reported for other ß-blockers such as metoprolol (Lennard *et al.*, 1983), propranolol (Walle *et al.*, 1984), bufuranol (Dayer *et al.*, 1986), penbutolol (Ochs *et al.*, 1986), and xibentolol (Honma *et al.*, 1985). Similarly, stereoselective hydrolysis by blood esterases has been reported for the beta-blocker esmolol (Quon *et al.*, 1988).

In addition to metabolism, acebutolol is also eliminated unchanged in urine and faeces. Urinary recovery of acebutolol accounts for 30-40% (Meffin *et al.*, 1977a) and 10-30% (Roux *et al.*, 1983a; Gulaid *et al.*, 1981; Gabriel *et al.*,1981) of a total dose after iv and oral administration, respectively. A greater amount of the S enantiomer was recovered in urine as compared to that of its antipode (Table 3-IX), suggestive of either a higher bioavailability or a greater renal clearance of this enantiomer. Renal clearances of the two enantiomers, however, were not significantly different from each other in healthy subjects (Table 3-IX). Furthermore, the enantiomeric ratio of acebutolol excreted in urine (ΣXu S:R,1.21±0.11) reflected the observed plasma ratio (AUC S:R = 1.24±0.08). Hence, it is likely that other pathways, such as hepatic metabolism is responsible for the observed differences in the cumulative amounts of enantiomers collected in urine.

Disposition of diacetolol was also found to be stereoselective, with greater amounts of the R enantiomer found in urine and plasma (Fig. 3-6; Table 3-X). As previously stated, this may imply that biotransformation of acebutolol to diacetolol is greater for the R enantiomer. The significantly longer $t_{1/2}$ and greater renal clearance of R-diacetolol as compared to S-diacetolol suggests that in addition to an apparent stereoselective formation of the R enantiomer of diacetolol, the clearance of diacetolol may also be stereoselective in favor of the R enantiomer. Indeed, renal clearance was significantly greater for R- than S-diacetolol (Table 3-X). Diacetolol is known to be excreted extensively through the kidneys, and values obtained were similar to previous reports (Zamon *et al.*, 1984; Roux *et al.*, 1980b; Zamon *et al.*, 1985; Flouvat *et al.*, 1981), therefore differences in renal clearance values could account for the significant differences in enantiomer half-lives. The stereoselective renal clearance may also account for the observed difference in the cumulative amount of diacetolol enantiomers collected in urine. However, higher renal clearances of R-diacetolol cannot account for observed differences in enantiomer plasma concentrations. Interestingly, whereas the t½ of S-diacetolol was significantly longer than that of the parent drug, this was not found to be the case with the R enantiomer. Considering that the t1/2 of R-diacetolol was similar to that of the parent drug, this may indicate that the time course of R-diacetolol is formation rate limited (Pang, 1985).

In regard to stereoselective renal clearance observed for diacetolol, as glomerular filtration is a passive process and protein binding of diacetolol is limited (6-10%; Coombs, 1980), it is likely that the renal clearance of diacetolol involves tubular secretion and/or reabsorption. At present we do not have evidence to support either an active reabsorption or secretion process. Nevertheless, such evidence does exist for other similar racemic drugs including pindolol (Hsyu & Giacomini, 1985), atenolol (Mehvar *et al.*, 1990) and metoprolol (Lennard *et al.*, 1983), as well as the antiarrhythmic disopyramide (Le Corre *et al.*, 1988).

An interesting observation in the majority of young and aged subjects (14 of 21), was the occurrence of multiple peaks for acebutolol plasma concentration curves. Although erratic absorption has been previously implied for acebutolol (Winkle *et al.*, 1977; Zaman *et al.*, 1984), the incidence of multiple peaking has not been reported for the drug. Nevertheless, in most cases, pharmacokinetic studies after oral doses have reported mean values which may mask fluctuations in plasma concentrations rather than examining concentration-time curves for individual subjects (Gulaid *et al.*, 1981; Kaye *et al.*, 1976; Roux *et al.*, 1980b, 1983a, 1983b; Zaman *et al.*, 1984, 1985). With other agents, this phenomenon has been attributed to a multitude of causes such as intestinal absorption windows, changes in gastrointestinal motility or enterohepatic recirculation

(Plusquellec *et al.*, 1987; Oberle & Amidon, 1987; Suttle *et al.*, 1992). For acebutolol, however, we cannot pinpoint the underlying mechanisms based on our human data.

4.2.2. Pharmacokinetics of acebutolol enantiomers in the elderly.

The prevalence of inflammatory disease is much greater in the elderly population. In these subjects, physiological properties affecting drug disposition are progressively altered (Roubenoff, 1993; Greenblatt *et al.*, 1982). Some of these physiological changes such as altered plasma proteins, decreased lean body mass and reduced organ function are similar to those observed in rheumatoid arthritis. For highly extracted beta-blockers such as propranolol, labetalol, and oxprenolol both alteration in liver blood flow and intrinsic metabolism in the elderly have been reported to affect their pharmacokinetics (Castleden *et al.*, 1975; Castleden & George, 1979; Hitzenberger *et al.*, 1982; Kelly *et al.*, 1982; Rigby *et al.*, 1985). We, however, did not observe any effect of aging on the AUC or oral clearance of acebutolol. This may be due to a less pronounced first-pass elimination of acebutolol as compared to drugs such as propranolol, clearances of which can dramatically change with small changes in liver blood flow. Furthermore, the metabolism of acebutolol has been reported to be relatively unaffected by chronic liver disease (Zaman *et al.*, 1985), indicating that the liver has a high capacity for metabolism of acebutolol. Accordingly, in healthy elderly subjects, changes in enzyme activity or liver blood flow may not be extensive enough to affect the metabolism of acebutolol. The lack of effect of aging observed by us is contrary to a previous study reporting higher AUC values and longer t½s in elderly subjects (Roux *et al.*, 1983b). However the elderly values were compared with those of a young population and whereas our subjects ranged from 60 to 75 years, the average age in their study was 79 years. Hence, it is possible that changes in clearance of acebutolol may only become apparent in much older subjects.

Despite the lack of significant effect on AUC, the observed significant age-related decrease in β of S-acebutolol (Fig. 3-8a) suggests that the pharmacokinetics of acebutolol is affected by age. Altered distribution may be responsible for the apparent age-related clearance changes of acebutolol as increases in Vd may subsequently incur similar increases in $t_{1/2}$. Nevertheless, it is unlikely that small and insignificant changes in the binding of minimally protein bound drugs such as acebutolol would substantially influence their distribution. On the other hand, it is possible that longer $t_{1/2}$ values in aged subjects is indicative of a reduction in the clearance of acebutolol. Oral clearance and AUC values may not reflect the reduction in systemic clearance if the oral bioavailability of acebutolol is also decreased in the elderly. As liver blood flow is generally reduced in the elderly, this may result in both a decreased bioavailability and reduced hepatic clearance of acebutolol. It has been determined that with moderate to highly extracted drugs, a decrease in liver blood flow produces exactly the same decrease in both bioavailability and systemic clearance so that there is no net effect on AUC_{oral} (Gibaldi & Perrier, 1982).

