NON-STEROIDAL ANTI-INFLAMMATORY DRUGS: PHARMACOKINETICS AND CLINICAL RESPONSE IN RHEUMATOID ARTHRITIS

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to the

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DECLARATION

The research work described in this thesis was carried out during my appointment as Research Assistant in the Department of Materia Medica, Stobhill General Hospital, Glasgow. This thesis would not have been possible without the cooperation and collaboration of medical and nursing colleagues. While all the analytical work presented in this thesis was carried out by myself, the rheumatological assessments were carried out by trained clinical metrologists.

Quotations have been distinguished and sources of information acknowledged. Some of the work has been presented to Learned Societies or accepted for publication and a list of presentations and publications is included. The writing of this thesis was entirely my own work.

It is important to note that all patients gave informed written consent before participating in the studies described in this thesis, and all studies were approved by the Research and Ethical Committee of Unit 1 North, Greater Glasgow Health Board.

ACKNOWLEDGEMENTS

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Finally, I am indebted to my husband, Greg, for his continuous support and encouragement.

SUMMARY

Comparative studies of non-steroidal anti-inflammatory drugs (NSAIDs) in rheumatoid arthritis indicate that patient response is variable and unpredictable. Although variability in pharmacokinetics might be implicated, no study has been able to demonstrate this. Changes in patient response to increments in dose or concentration have been difficult to detect, possibly due to the variable nature of the disease, to individual differences in disease severity and to the subjective nature of the rheumatological measurements.

In this thesis the response to increments in dose or concentration of two NSAIDs, fenclofenac and naproxen, were investigated in patients with rheumatoid arthritis. In both cases three doses were given to all patients in a randomised double-blind design. Attention was focused on the determination of pharmacokinetic variability and the utility of plasma concentrations in the explanation of clinical response. In addition, the disposition of indomethacin in plasma and synovial fluid was studied.

Analytical techniques were developed for the accurate measurement of plasma concentrations by high performance liquid chromatography and for the determination of the concentration of these drugs not bound to plasma proteins using equilibrium dialysis.

The variability in the pharmacokinetics of the NSAIDs was assessed by performing single dose studies. There was considerable variability in the clearance of both

fenclofenac and naproxen. The clearance of fenclofenac appeared to be reduced in patients with raised alkaline phosphatase and with increasing age. The clearance of naproxen was also reduced in the elderly and appeared to be lower in female patients.

Linearity or non-linearity in the kinetics was determined from trough samples taken at steady state on each dose. The kinetics of fenclofenac (free plus bound) over the dose range 600 to 1800mg/day were consistent with linearity, but the kinetics of naproxen over the dose range 500 to 1500mg/day were non-linear in all patients.

Protein binding studies confirmed that the non-linearity could be explained in terms of saturation of naproxen binding sites on plasma proteins. Saturation of binding also occured with fenclofenac but the increase in the free fraction with increasing total concentration was less dramatic for fenclofenac than for naproxen and did not appear to have a significant effect on the kinetics of total fenclofenac. There was, however, a linear increase in free concentrations.

The indomethacin study showed that there was some variability in the concentrations of indomethacin achieved in synovial fluid. There were also variations in the rate of input and output from synovial fluid. These factors may also be important in determining the variability in clinical response to NSAIDs. During the elimination phase a concentration gradient between plasma and synovial fluid was identified. The concentration in synovial fluid was in

general at least twice that in plasma at later times and may explain the extended clinical effect produced by NSAIDs.

The relationship between dose or plasma concentration (total or free) of fenclofenac and naproxen and clinical response (Ritchie Articular Index, duration of morning stiffness, mean grip strength and the analogue pain score) was in general most appropriately described in terms of a simple linear model which took account of inter-individual disease severity (individual intercept). The improvement in symptoms (if any) which occured with increments in dose or concentration was described by a common slope.

In general knowledge of the inter-individual variability in total or free concentration at steady state added little to the explanation of the clinical response if the dose was known. As a result of the considerable inter-individual variability in response, the average clinical improvement with increments in dose (or concentration) were not dramatic. Often the greatest improvement was observed between no treatment and the lowest dose. Further increases in dose were not associated with a proportional improvement in response. This suggested that the initial (no treatment) state was exaggerated (assessments carried out under non-blinded conditions) or that the doses used currently in clinical practice are close to those necessary to achieve a maximum response.

If assessments carried out after withdrawal of previous

therapy were included in the analysis, a hyperbolic or E_{max} model was more appropriate in some cases. This was more apparent for naproxen (analysed in terms of dose or free concentration) than for fenclofenac. The relationship between total naproxen and response was still most appropriately described by a linear model as both response and concentration tended to plateau. In both studies the analogue pain score appeared to be the most sensitive measure of changes in response.

There were no serious side-effects experienced with either of these drugs. The small number of patients precluded any formal study of the relationship between side-effects and concentration.