Our results indicate that the renal clearances of acebutolol enantiomers, however, were clearly influenced by aging. The progressive reductions in the CL_{renal} and ΣXu of acebutolol enantiomers with increasing age were significant. As our elderly subjects had reduced renal function and CL_{cr} was also highly correlated with Cl_{renal} of acebutolol (Fig. 3-9c), these changes are mainly due to a progressive age-related decline in renal function. That is, decreased CL_{renal} in the elderly rather than the age *per se*, is mainly due to their reduced renal function. Previous studies (Roux *et al.*, 1980b; Smith *et al.*, 1983) have also established a strong correlation between renal clearance of acebutolol and creatinine clearance in patients with all grades of renal failure.

As in young patients, plasma concentrations of the S enantiomer of acebutolol were slightly but significantly greater than those of its antipode (Fig. 3-7). Interestingly, age appears to have an effect on stereoselective disposition as the S:R ratio of acebutolol in urine was observed to decline with increasing age. (Fig. 3-10b). A stronger relationship of the ΣXu S:R ratio with Clcr (Fig. 3-10f) may indicate that this is mainly due to the age-related decline in renal function. Renal clearances of acebutolol were higher than calculated creatinine clearance values in all young and elderly subjects indicating that in addition to filtration, tubular secretion and/or reabsorption may be involved in the renal elimination of acebutolol. Therefore the progressive approach of the ΣXu S:R ratio towards unity may indicate that an active renal process such as tubular secretion and/or absorption exists and is detrimentally affected by diminished renal function.

On the other hand, changes in the ΣXu S:R ratio of acebutolol may reflect alterations of the enantiomer disposition in plasma rather than a declining stereoselectivity of CL_{renal}. Indeed, a strong relationship was observed between the AUC S:R ratio with the urinary S:R ratio in these subjects (r=0.85). Furthermore, the disposition of acebutolol enantiomers in plasma also appears to be affected by aging as we observed a decrease in the AUC S:R ratio with declining Cl_{cr} (Fig. 3-10e). However, as CL_{renal} only accounted for 5-10% of acebutolol oral clearance and reduced renal function did not affect the AUC of the individual enantiomers, the change in the plasma enantiomer ratio may be indicative of another stereoselective pathway such as metabolism or biliary clearance which may also be altered in aged subjects. Further studies are required to determine the cause of this effect of aging on the stereoselective disposition of acebutolol. Nevertheless, the clinical relevance of this observation may not be significant as the degree of stereoselectivity of acebutolol is minimal.

The pharmacokinetics of the metabolite diacetolol after administration of acebutolol has been previously reported to be affected by age (Roux *et al.*, 1983b). The authors speculated that higher plasma levels and prolonged elimination in the elderly subjects could be due to diminished renal function, as diacetolol is primarily excreted renally. Similarly, we observe declining β with age (Fig. 3-8b). However, although the elimination rate constants of R- and S-diacetolol were highly correlated with age, no relationship was observed with creatinine clearance (Fig. 3-8d). This may suggest that a non-renal route of elimination for diacetolol is affected by age. This appears plausible as it has been reported that the non-renal route of elimination is significant for diacetolol, and may account for 1/3 of its total body clearance (Flouvat *et al.*, 1981). Furthermore, only 16% of the acebutolol dose was recovered as either acebutolol or diacetolol in urine. In other younger subjects, recovery was slightly higher (27±8.4%) which is consistent with previous reports (Roux *et al.*, 1983b; Gulaid *et al.*, 1981). This may reflect incomplete absorption of acebutolol or biliary excretion of acebutolol and/or diacetolol. On the other hand, the low recovery may be due to formation of other metabolites.

As expected, CL_{renal} of diacetolol enantiomers were observed to diminish with age (Fig. 3-9b). Since CL_{renal} and ΣXu were highly correlated with Cl_{cr} (Fig. 3-9d), declining renal clearance in the elderly can mainly be attributed to diminished renal function. Previous studies in patients with renal failure have also reported a similarly high correlation between creatinine clearance and renal clearance of diacetolol (Smith, 1983; Kirch 1982; Roux 1980b). Diminished renal clearance of the beta-blockers sotalol and pindolol has also been observed in elderly subjects (Ishizaki *et al.*, 1980; Gretzer *et al.*, 1986).

In these subjects, a stereoselective renal clearance of R-diacetolol was observed. Furthermore, the enantiomeric S:R ratio of diacetolol collected in urine was linearly related to Cl_{cr} (Fig. 3-10f). Thus diminishing renal function also affects the stereoselective disposition of diacetolol. This further supports the notion of an active tubular secretion and/or reabsorption of diacetolol occurring and it is these processes that may be affected by declining renal function. The active renal tubular secretion of the basic drug procainamide and its acetyl metabolite has been shown to decline with age (Reinberg *et al.*, 1980). A similar active carrier-mediated renal transport system may be responsible for age-related stereoselectivity observed with acebutolol and its acetyl metabolite, diacetolol.

4.2.3. Pharmacokinetics of acebutolol in arthritic subjects

The effect of arthritis on the pharmacokinetics of acebutolol was found to be minimal in the patients examined. Although a non-significant trend in which slightly higher AUCs of R-acebutolol were present in arthritic subjects, it is unlikely that there is an appreciable effect of arthritis on the clearance of R-acebutolol. On the other hand, the significantly reduced oral clearance and prolonged $t_{1/2}$ values of S-acebutolol suggests that changes in the pharmacokinetics of acebutolol occur in inflammation.

The lower oral clearance and higher AUC values of S-acebutolol in arthritic as compared to healthy subjects indicates either an increase in the absorption and/or a decrease in the elimination of the drug in arthritis. It is unlikely that altered absorption is responsible for the observed changes. Acebutolol is almost completely absorbed in healthy subjects (Meffin *et al.*, 1978; Gabriel *et al.*, 1981), therefore absorption would not be expected to increase any further in arthritic subjects. Furthermore, as absorption is a passive process, it is not likely that an enhanced absorption of acebutolol would result in a significant increase in the AUC of the S but not the R enantiomer.

A reduction in clearance may arise as a function of renal or nonrenal pathways. However, the CL_{renal} was only found to have a minor ($\approx$10%) contribution to the CL_{oral} of acebutolol, and was not significantly different in arthritic as compared to healthy subjects. Therefore, it is reasonable to assume that in arthritic subjects the reduction in clearance is primarily a result of decreases in elimination through non-renal pathways such as hepatic metabolism rather than changes in renal excretion.

Arthritic-induced changes to the AUC of S-acebutolol could result from a reduction in presystemic and/or systemic metabolism of the drug. However, if one assumes that increases in $t_{1/2}$ is indicative of the systemic clearance of acebutolol, then the increased AUC or decreased CL_{oral} may more likely be due to reduced systemic rather than presystemic metabolism. Furthermore, based on urinary data, it is unlikely that altered presystemic availability is responsible as the amount of acebutolol enantiomers recovered in urine were similar between healthy and arthritic subjects. Nevertheless, bioavailability based on the urine collection is not conclusive, as although the period of urine collection (24 h) equaled approximately five $t_{1/2}$ in healthy subjects, this period only equaled three to four $t_{1/2}$ in arthritic subjects.

On the other hand, prolonged $t_{1/2}$ values of acebutolol and diacetolol could also reflect an increased distribution of the drugs. The unlikeness of this suggestion stems from the fact that inflammation is normally associated with increased protein binding of basic drugs which results in a decrease rather than increase in its Vd. Furthermore, increases in the limited (6-10%) binding of acebutolol and diacetolol (Coombs *et al.*, 1980) would not cause an appreciable change in their distribution.

Inflammation-induced changes in the metabolism of drugs with limited protein binding are thought to result from a reduced intrinsic clearance. As plasma concentrations of the metabolite, diacetolol were not significantly altered in arthritic subjects, nor the metabolite/drug ratio, it would appear that this metabolic route of acebutolol was unaffected by arthritis. However, subsequent changes in the clearance of the metabolite, diacetolol, may mask alterations in its formation. Indeed, the trend of lower peak plasma concentrations of R and S-diacetolol combined with their significantly prolonged $t_{1/2}$

values in arthritis may indicate a reduction in both the formation and clearance of diacetolol. As the CL_{renal} of diacetolol was not significantly different in arthritic patients, longer $t_{1/2}$s may result from a decreased non-renal route of elimination, similar to that hypothesized for elderly subjects. Administration of the pure metabolite would be required to determine the effect of arthritis on formation and clearance parameters of diacetolol.

Interestingly, in arthritic patients, the renal clearance of acebutolol was found to be stereoselective in favour of the R enantiomer. This was not the case in the healthy subjects. This finding, combined with the observation that in elderly subjects there is an age-related decline in the urinary enantiomer ratio of acebutolol, appears to implicate involvement of an active renal process. Altered physiological states such as arthritis likely results in minor changes to the stereoselective nature of this carrier mediated transport system.

Overall, the differences between the pharmacokinetics of acebutolol and diacetolol enantiomers in arthritic and healthy subjects were minimal and not therapeutically relevant. It is possible that arthritis-induced changes in drug disposition are minimized or suppressed in these patients, as our arthritic subjects did not have active disease due to concurrent use of medication (Table 1-III). In fact, in 3 of the arthritic volunteers, sedimentation rate values were within the normal range. In untreated patients or those severely afflicted, arthritis-induced changes may result in more appreciable changes in acebutolol disposition. Therefore, we tested this possibility in an animal model.

4.3 Disposition Studies of Acebutolol Enantiomers in Rats

4.3.1. Pharmacokinetics of acebutolol in healthy rats.

To date, virtually no information on the disposition of acebutolol in the rat is available. As the effect of arthritis was to be studied in female healthy and adjuvant-induced arthritic Sprague-Dawley rats, it was necessary to first delineate the pharmacokinetics of acebutolol in this species. Overall, our results indicate that the rat is a good animal model for studying the pharmacokinetics of acebutolol. In our rats, the 9.5% biliary and 25% urinary excretion of unchanged acebutolol are comparable to humans, in which 2.7-8.5% and 13-25% of an orally administered dose is collected unchanged in bile (Kaye & Oh, 1976) and urine (Roux *et al.*, 1983a; Meffin *et al.*, 1978; Ryan, 1985), respectively. After iv administration, plasma levels of acebutolol decline with a t½ of about 3 hr in humans (Roux *et al.*, 1983a; Roux *et al.*, 1980a); this compares favourably with the value of 2.0±0.5 hr in the rat. With regards to stereoselectivity, slightly but significantly higher amounts of the S as compared to the R enantiomer of acebutolol are seen in urine and plasma after a 10 mg/kg iv dose (AUC S:R, 1.06±0.02), which is similar to what we observed in humans (S:R AUC, 1.20±0.10; ΣXu 1.17±0.05). Larger amounts of the S isomer as compared to its antipode were found in the urine of all rats (ΣXu S:R, 1.12-1.16), regardless of dose or dosage route. Since the CL_{nr} of R-acebutolol was significantly greater than S-acebutolol after both oral and iv doses, it is possible that a stereoselective hepatic clearance in favour of R-acebutolol is responsible for this observation. Furthermore, stereoselectivity was dose-dependent, in that CL_{sys} of the R but not the S enantiomer significantly decreased with increasing dose, resulting in lower S:R ratios with the higher dose. Although the extent of stereoselectivity was minimal in the rat, it was consistently observed and thus offers insight to the similar enantiomer disposition observed in humans.

Metabolism of acebutolol proceeds in the same way, qualitatively, in man, dog and rats (Maxwell & Collins, 1974). The metabolic precursor of diacetolol, acetolol, has been collected as a major metabolite (30%) in rat urine (Andresen & Davis, 1979), yet is only found in minute quantities in human urine (Gulaid *et al.*, 1981). Hence it appears that quantitative differences in acebutolol metabolism exist between the two species. Indeed after oral doses, diacetolol levels in rats (AUC diacetolol:acebutolol $\approx$ 0.2) were much lower than those in humans (AUC diacetolol:acebutolol $\approx$ 2.0). Nevertheless, presystemic diacetolol formation likely occurs in the rat as the AUC of diacetolol was found to be twice as high after oral doses (Table 3-XII). Consequently, it is likely that presystemic metabolism of acebutolol in rats occurs in the liver and to a lesser extent than that reported for humans.

Following iv administration, CL_{sys} values were observed to exceed (> 30 mL/min/kg) reported liver plasma flow rates (Harashima *et al.*, 1985). Renal and biliary clearance alone, however, can not completely account for elimination through non-hepatic routes. After subtraction of CL_{renal} and CL_{bile} from CL_{sys}, clearance values were still found to exceed the hepatic plasma flow rate. This suggests involvement of an extra-hepatic pathway of elimination for acebutolol (e.g., gut or renal metabolism, intestinal exsorption).

Another interesting point was that after ip doses, the availability of acebutolol was much greater than that seen after oral doses. As portal concentrations are higher after ip as opposed to oral doses, differences in availability may be due to a saturation of first-pass metabolism. This is plausible as in rats, we observed a decrease in CL_{po} of acebutolol with increasing dose. Furthermore the dose-dependency of acebutolol after oral doses has been previously established in humans (Meffin *et al.*, 1978). However, assuming linear kinetics and that the entire ip dose reaches the portal circulation, the lower than expected extraction after ip doses suggests gut involvement. We tested for the possibility of gut metabolism. However, intestinal metabolism of acebutolol was not observed after incubation with either various gut segments or gut contents. Nevertheless, others have suggested that presystemic metabolism, namely hydrolysis, of acebutolol occurs in the rat gut as perfusion of the isolated rat liver with acebutolol has not been found to result in hepatic transformation to diacetolol (Alexander & Andresen, 1982). Therefore, it is possible that acebutolol is metabolized in the gut but this biotransformation was not detected due to the conditions of our experiment, which only involved aerobic metabolism. On the other hand, it is possible that incomplete absorption rather than gut metabolism is responsible for the low availability of acebutolol after oral doses.

Despite a clear cut evidence for gut (presystemic) metabolism, the gut is likely involved in elimination of acebutolol since active transport of acebutolol across the intestinal wall into the gut has been reported *in vivo* in dogs and *in vitro* with everted rat gut (George & Gruchy, 1979). In dogs, elimination via the small intestine accounts for 8% of CL_{sys} (Collins & George, 1975). This may also be the case in humans; after iv doses, 16% of unchanged acebutolol is recovered in the faeces of man (Gabriel *et al.*, 1981) which is more than that reportedly collected from bile (Kaye & Oh, 1976). Contribution of clearance along the gut could be responsible for this discrepancy, and may also partly explain the low recovery (less than 40% of iv dose) of acebutolol as either the acetylated or unchanged drug from urine and bile. In addition, as acebutolol is cleared into the intestine, some reabsorption may occur, which could partially explain fluctuation in plasma concentrations. The absence of erratic peaking after iv and ip doses (Fig. 3-12), however, suggests that reabsorption alone can not account for the multiple peaks observed after oral administration.