In the absence of concentration related toxicity, the findings presented in this thesis suggest that if the dose of the NSAID is increased, on average an improvement in response can be expected. Drug concentrations measurements (in plasma) appear to be unnecessary in clinical practice. NSAID pharmacokinetic variability appears to contribute little to the total variability in clinical response to these drugs.

CHAPTER 1

GENERAL INTRODUCTION AND BACKGROUND

1.1 GENERAL INTRODUCTION

The work described in this thesis arose from a desire to optimise the therapeutic use of non-steroidal anti-inflammatory drugs (NSAIDs) in the treatment of rheumatoid arthritis. The number of NSAIDs available to the rheumatologist has increased dramatically during recent years. The choice and dose of drug remains, however, relatively empirical. This may in part be a result of the lack of good data concerning the variability in the pharmacokinetics of these drugs, or conclusive data relating plasma or synovial fluid concentration and clinical response.

The withdrawal of benoxaprofen, after its use in the elderly was associated with fatal hepatic toxicity highlighted the potential risks associated with the use of drugs of this class. Indeed, one of the drugs studied in this thesis (fenclofenac) was discontinued recently because of a high incidence of skin rashes.

There is a need to investigate the variability in the pharmacokinetics of these drugs and to determine whether differences can be explained in terms of patient specific factors. Together with information regarding the relationship between concentration and clinical effect or toxicity, this would allow a more rational use of these drugs.

In this thesis Chapter 1 gives a general introduction. The analytical techniques used to determine total and free drug concentrations are presented in Chapter 2. Chapter 3 describes the traditional rheumatological assessments, the assessments chosen for the subsequent studies and introduces

some of the newer objective measurements which might prove useful in the future. This is followed in Chapter 4 by an outline of the general approach used in the analysis of data.

Chapters 5 and 6 present two controlled studies of naproxen and fenclofenac in patients with rheumatoid arthritis to determine whether knowledge of inter-individual differences in the pharmacokinetics of these NSAIDs can contribute to the explanation of the clinical response. Chapter 7 presents a single dose study of a slow release preparation of indomethacin to elucidate the relationships between concentrations in plasma and synovial fluid.

The final chapter presents a general discussion of the results and clinical implications.

1.2 RHEUMATOID ARTHRITIS AND ITS TREATMENT

Rheumatoid arthritis is a chronic systemic disease characterised by inflammatory arthritis of the peripheral joints. The aetiology of the disease is largely unknown, although there appears to be some immunological basis for it which results in a chronic inflammatory response. There is evidence that the disease is an autoimmune disorder which has to be triggered by some genetic or environmental factor.

Inflammation is the normal response to tissue injury and is characterised by heat, redness, swelling, tenderness and pain. During the inflammatory response, chemical mediators such as 5-hydroxytryptamine (5-HT), slow-reacting substance of anaphylaxis (SRS-A), various chemotactic factors, bradykinin and prostaglandins are

liberated locally. Phagocytic cells migrate into the area and cellular lysosomal membranes may be ruptured, releasing lytic enzymes.

The inflammatory response in rheumatoid arthritis probably occurs due to the combination of an antigen (gamma globulin) with an antibody (rheumatoid factor) and complement, causing the local release of chemotactic factors that attract leucocytes. The leucocytes phagocytose the complexes of antigen, antibody and complement and in doing so release lysosomal enzymes. This leads to a continuous inflammatory reaction and eventually to extensive tissue damage.

The drugs available to treat rheumatoid arthritis are generally divided into two types:

1. NSAIDs which provide symptomatic relief by reducing the inflammation. For most patients, relief from pain and stiffness caused by inflammation develops within a week of treatment.

2. 'Second-line' drugs such as gold (sodium aurothiomalate) and penicillamine which appear to have some disease modifying effects which develop much more gradually over several months.

Steroids, have features of both types of drug and in addition claims have been made recently that some of the newer NSAIDs may have some disease modifying properties (eg benoxaprofen and fenclofenac). The use of steroids and 'second-line' drugs is , however, restricted by the serious toxic effects that often develop during treatment. Thus emphasising the desire to obtain the maximum therapeutic effect from the less toxic NSAIDs.