The food-related decrease in bioavailability of acebutolol could be thought to result from either an incomplete absorption resulting from the interference of proteins and fats in food or an increased first-pass metabolism due to increased liver blood flow in fed rats. The 48% decrease in F_{gut} and proportionally smaller AUC of diacetolol (Table 3-XII) in fed rats suggests that the decreased bioavailability of acebutolol likely stems from decreased absorption. Food has not been found to significantly influence the pharmacokinetics of acebutolol in humans (Zamon *et al.*, 1984). The effect of fasting on the CL_{sys} of acebutolol was not investigated in this study as we were concerned with determining effects of food on oral bioavailability primarily for subsequent study design.

It is interesting that we observed a similar incidence of multiple peaks in the absorptive phase in our orally dosed rats (10 of 15 rats) as compared to what we observed in humans (14 of 21 healthy subjects). The phenomenon of multiple peaking has often been attributed to enterohepatic recycling. In this case, it is unlikely that recirculation plays an important role because fluctuations in plasma concentration time patterns were not observed after ip and iv doses (Fig. 3-12). Furthermore, although dependency on administration route could be due to a saturable first pass elimination into bile, there was no difference in the CL_{bile} between iv and id doses. In fact, a significantly smaller quantity of the acebutolol dose was collected in bile after id dosing. Incomplete systemic availability after id doses of acebutolol can account for this difference. Although animals were under urethane-anaesthesia which may result in physiological changes to liver blood flow and gastrointestinal motility, the absolute F after id doses was approximately 55% for both enantiomers, which is close to the 59-63% oral bioavailability observed in the unanesthetized rats.

Various models of discontinuous gastrointestinal absorption have been developed to describe the multiple peaks seen with other drugs (Plusquellec *et al.*, 1987; Brockmeier *et al.*, 1986; Oberle & Amidon, 1987; Suttle *et al.*, 1992). For instance, it has been suggested that with cimetidine double absorption peaks may occur due to variable gastric emptying rates (Oberle & Amidon, 1987). However these theories are based on hypothetical models and are yet to be proven either *in vitro* or *in vivo*. Existence of a classical absorption window such as that described by Plusquellec *et al.* (1987) and others (Suttle *et al.*, 1992) incorporates a nonabsorbing gastrointestinal segment between one or more absorption sites. The cause of such discontinuous absorption includes site specific transport proteins. Concentration dependency and saturation of acebutolol absorption would be indicative of an active transporter. With respect to acebutolol, this is unlikely, because acebutolol readily crossed the intestinal wall in all of the gut segments examined. Furthermore, when the extent of transport was compared between 1 and 250 μg/ml, no concentration dependency of the transfer process was noticed (Fig. 3-13).

Another plausable explanation to describe multiple absorption peaks involves the formation of an poorly absorbed micelle complex of drug with bile salts. Such a phenomenon has been recently investigated for the ß-blocker, pafenolol (Lennernas & Regardh, 1993a, 1993b). The authors suggest that the initial pafenolol peak is formed after rapid micelle formation in the small intestine results in cessation of absorption. Subsequently, dissociation of the micelles in the ileum due to active reabsorption of bile salts results in the second, major drug plasma concentration peak. A similar process may exist for acebutolol. The food-related reduction in acebutolol absorption may result from increased micelle formation due to the stimulation of bile acid secretion by food. Furthermore, rapid intestinal absorption of acebutolol was noted in the bile-duct cannulated rats. Nevertheless, further studies are required to confirm or rule out this possibility. In situ intestinal formation of a micelle complex has also been described for the ß-blocker, nadolol (Yamaguchi *et al.*, 1986).

4.3.2. Pharmacokinetics of acebutolol in arthritic rats

One of the most striking observations in this study was the significantly raised plasma levels of R- and S-acebutolol in AA. This elevation was more noticeable than that observed in human arthritic subjects. An increased plasma drug concentration may stem from altered absorption, distribution and/or elimination processes. Arthritis-associated alterations in drug plasma concentrations are often ascribed to decreased distribution as inflammation results in the elevation of AAG, a protein responsible for the binding of basic drugs [Belpaire *et al.*, 1982; Bishop *et al.*, 1981; Walker *et al.*, 1986)]. However, acebutolol has limited protein binding (7-12%) which did not significantly increase in AA. Therefore, alterations in protein binding cannot explain the three to ten fold higher plasma concentrations we observed for AA rats between 0.5 to 2 h.

It is thought that changes in intestinal integrity in inflammatory conditions may influence the absorption of xenobiotics (Mielants *et al.*, 1991). Specifically, permeability changes in AA rats may lead to an enhanced absorption and hence increased plasma levels of acebutolol. Nevertheless, it is unlikely that observed increases in plasma concentrations would be due, solely, to increased absorption. In the fasting rat, absorption of acebutolol (F_{gut}) was determined to be greater than 90%, and thus would not be expected to increase any further in rheumatic diseases. Furthermore, previous *in vivo* investigations using perfused jejunum from healthy and treated rats failed to find any affect of AA on the oral absorption of another *β*-blocker, propranolol (Key *et al.*, 1986). Decreased intestinal motility in rheumatoid arthritis (Harris, 1981), may also influence drug absorption. However, the T_{max} of acebutolol was unaffected in AA, suggesting no change in its absorption rate.

The effect, if any, AA would have on the exsorption of acebutolol across the intestinal wall into the gut, is unknown. Arthritic-induced changes to the proteins responsible for this intestinal transport, resulting in either a decreased excretion into the GI tract or an increased reabsorption of acebutolol, could possibly account for higher plasma levels. However, the elimination phase of acebutolol was parallel in the two groups despite an elevation of plasma concentrations during the absorptive phase in AA, suggesting changes to a presystemic rather than systemic pathway.

Alternately, a decreased metabolism may provide a more plausible explanation for the observed differences in AA. Extraction of acebutolol upon first pass ($1\text{-}F_{liver}$) was calculated to be approximately 0.15 for R- and S-acebutolol in the ip dosed rat. The hepatic clearance of drugs possessing such extraction ratios are mainly influenced by changes in intrinsic clearance. Decreased intrinsic clearance of antipyrine, propranolol and metoprolol has been reported both *in vivo* and *in vitro* for rats with turpentine-induced inflammation (Belpaire *et al.*, 1989; Chindavijak *et al.*, 1987a). Thus, a similar decrease in enzyme activity in AA may explain observed changes to acebutolol disposition. Assuming complete absorption, the estimated oral clearance of R- and S-acebutolol would be 6.8 and 6.4 L/h/kg in AA and 14.5 and 13.4 L/h/kg in controls, respectively. This is higher than values obtained in our jugular vein cannulated rats (Table 3-XII). Observed differences between these controls and those used for the earlier pharmacokinetic studies may be due to a surgery-related reduction in intrinsic clearance in the cannulated rats, similar to that described for propranolol and antipyrine (Chindavijak *et al.*, 1988) or may be due to the dose-dependent kinetics of acebutolol, as much lower doses (50%) were used in these rats. Since oral clearance is an estimate of intrinsic metabolism, this would indicate a 50% decrease of enzyme activity in AA. Similarly, a 79-87% decrease in oral clearance has been reported for metoprolol and propranolol in rats with turpentine-induced inflammation (Belpaire *et al.*, 1989). Moreover, increases in t½ were not observed in these rats. As a prolonged t½ would be expected with such a decline in systemic clearance, the authors speculated that inflammation affects the first-pass metabolism of these highly extracted drugs. Likewise, as the t½ of acebutolol was not affected in AA, the presystemic clearance of the drug is likely affected. This is contrary to the prolonged $t_{1/2}$ and relatively unchanged bioavailability observed in human arthritic subjects. As previously discussed, the relatively lower hepatic metabolic capacity in rats as compared to humans may play a factor in this discrepancy.

A trend of higher diacetolol levels was also evident in AA, but this was only significant at 2 h likely due to large variability in diacetolol plasma concentrations. Similar diacetolol:acebutolol ratios in the two groups of animals may suggest that the metabolism of acebutolol to diacetolol was unaffected by AA. Alternately, it could also indicate that clearances of both acebutolol and diacetolol were affected to the same extent. As previously stated, in arthritic humans, although the formation of diacetolol was possibly affected, its clearance was also reduced consequently resulting in relatively unchanged plasma concentrations. Another possibility exists that metabolism of acebutolol to another product such as acetolol may have been reduced in AA, and thus could be responsible for the reduced clearance of acebutolol. Unfortunately, this metabolite was not available to us for development of the analytical procedure.

With regards to stereoselectivity, at early sampling times (0.5-2 h), the enantiomer ratio of acebutolol was found to be concentration dependent in AA as it was for controls (Fig. 3-17). This also indicates that a saturable, stereoselective pathway of elimination for acebutolol is present in these rats, similar to that observed in cannulated rats. The S:R ratio of acebutolol enantiomers was not significantly changed in AA despite the elevated plasma concentrations. However, concentration-dependency of the S:R ratio appears to be slightly altered in AA (Fig. 3-17). The observed reduced S:R diacetolol ratio in AA may also indicate a disease-induced change in either formation or clearance of diacetolol .

Overall, the disposition of acebutolol was affected by arthritis to a greater extent in rats as compared to humans. This may stem from the fact that the majority of human subjects did not have active disease due to concurrent use of medication. Nevertheless, it is important to note that although these rats were severely afflicted with arthritis, the extent of inflammation-induced dispositional changes was still relatively minor. Thus large sample sizes would be required in further studies to overcome the inherent intersubject variability. Therefore we chose to continue our studies with the ß-blocker propranolol, the pharmacokinetics of which is reportedly affected to a much greater extent in inflammation.

4.4 Disposition Studies of Propranolol Enantiomers in Rats

4.4.1. Pharmacokinetics of propranolol enantiomers in arthritis.

As previously reported (Guttendorf *et al.*, 1991), disposition of propranolol is stereoselective in rats. This stereoselectivity is dependent upon the route of administration: in controls, plasma levels of the active S- enantiomer accounts for only 27% of total (S + R) plasma concentrations after i.v. and 18% after oral doses. This likely reflects both a greater systemic and presystemic clearance of S-propranolol. Our data corresponds to a 16% bioavailability for total (S + R) propranolol which is consistent with previous reports (Iwamoto, 1985). In addition, a stronger binding affinity of R-propranolol to plasma proteins, as seen by our results and that of others (Takahashi *et al.*, 1990a, 1990b), is most likely responsible for differences in enantiomer distribution.

In AA, plasma concentrations of both propranolol enantiomers were found to be significantly raised after oral but not after iv doses. The approximately twelve fold increase in total (R+S) propranolol AUC after oral doses is consistent with previous non-stereospecific studies in AA (Bishop & Schneider, 1981; Walker *et al.*, 1986). On the other hand, earlier reports of propranolol in experimentally-induced inflammation have also observed significantly higher plasma concentrations after iv administration (Belpaire *et al.*, 1989; Bishop & Schneider, 1981; Walker *et al.*, 1986). It is unclear as to why our results indicated only a 25% increase in total propranolol AUC in AA after iv doses, which did not reach statistical significance, whereas others have seen approximately 100-

175% greater AUCs of propranolol in inflammation (Belpaire *et al.*, 1989; Bishop & Schneider, 1981). As the degree of paw swelling was rather limited in our animals, it is likely that differences in the relative severity of inflammation between our study and that of others may be responsible for this discrepancy. This possibility, however, could not be confirmed as indices of disease activity were not given by the authors of these initial studies.

The route-related effect of elevated plasma concentrations in AA, indicates that presystemic rather than systemic clearance is responsible for the observed disposition changes. Systemic metabolism of propranolol, a highly cleared drug, is dependent mainly upon the liver blood flow (Q). It has been shown that Q remains unchanged in AA (Walker *et al.*, 1986). On the other hand, decreases in intrinsic clearance of highly extracted drugs generally result in a decreased presystemic rather than systemic clearance (Gibaldi & Perrier, 1982). Presystemic changes may be attributed to absorption and/or metabolism processes. An increased absorption is unlikely to be the cause of the elevated plasma concentration of propranolol as it has been shown that in both control and AA rats the drug readily crosses the jejunum (Key *et al.*, 1986). In addition, absorption is generally a passive process yet arthritic-induced increases in the plasma concentrations of propranolol were more pronounced with the R enantiomer. Hence, the increased plasma enantiomer ratios in AA suggest involvement of a stereoselective pathway. Therefore, the increased bioavailability of propranolol enantiomers likely reflects an enantioselective decrease in their intrinsic metabolism.

In addition to the elevated plasma concentrations of propranolol observed in AA rats, our *in vitro* study also demonstrated a reduction in the disappearance rate of propranolol in arthritic rats. Furthermore, stereoselective differences in the metabolism of the two enantiomers were only evident in the microsomal fractions we obtained from arthritic rats. Provided that the results of the microsomal study reflect those of the intact animal, it suggests a non-parallel decrease in the oxidative metabolism of propranolol enantiomers. This is consistent with earlier nonstereospecific studies which report marked decreases in the oral clearance (Bishop *et al.*, 1981; Walker *et al.*, 1986) and *in vitro* metabolism (Chindavijak *et al.*, 1987; 1988) of propranolol in experimentally-induced inflammation. Very recently it has been observed that pre-treatment of rats with interleukin 1ß, resulting in an acute inflammatory response, also causes an enantioselective increase in plasma concentrations of propranolol (Vermeulen *et al.*, 1993), similar to our findings. Likewise, the authors felt that a 3-7 fold decrease in the intrinsic clearance of R-propranolol was responsible for the stereoselective changes in propranolol bioavailability.

Interestingly, the stereoselectivity *in vitro* was opposite to that observed *in vivo*. Propranolol is metabolized through a multitude of pathways including N-dealkylation, hydroxylation and glucuronidation. Several enzyme systems for each of these pathways have been proposed, many of which have been found to be stereoselective for different enantiomers (Nelson & Bartels, 1984; Nelson & Shetty, 1986; Thompson *et al.*, 1981). Thus it is plausible that the stereochemical ratio observed in AA *in vivo* may be influenced by impairment of additional pathways of metabolism such as glucuronidation.