1.3 NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

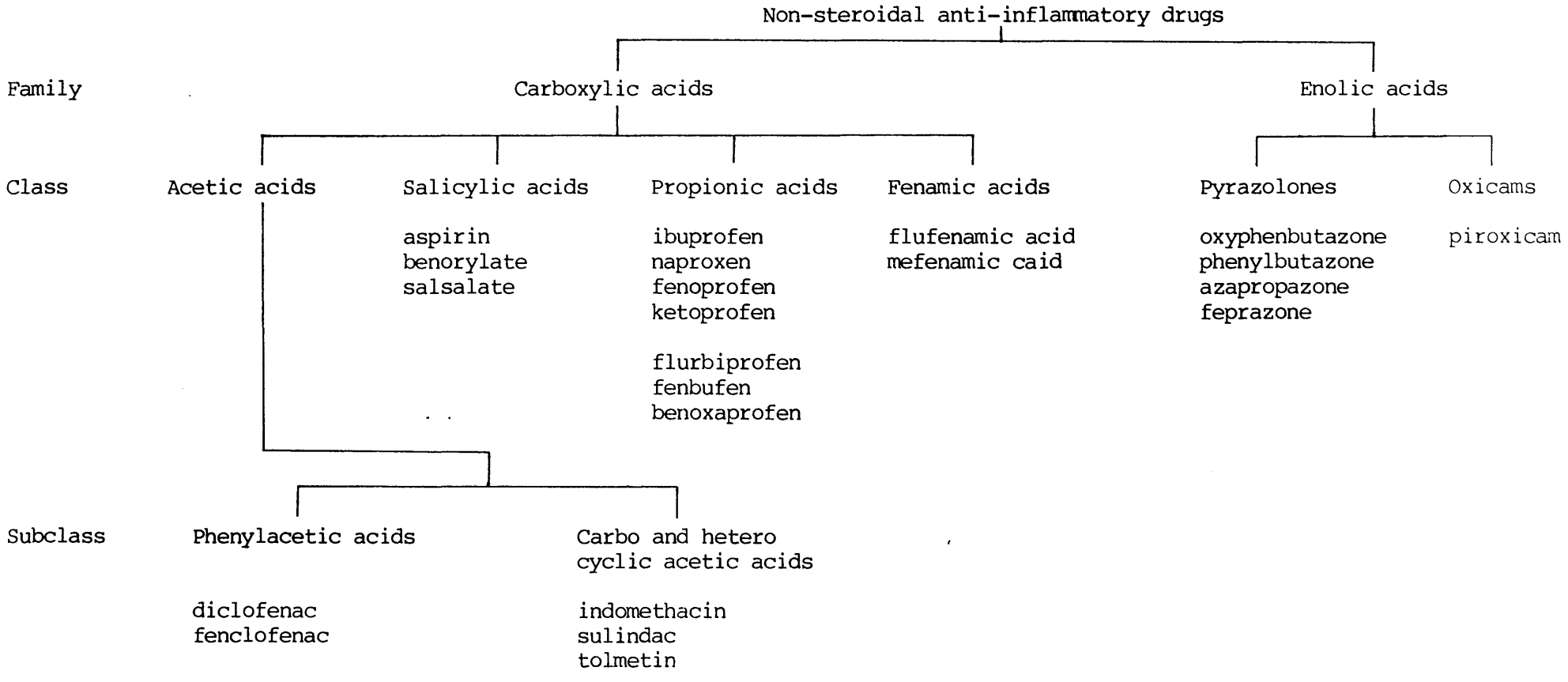
1.3.1 Mechanism of action of NSAIDs

The NSAIDs are a diverse group of compounds (although in general they are all carboxylic acids) which share certain therapeutic actions and side-effects (Table 1.1). Salicylic acid had been used for a number of years to reduce the symptoms of inflammation, but until recently the mode of action was largely unknown. In 1971 Vane and others discovered that low concentrations of aspirin and indomethacin inhibited the enzymatic production of prostaglandins (Vane, 1971; Smith & Willis, 1971; Ferreira, Moncada & Vane, 1971). This, together with the evidence that prostaglandins contributed to the pathogenesis of inflammation led Vane to propose that inhibition of prostaglandin synthesis explains the therapeutic and some of the toxic effects of the NSAIDs. Prostaglandins generally act as vasodilators, and they potentiate the pain and oedema induced by other mediators such as bradykinin and histamine which are also released during inflammation.

Correlations between the relative anti-inflammatory potency in animal models of inflammation and the reduction in prostaglandin concentrations support the view that the major effect of these drugs can be accounted for by the inhibition of prostaglandin synthesis (Higgs, Moncada & Vane, 1980).

Two enzymes convert phospholipid derived arachidonic acid to a number of substances known as 'eicosanoids' (Figure 1.1). The NSAIDs in general selectively inhibit the enzyme

TABLE 1.1 Classification of non-steroidal anti-inflammatory drugs



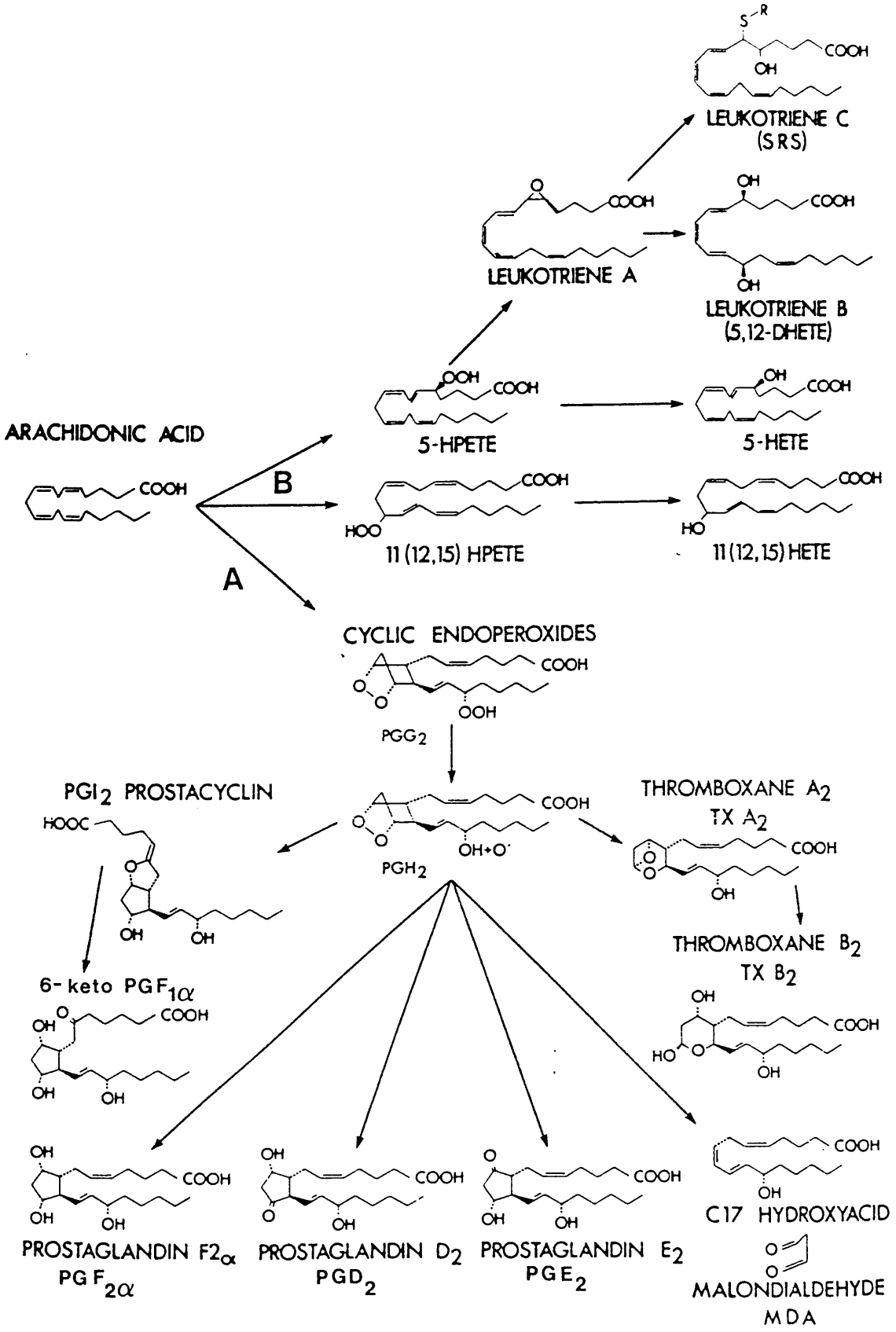


FIGURE 1.1 The formation of prostaglandins, thromboxanes and leucotrienes from arachidonic acid

A Cyclo-oxygenase pathway
B Lipoxygenase pathways

cyclo-oxygenase which catalyses the conversion of arachidonic acid into endoperoxides and then by the action of isomerases to the prostaglandins and thromboxane. The second pathway is catalysed by lipoxygenase(s), arachidonic acid is converted to an unstable hydroperoxy acid and eventually to non-cyclysed hydroxy acids (HETE), and leucotrienes. All tissues except red blood cells are capable of producing prostaglandins in response to injury. Prostaglandins are not stored, and their release reflects de novo synthesis. The cyclo-oxygenase enzyme appears to be tissue specific; the potency of inhibition by NSAIDs varies from tissue to tissue (Flower & Vane 1974). In addition different tissues produce different profiles of prostaglandin products possibly due to different isomerase enzyme activities present in the tissue. The major prostaglandins identified in synovial effusions of patients with rheumatoid arthritis were PGE_2, TXB_2 (the stable breakdown product of TXA_2) and 6-keto-$PGF_{1\alpha}$ (the stable breakdown product of PGI_2). However, the ratio of the different cyclo-oxygenase products showed considerable inter-subject variability indicating a heterogeneous cellular origin (Bombardieri et al, 1981). In addition, the extent of inhibition of prostaglandin synthesis by indoprofen ranged from 33% for 6-keto-$PGF_{1\alpha}$ to 90% for PGE_2.

Most NSAIDs are reversible inhibitors of cyclo-oxygenase and the effect of indomethacin on prostaglandin synthesis in vivo decreases as the drug is eliminated (Kane et al, 1978).

The NSAIDs also inhibit or interfere with a variety of other enzyme or cellular systems, and these effects may also contribute to their clinical effects. The concentrations required, however, tend to be considerably higher than those necessary to produce a therapeutic effect. Inhibition of the migration of leucocytes or monocytes into inflamed sites has been reported for some NSAIDs, doses required to reduce leucocyte migration are in general considerably higher than those which prevent oedema and the effects are species specific (Higgs et al, 1980).