Impaired drug metabolism in AA has been reported as early as 1974, when Beck and Whitehouse observed increased sleeping times with hexobarbital. The mechanism of inflammation induced changes in hepatic enzyme activity is thought to stem from the release of inflammatory mediators (Ishikawa *et al.*, 1991). In response to tissue injury, hormonal like proteins called the cytokines are released from macrophages. Activity of these mediators, specifically interleukins 1 and 6, on hepatocytes results in both a decreased production of drug metabolizing enzymes and increased synthesis of the acute phase proteins (Ferrari *et al.*, 1993a). This is supported by the observation that cytochrome P-450 content is significantly decreased in livers of rats with experimental inflammation (Ishikawa *et al.*, 1991) as well as in hepatocytes incubated with interleukin-1 (Sujita *et al.*, 1990). Although an acute inflammatory response may also temporarily result in altered hepatic metabolism, it has been shown by Beck and Whitehouse (1974), that the effect of non-arthrogenic adjuvant injection on drug metabolism lasts for less than 14 days. As we studied our animals 21 days after the *M. butyricum* injection, the diminished propranolol metabolism is more likely to be resulting from a systemic involvement of the disease rather than from a local effect of the adjuvant.

Basic drugs such as propranolol are mainly protein bound to the acute phase reactant AAG, levels of which are greatly increased in inflammatory conditions (Belpaire et al., 1982). Indeed, significant relationships between protein binding and AAG levels have been observed with the highly bound ß-blockers, propranolol and oxprenolol and this was thought to be responsible for the increased bioavailability observed in human inflammation (Schneider *et al.*, 1981; Kendall *et al.*, 1979). Our data do not exclude the possibility that changes in free fraction may affect propranolol disposition. After iv administration, a 20-30% reduction in Vd was observed in AA, and although not significant, it may suggest a trend towards altered protein binding. However, for highly protein bound drugs such as propranolol, changes in protein binding are often associated with significant changes in Vd. The non-significant reduction of Vd of propranolol may suggest that protein binding changes were minimal. Hence, it is unlikely that the dramatically reduced oral clearance of propranolol was solely due to altered protein binding. Indeed, in the worst case scenerio, the % unbound of R and S-propranolol was found to change from 7% and 33% in control rats to 2.7% and 8.7% in severely afflicted AA rats (Table 3-XIX), respectively. Assuming: F = Q/Q+(Dose/AUCpo) (Gibaldi *et al.*, 1971); CL_{total} = fu· Cl_{int} and Q = 3.5 L/h/kg, such an increase in protein binding without a change in CL_{int} should result in only a 70% and 175% increase in F of R- and S-propranolol, respectively. This is not in agreement with our results as F of R- and S-propranolol was increased by 640% and 280%, respectively. Thus, in addition to an increased protein binding in AA, a suppressed CL_{int} is likely to play a significant role in the increased bioavailability of propranolol.

The significantly prolonged t½ of R-propranolol after oral (2.8±0.94 h) as compared to iv (0.40±0.14 h) in arthritic animals is suggestive of non-linear kinetics. As a dose-dependent presystemic elimination has been described for propranolol (Iwamoto & Watanabe, 1985), it is plausible that a decrease in the microsomal enzyme capacity in AA could result in saturation of both presystemic and systemic metabolism of R-propranolol after oral administration. However, this reduction in enzyme capacity in AA may not affect CL_{sys} when given intravenously as much lower concentrations are presented to the eliminating organ; levels which may not exceed the liver saturation threshold. Consistent with this hypothesis are our bioavailability data which indicate greater than expected F values after oral doses in AA (Table 3-XVII). Such an effect was not observed for S-propranolol.

It appears that arthritis influences the bioavailability of the highly protein bound, extensively metabolized ß-blocker, propranolol to a greater extent than that seen with acebutolol. With both drugs, depression of metabolic activity in inflammatory conditions results in diminished intrinsic clearances and hence higher plasma concentrations after oral doses. Limited increases in acebutolol enantiomer concentrations in arthritic rats may stem from the fact that, in the rat, acebutolol undergoes only moderate extraction upon first pass and protein binding is limited. Thus the dramatically elevated plasma concentrations of propranolol enantoimers likely stems from a combination of disease induced changes in both protein binding and hepatic enzyme activity.

4.4.2. Influence of inflammation severity and NSAID therapy on propranolol disposition in arthritis

As the clearance of propranolol was found to be dramatically decreased in AA, we subsequently examined the influence of inflammation severity on its disposition. Despite the large variability, we observed higher plasma concentrations of both propranolol enantiomers in all groups of AA rats examined (Table 3-XVIII) as compared to those in healthy control rats (Table 3-XVII) .

The observed variability in plasma concentration elevation may well be explained by considering the degree to which individual animals were afflicted with arthritis. Development of arthritis in AA is dependent on the dose, vehicle and route of administration of adjuvant (Whitehouse, 1988). Tail base inoculation of *M. butyricum* resulted in significantly higher arthritis scores and paw swelling as compared to ip injection. Significantly lower CL_{oral} of propranolol enantiomers were also seen in severe AA as compared to mild AA. As clearance of the R enantiomer was affected to a greater extent, the enantiomeric plasma ratio was higher in severe as compared to mild AA, which is consistent with our previous study (Piquette-Miller & Jamali, 1993). More importantly, however, was the significant correlations between measurements of disease severity including AI and physical hindpaw swelling (paw diameter and volume changes) with AUCs or CL_{oral} of propranolol enantiomers in all treatment groups (Fig. 3-22). Therefore the progressively elevated AUCs of propranolol with increased inflammation indicate that a strong relationship exists between disease severity and decreased propranolol clearance. It is likely that a similar relationship between the magnitude of inflammation and altered propranolol disposition exists in humans. Schneider and Bishop (1979) noted that some high and low plasma concentrations of propranolol occurred in rheumatoid arthritic patients. Despite the lack of a significant correlation between AUCs of propranolol and biochemical indices of inflammation, an arbitrary subdivision of patients into two groups based on their ESR showed marked differences: whereas elevated ESRs (>20 mm/hr) were associated with raised AUCs of propranolol, plasma concentrations in subjects with lower ESRs (<20 mm/hr) were not significantly different from controls. Hence, it may be possible to predict arthritic-induced changes in drug disposition on the basis of an appropriate measure of inflammation in humans.

Inflammation in arthritic patients is often controlled by drugs such as the NSAIDs. The influence of NSAID therapy on joint inflammation and arthritic-induced changes in drug disposition was examined by administering ketoprofen to animals injected with adjuvant. Ketoprofen did not appear to have a significant effect on either the symptoms of inflammation or pharmacokinetic parameters of propranolol in mild AA. However, in light of the large interanimal variability, the lack of significant effect of ketoprofen on both disease severity and propranolol disposition in mild AA could be due to the fact that the animals were relatively healthy, thus it is possible that slight improvements were not detectable in the treated group. Indeed a nonsignificant trend of lower AIs was observed in the ketoprofen-treated group. On the other hand, a significant decrease in the degree of inflammation was seen in the severe model of AA. This decrease in AI scores coincided with a significant decrease in the AUC of propranolol as compared to the untreated group. For extensively metabolized drugs such as propranolol, reductions of AUC after oral doses reflect alterations in their intrinsic clearance. Furthermore, the drug metabolic capacity is diminished in arthritis. Hence, ketoprofen therapy likely influences the pharmacokinetics of propranolol by exerting a protective effect on liver enzymes in arthritic animals. Thereby a partial reversal of the inflammation-induced depression of first-pass metabolism is apparent in treated rats.