Some NSAIDs appear to have a differential effect on leucocyte migration *in vivo*. Indomethacin, aspirin and flurbiprofen enhance the accumulation of cells in inflammatory exudates at doses which significantly reduce prostaglandin production, but inhibit cell migration at higher doses (Higgs et al, 1980). This observation can be explained if the inhibition of cyclo-oxygenase diverts substrate towards the production of chemotactic lipoxygenase products, which then account for increased leucocyte migration. The subsequent inhibition of leucocyte migration at higher doses may be explained by a non-specific inhibition of arachidonic acid peroxidation.

In addition, prostaglandins released by macrophages *in vitro* appear to have a negative feedback effect on the production of lymphokines by T lymphocytes (Gordon, Bray & Morley, 1976). If a negative feedback on T cell function by prostaglandins is important *in vivo* and if the enhancement of the production of chemotactic lipoxygenase products

occurs with the inhibition of cyclo-oxygenase, NSAIDs, although alleviating the symptoms of inflammation, may enhance certain features of chronic inflammatory disease.

1.3.2 NSAIDs investigated in this thesis

(i) Fenclofenac

Fenclofenac, a phenyl acetic derivative, was developed in the mid 1970's for the treatment of chronic inflammatory disorders. In animal models of inflammation, Atkinson & Leach (1976) found that the anti-inflammatory profile of fenclofenac was different from other common NSAIDs. Fenclofenac was only slightly effective in an acute inflammation model (rat carrageenan paw oedema), while it was relatively more effective in a chronic model of inflammation (adjuvant arthritis in the rat).

The efficacy of a standard 1200mg daily dose was shown to compare favourably with 150mg of indomethacin daily (Aylward et al, 1980) and to be more effective than 750mg of naproxen daily (Tiselius, 1980). In long term trials of fenclofenac the frequency of gastrointestinal side effects compared favourably with other NSAIDs (Smith, 1977). The observation that there was a reduction in the ESR during long term treatment suggested that fenclofenac possessed some disease modifying effects. One study indicated that there were significant improvements in both clinical and laboratory indices (eg C-reactive protein) after 6 months when no significant effects had been observed after 3 months (Berry et al, 1980).

Fenclofenac, however, was withdrawn from clinical use by the Committee on Safety of Medicines, shortly after the completion of the study described in Chapter 5 because in their view, the use of fenclofenac was associated with an unacceptably high incidence of skin rashes. In earlier clinical studies, the incidence of skin rashes was approximately 14% during long term treatment (Smith, 1977).

(ii) Naproxen

Naproxen, a propionic acid derivative, was introduced in 1973 and has subsequently become a standard in this class of NSAID. Like other alpha substituted propionic acids, naproxen is a chiral compound. Only the S(+)-enantiomer is anti-inflammatory and an inhibitor of cyclo-oxygenase. The pharmaceutical preparation contains only the active isomer.

In rheumatoid arthritis, naproxen is effective and well tolerated. In a study of four propionic acid derivatives, naproxen (500mg/day) combined the greatest efficacy with the lowest incidence of side-effects (Huskisson et al, 1976). Naproxen is generally prescribed in doses of 500 to 1000mg daily (two divided doses).

(iii) Indomethacin

Indomethacin has been used in the treatment of rheumatoid arthritis for over 20 years. After, the discovery that the therapeutic action of indomethacin could be explained in terms of prostaglandin synthetase inhibition, it became a reference for comparison with newer agents. Side-effects tend to be more common for

indomethacin than for the newer NSAIDs. Headache was reported in over 10% of patients treated with indomethacin (Rhymer & Gengos, 1979). A relationship between central nervous system side effects and high peak concentrations of indomethacin has been noted (Baber et al, 1978).

Indocid-R, marketed as a 'slow release' preparation of indomethacin, has been designed to give flatter concentration profiles, theoretically minimising the side-effects often associated with high peak concentrations.

1.4 PHARMACOKINETICS OF NSAIDs

The important factors which determine the concentration of a drug in plasma are:

(a) the presence or absence of linear kinetics, and

(b) the extent of inter-subject variability in the kinetics.

The following background to the pharmacokinetics of NSAIDs concentrates on the specific drugs investigated in this thesis.

1.4.1 Absorption

In general the absorption of NSAIDs is rapid and bioavailability is close to 100%. Although food reduces the rate of absorption and the peak concentration, the overall bioavailability appears to be unaltered. This has been observed for fenclofenac (Henson et al, 1980) and naproxen (Runkel et al, 1972).

The absorption of indomethacin after oral administration was complete as judged from material balance studies (Duggan et al, 1972). However, subsequent studies have suggested that indomethacin undergoes significant enterohepatic recirculation (Kwan et al, 1976). Published bioavailability studies must be interpreted with caution.