Furthermore, as we observed significant relationships between AI and AUCs of propranolol enantiomers among the ketoprofen-treated animals (Fig. 3-22), it appears that the reduction of arthritic-induced changes in hepatic enzyme activity is dependent on the effectiveness of drug treatment. In animals in which inflammation is adequately controlled by NSAID treatment, plasma concentrations of propranolol are likely to be closer to normal values than for animals in which drug therapy is ineffective. Clinical efficacy of the NSAIDs is due, in part, to a reduction in the formation of the prostaglandins and thromboxane via inhibition of cyclooxygenase (Harris, 1981). Neither prostaglandins nor thromboxane have been shown to significantly influence production of hepatic enzymes. Thus the protective effect of NSAID therapy on liver metabolism is likely an indirect one. Presumably, due to diminished joint swelling, a reduction in the release of other inflammatory mediators (ie. interleukin 1) may in turn affect liver enzyme production.

Contrary to what we have observed with propranolol and ketoprofen, others have reported that therapy with other NSAIDs, namely indomethacin and phenylbutazone, do not significantly alter the depression of hexobarbital metabolism in inflammation (Beck & Whitehouse, 1974; Di Pasquale *et al.*, 1984). This could be due to the fact that NSAIDs are heterogeneous compounds and for all of them prostaglandin synthesis inhibition has been recognized as the common mechanism of action (Harris, 1981). It is presently clear that other pathways are involved and dissimilarities exist as to the mechanisms and extent of activity different NSAIDs influence in inflammation (Abramson & Weissman, 1989). Another possibility is that this apparent discrepancy might be explained by considering the duration of drug therapy in these studies. In previous reports, NSAIDs were administered to animals with established arthritis, whereas we began ketoprofen therapy upon induction of the disease. Accordingly, in addition to providing symptomatic relief in AA, early administration of NSAIDs has been shown to have disease modifying properties (Palylyk & Jamali, 1993). Treatment of AA with disease-modifying drugs such as cyclosporin A (Pollack *et al.*, 1989) as well as with the steroidal anti-inflammatory agents, dexamethasone, corticosterone (Beck & Whitehouse, 1974) and pregnenolone 16α-carbonitrile (Kourounakis & Hadjipetrou-Kourounakis, 1984) are associated with an improvement of hepatic metabolic activity and reversal of arthritic induced pharmacokinetic changes.

Disease severity-related changes in protein binding were also found. In ketoprofen and control rats with severe AA, the fraction unbound of both propranolol enantiomers were significantly related to AI (Fig. 3-23). Protein binding influences the Vd of highly protein bound drugs such as propranolol. Thus a severity-related influence of arthritis on protein binding may explain why others have found a two fold decrease in the Vd of propranolol in experimental inflammation (Bishop & Schneider, 1981; Walker *et al.*, 1986; Belpaire *et al.*, 1989), whereas we could not detect any significant differences in AA as compared to controls (Table 3-XVII). Paw swelling and inflammation were limited in the animals used for our experiments. Consequently, as results indicate that the free fraction of propranolol is related to disease severity (Fig 3-23), minimal changes in protein binding and Vd would be expected in our animals. On the other hand, it is likely that the animals used in previously cited studies were afflicted to a greater degree. Therefore a moderate to severe degree of inflammation, resulting in a greater reduction in the free fraction, likely influences the Vd of propranolol to a significant extent.

Therefore, a difference in disease severity between our study and those of others would likely explains the discrepancy between our findings and that of others on the Vd changes of propranolol in inflammation.

Surprisingly, although the hepatic uptake and subsequent metabolism of propranolol is reportedly non-protein restricted (Gariepy *et al.*, 1992), a significant relationship was found to exist between fu and CL_{oral} of R and S-propranolol in the severely afflicted animals (Fig. 3-24). In the isolated perfused liver, extraction of propranolol is unaffected by changes in AAG and albumin concentration unless the unbound fraction is reduced to less than 2% (Gariepy *et al.*, 1992). Therefore as the fu of S-propranolol is greater than 10% in both treated and control AA rats, its oral clearance should be independent of protein binding. Nevertheless, a substantial reduction in the metabolism capacity of liver may render the drug more sensitive to changes in factors such as protein binding. Hence, it is possible that intrinsic clearance has decreased to such an extent that propranolol no longer acts as a very high extraction drug. Similar suggestions were made by Walker *et al* (1986) and Belpaire *et al* (1989) to explain the decreased systemic clearance after iv doses of propranolol observed in rats with inflammation. However, propranolol displays saturable protein binding. At high concentrations of drug, such as those found in the portal vein after oral doses, there is proportionately less available binding sites. Hence as the unbound fraction increases, more free drug is available for metabolism by hepatic enzymes. Therefore, considering the saturable protein binding and extensive liver capacity for metabolism of propranolol, it is unlikely that a change in plasma protein binding in AA rats would result in altered first pass metabolism for the drug.

Another explanation for the apparent dependency of propranolol CL_{oral} on its free fraction can be due to the fact that CL_{oral} = Dose/AUC and both AUC and fu are significantly related to disease severity (Fig. 3-22, 3-23). Therefore, the relationship between fu and CL_{oral} may not indicate a dependency of CL_{oral} on protein binding but rather may be coincidental. In arthritis, there is a simultaneous increase in the production of specific acute phase proteins such as AAG along with a decreased formation of hepatic enzymes. The result is altered protein binding of propranolol along with a concurrent decrease in intrinsic clearance.

Nevertheless, although changes in the pharmacokinetics of propranolol in AA cannot be attributed soley to either an increased protein binding or a decreased intrinsic metabolism, the combination of the two events are likely responsible for the dramatic elevations in propranolol plasma concentrations seen after oral doses. Furthermore, as disease severity and therapeutic intervention influence arthritic-induced changes in the pharmacokinetics of a drug such as propranolol, it is apparent that these factors must be kept in mind when examining drug-disease interactions.

5. CONCLUSIONS

The purpose of this investigation was to examine the effect of inflammatory diseases such as arthritis on the stereoselective disposition of two model drugs. The methods developed to separate and quantitate enantiomers of acebutolol, diacetolol and propranolol were found to be convenient and suitable for use in pharmacokinetic studies. By utilizing these assays we were able to study the disposition of the individual enantiomers after administration of the racemate in humans and rats.

As the disposition and elimination characteristics of acebutolol enantiomers are comparable to human, it appears that the rat is an appropriate animal model in which to study the pharmacokinetics of acebutolol. In both humans and rats, there is a slight but significant stereoselective disposition of acebutolol and its metabolite. Similarities exist between the two species, in that the nonrenal clearance of R-acebutolol was greater than that of the S enantiomer. A stereoselective renal and biliary excretion of acebutolol in favour of the S enantiomer in the rat compensates for its lower hepatic clearance resulting in minimal differences in plasma enantiomer concentrations.