1.4.2 Metabolism

NSAIDs are generally eliminated entirely by hepatic metabolism, little parent drug is eliminated in the urine unchanged. In general the drugs are excreted as conjugates of the parent drug or any oxidative metabolites.

The oxidative metabolism of fenclofenac is illustrated in Figure 1.2. More than 93% of an oral dose has been shown to be excreted in the urine (>93%) in the form of conjugates of the parent compound and the hydroxylated metabolites (Hucker, Kwan & Duggan, 1980).

The only oxidative reaction identified for naproxen is O-demethylation to give desmethyl naproxen (DMN). The parent drug and DMN are conjugated, mainly with glucuronic acid (Figure 1.3) and the major metabolite recovered in urine is naproxen glucuronide (Runkel et al, 1972, 1976). These earlier metabolic studies suggested that about 10% of a dose was excreted unchanged in urine (Runkel et al, 1976), however, subsequent studies have indicated that negligible naproxen is excreted unchanged, and that naproxen is liberated from the glucuronide in urine while stored frozen

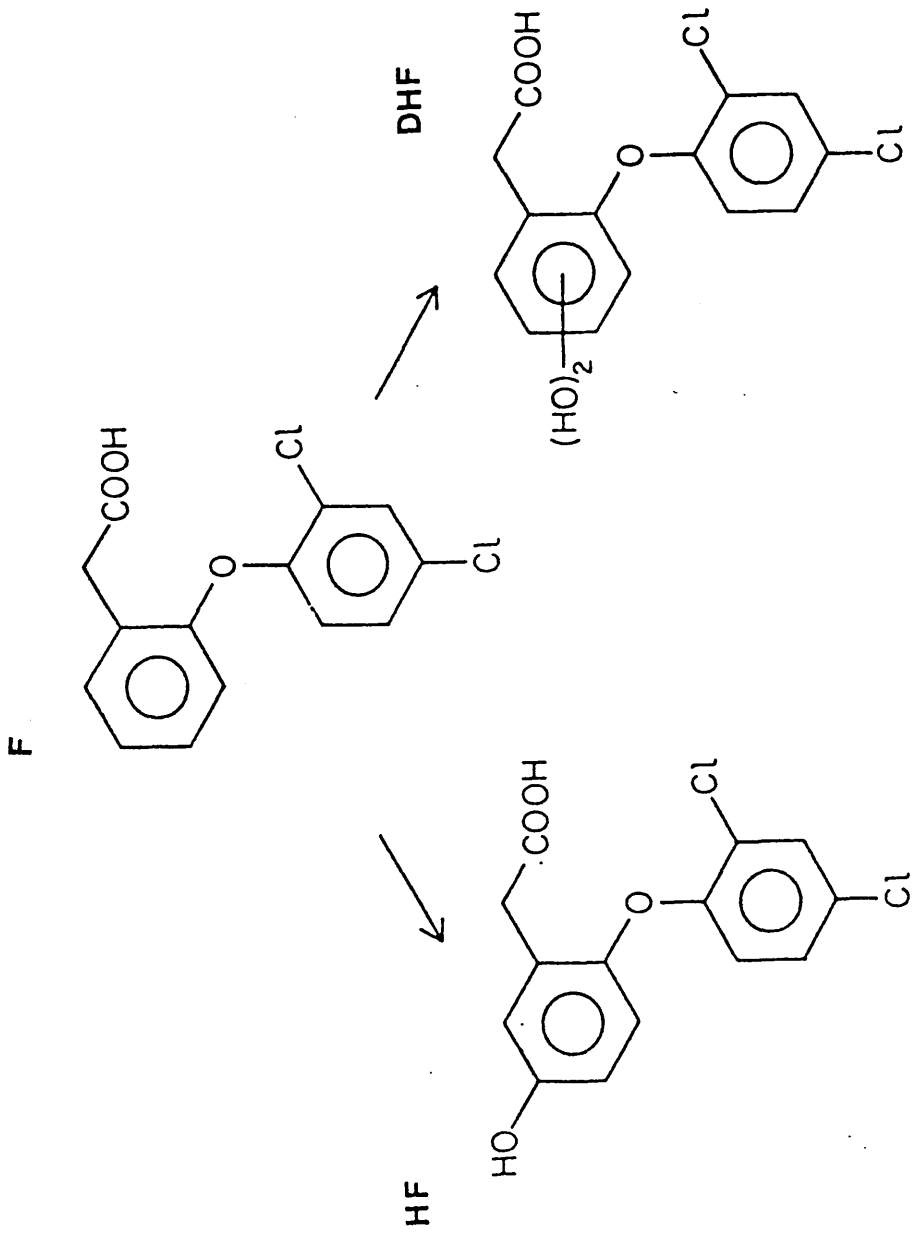


FIGURE 1.2 The oxidative metabolism of fenclofenac

Key: F fenclofenac
HF hydroxyfenclofenac
DHF di-hydroxyfenclofenac

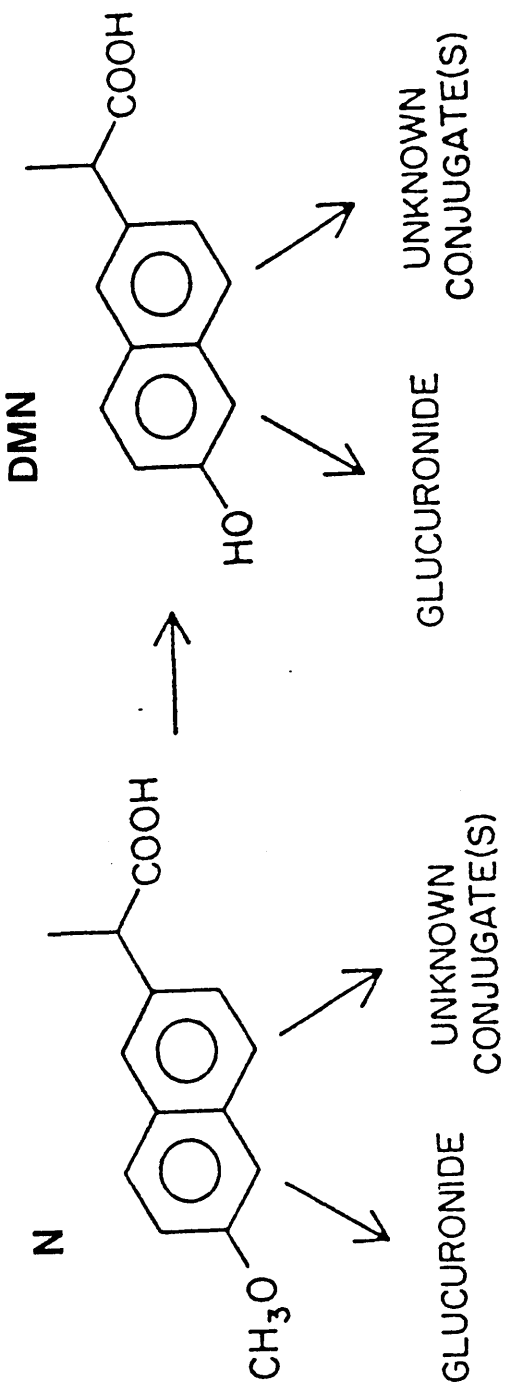


FIGURE 1.3 The metabolism of naproxen

Key: N naproxen
DMN 6-O-desmethylnaproxen

(Upton et al, 1980b).

Indomethacin is metabolised extensively (Figure 1.4). O-demethylation and N-dealkylation to give desmethyl (DMI), desbenzoyl (DBI) and desmethyl-desbenzoyl (DMBI) metabolites followed by conjugation (Duggan et al, 1972). The major pathway is demethylation followed by dealkylation.

1.4.3 Distribution and elimination

The clearance of NSAIDs is generally less than liver blood flow and is therefore affected by differences in protein binding and hepatic metabolic activity (Wilkinson & Shand, 1975). There is therefore scope for considerable inter-subject variability in the elimination of these drugs. The volume of distribution of NSAIDs is small and of the order of 10 to 20 l due to the high degree of plasma protein binding. In general around 99% of the total drug in plasma is bound to protein.

(ii) Naproxen

The elimination half-life of naproxen in healthy volunteers is about 14 hours (Runkel et al, 1974 & 1976). In healthy young male volunteers, the clearance of total naproxen at steady state on 375mg twice daily was 0.547±0.083 l/h (Upton et al, 1984). Early pharmacokinetic studies indicated that with single doses of naproxen over 500mg, there was a less than proportional increase in the AUC with further increments in dose up to 4g (Runkel et al, 1974 & 1976). In a study where radiolabelled naproxen was