In humans, the decreased systemic availability of R-acebutolol and increased formation of R-diacetolol is likely a consequence of stereoselective first-pass hepatic metabolism. In rats, however, the enantioselective clearance was more pronounced after iv rather than oral doses, suggesting that systemic rather than presystemic metabolism is selective for the R enantiomer. Studies involving rapid and slow acetylator phenotypes may shed some information as to whether the hydrolysis or acetylation pathway is involved. Nevertheless, differences in enantioselectivity between the two species may be, in part, due to the quantitative differences in their presystemic formation of diacetolol.

Stereoselective renal elimination of R-diacetolol seen in both species appeared to be partly responsible for observed differences in the disposition of diacetolol enantiomers. The therapeutic consequence of the stereoselectivity in acebutolol and diacetolol disposition has yet to be determined, as although minor differences are seen in healthy subjects, altered physiological function in disease states may result in changes to enantioselectivity.

From the results obtained in elderly subjects, it appears that aging has an effect on the disposition of acebutolol and its metabolite, diacetolol. Diminishing renal function is associated with lower renal clearance values of acebutolol and diacetolol whereas age-related decrease in β of S-acebutolol, R- and S-diacetolol may be due to a decreased non-renal elimination. As reduced renal function was found to influence the enantiomeric ratio of acebutolol and diacetolol collected in urine as well as the S:R ratio of acebutolol in plasma, an active stereoselective pathway such as tubular secretion may also be affected in the elderly. Nevertheless, when comparing mean values from young subjects to those obtained in the elderly, only minor differences were observed. In an older population, however, these age-related changes in the disposition of acebutolol and diacetolol may be enhanced. It is, therefore, imperative to keep age in mind and to use age-matched controls when studying the effect of diseases such as arthritis on the pharmacokinetics of acebutolol.

Adjuvant arthritis was found to significantly influence the pharmacokinetics of the enantiomers of the ß-blockers, acebutolol and propranolol. After oral administration to severely afflicted arthritic rats, AUCs of R- and S-acebutolol were increased by 2.5 fold; whereas the AUCs of R and S-propranolol were 67 and 16 fold greater than controls, respectively. Differences in the extent to which propranolol and acebutolol were affected by arthritis may reflect dissimilarities in the extent to which these agents are subject to first-pass metabolism and/or their degree of protein binding. Propranolol is extensively cleared through first pass through the liver, and although acebutolol is also subject to presystemic metabolism, the magnitude of this clearance route is much less than that seen with propranolol. Furthermore other routes of elimination such as renal and biliary clearance significantly contribute to the clearance of acebutolol. Thus it is reasonable to assume that the decreased hepatic enzyme activity found in inflammation would have more pronounced effects on a drug such as propranolol. Likewise, although increased protein binding in arthritic rats cannot solely account for their dramatically elevated plasma concentrations of propranolol, it is conceivable that altered binding has a notable contribution. On the other hand, protein binding of acebutolol is limited and not significantly influenced by arthritis. Consequently, with both drugs, elevated plasma concentrations reflecting decreased metabolism are likely due to reduced intrinsic clearance.

Factors which have not been previously addressed, such as enantioselectivity, NSAID therapy and disease severity were all found to play a role in inflammation-induced changes in drug disposition. With regards to stereoselectivity in human arthritic subjects, the oral clearance of the S- but not the R enantiomer of acebutolol was significantly reduced. Oral clearances of R- and S-acebutolol were reduced by 22 and 25%, respectively. However, it is questionable as to whether this effect was truly enantioselective as arthritis did not significantly influence the plasma and urine S:R ratios. Nevertheless, the metabolism of acebutolol is stereoselective in both healthy and arthritic subjects, thus it is conceivable that there may be dissimilar effects of disease on the metabolic transformation of individual enantiomers. An interesting point, however, was that renal clearance of acebutolol was stereoselective in arthritic but not control patients. This suggests that inflammation possibly results in minor changes to the stereoselective nature of the renal transport system.

The stereoselective nature of arthritic-induced changes in drug disposition was more apparent with the agent, propranolol. Dissimilarity in the extent of effect that adjuvant arthritis had on the clearance of the individual enantiomers resulted in marked changes to the plasma enantiomer ratios. More importantly, in adjuvant arthritis measurement of total propranolol concentrations was more indicative of plasma levels of the inactive R- enantiomer, as S-propranolol only accounted for 7% of total (R+S) AUC after oral administration. However, in human arthritic subjects, it is possible that inflammation will influence the plasma concentrations of the therapeutically active enantiomer to a greater degree than those of the inactive enantiomer. Whereas plasma levels of the R- enantiomer are greater in rats, it is the active S- enantiomer concentration which is dominant for humans. In addition, in humans, binding to AAG is greater for the S isomer. This underlines the importance of measuring individual enantiomers when examining the effect of disease or drug interactions on the disposition of racemic drugs.

Disease severity and/or NSAID therapy is another factor which should be kept in mind. Therapeutically, the consequences of the reduction in the oral clearance of acebutolol were likely negligible as disposition changes were minor and plasma concentrations were still well within therapeutic range. This suggests that the liver has a high capacity for the metabolism of AC, thus a reduction in enzyme activity in arthritic subjects results in only subtle changes to acebutolol disposition. However, it is also possible that the pharmacokinetics of acebutolol were relatively unchanged due to a combination of the moderate degree of inflammation involved and NSAID therapy in these subjects. Hence, plasma concentrations of acebutolol may be further increased in an acute flare up or uncontrolled arthritis.

Indeed, in rats which were severely afflicted with adjuvant arthritis, peak plasma concentrations and the AUC of acebutolol were elevated to a greater degree than that observed in humans. The higher bioavailability of acebutolol resulting from decreased metabolism is likely due to a reduction in intrinsic clearance in arthritis. Nevertheless, given the severity of disease inflicted in these animals, it would appear that alterations in the pharmacokinetics of acebutolol and diacetolol were relatively minor. As there are numerous pathways of elimination for acebutolol it is possible, although there is a reduction in the hepatic clearance of acebutolol, that other non-hepatic clearance routes compensate in arthritis.

Increased plasma concentrations of the propranolol enantiomers were found to be significantly related to the degree of inflammation. This indicates that a strong relationship exists between the reduced hepatic enzyme activity associated with arthritis and the severity of the disease. Furthermore, treatment with the NSAID ketoprofen was shown to lower the arthritic-induced changes in propranolol disposition in line with clinical improvement of arthritic scores. Consequently, we found that NSAID therapy had a protective effect on liver metabolism in addition to symptomatic relief in adjuvant arthritis. The effectiveness of NSAID therapy was apparent primarily in severe arthritis, in which dramatic improvements in arthritic scores were observed. Thus measurement of physical and biochemical markers of inflammation in severe or uncontrolled arthritis may be helpful in planning dosage guidelines for arthritic patients. In mild arthritis, clinical improvement with NSAID therapy may be difficult to quantify. Furthermore in mild arthritis, other factors such as age, liver and renal function may influence drug disposition to a greater extent than the relative degree of inflammation. Altered protein binding, likely due to increased AAG levels, also reflects disease activity. This may, in part, explain the intersubject variation in pharmacokinetics of drugs in disease states. In light of this finding, studies which examine the effect of diseases such as inflammation on drug disposition must consider the patients disease severity and treatment.

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