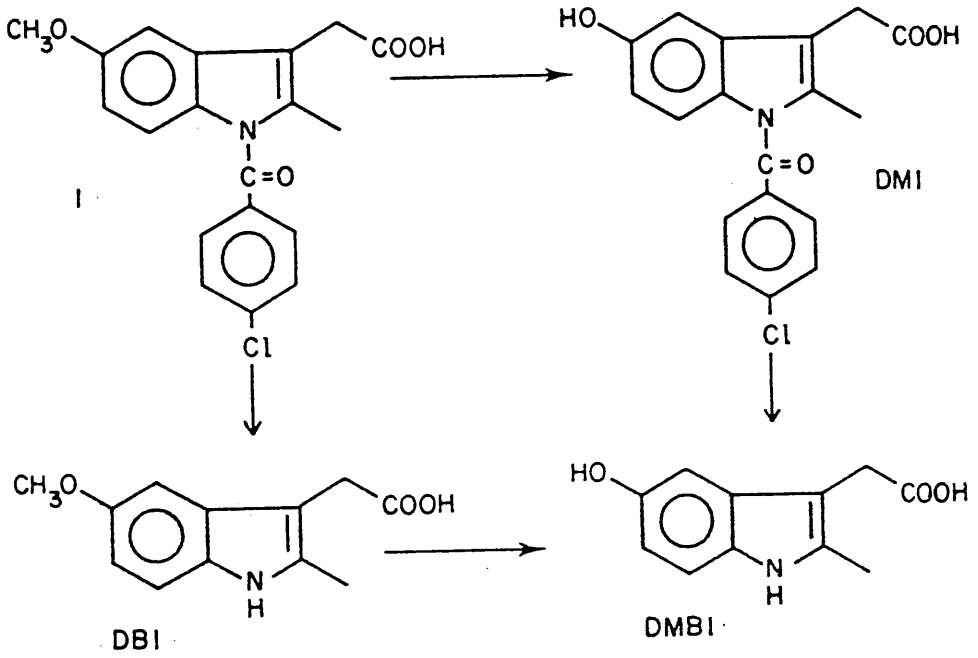


FIGURE 1.4 The metabolism of indomethacin

Key: I indomethacin
DMI desmethyl indomethacin
DBI desbenzoyl indomethacin
DMBI desmethyl-desbenzoyl indomethacin

given, recovery of drug in the urine indicated that this effect could not be due to reduced absorption of the larger doses (Runkel et al, 1974): there was little difference in the percentages of the various metabolites recovered in the urine.

Naproxen is bound principally to albumin. At a total concentration of 100µg/ml the percentage bound to human plasma and isolated human plasma albumin was 99% and 96% respectively (Calvo & Dominguez-Gil, 1983). At a total concentration of 5µg/ml, however, the percentage bound was higher in isolated human serum albumin (99.91%) than in plasma (99.79%) (Piafsky & Borga, 1977). These workers also found that naproxen showed little affinity for α_1 acid glycoprotein (α_1AGP).

Scatchard analysis of the binding of naproxen to solutions of human albumin, bovine serum albumin and human plasma indicate that naproxen is bound to at least two distinct binding sites (Calvo & Dominguez-Gill, 1983; Kaneo et al, 1981; Runkel et al, 1976).

The dose dependent kinetics of naproxen and other NSAIDs such as ibuprofen (Lockwood et al, 1983) have been explained in terms of the non-linear binding to plasma proteins which occurs at concentrations achieved clinically. In vitro studies indicate that the percentage of free naproxen ranged from 0.37 at a total concentration of 23µg/ml to 0.95 at a total concentration of 150µg/ml (Runkel et al, 1974). Any changes in the binding to plasma protein will affect the apparent clearance of total drug and the

volume of distribution.

(ii) Fenclofenac

There are few data on the pharmacokinetics of fenclofenac. Two studies have been published, one in healthy volunteers and the other in children with juvenile rheumatoid arthritis (Henson et al, 1980 & Makela et al, 1983). In healthy male volunteers the mean elimination half-life was 27 hours and ranged from 20-38 hours. The mean apparent clearance of fenclofenac (±SEM) was 0.38 (±0.04) l/h after a single 600mg oral dose. The hepatic extraction ratio was of the order of 0.007.

Up to 100µg/ml, the free fraction of fenclofenac was 0.3%. Above this the free fraction increased with increasing total concentration (Brewster & Muir, 1978). There was a suggestion from the data in healthy individuals that there was a non-linear increase in plasma concentrations on multiple dosing as assessed by differences in observed and predicted concentrations at steady state. Although the elimination half-life was comparable the clearance and volume of distribution were apparently higher after multiple dosing, suggesting the presence of non-linear kinetics due to saturation of binding to plasma protein (Henson et al, 1980).

In 17 children aged 4-14years, fenclofenac was given in doses of 10-25mg/kg body weight (in two divided doses) for up to 3 weeks. The mean elimination half-life was 25.4 hours and ranged from 15-39 hours (Makela et al, 1983). There was a linear relationship between peak plasma concentrations and

dose and between trough plasma concentration and dose.

(iii) Indomethacin

Alvan et al (1975) reported that the elimination of indomethacin could be approximated by a two compartment model and that this model was adequate to predict plasma concentrations at steady state after repeated dosing. The elimination half-life ranged from 2.6 to 11.2 hours and the plasma clearance ranged from 0.044 to 0.109 l/hr/kg. Despite variable kinetics suggested by the single dose study, plasma concentrations at steady state were quite similar between subjects. It was noted that terminal concentration time points did not decline exponentially, possibly due to enterohepatic recirculation and subsequently Kwan et al (1976) found that the two compartment model was inadequate. A more complex model was proposed to account for enterohepatic recirculation. There was no evidence of dose dependent kinetics (Alvan et al, 1975).

1.4.4 Pharmacokinetics of NSAIDs in age and disease

There are no data on the kinetics of fenclofenac in elderly patients or in renal or hepatic disease.

Upton et al (1984) found there was no significant difference in the clearance of total naproxen in young and elderly healthy male volunteers at steady state. However, in the elderly group, reduced binding to plasma protein masked a 50% decrement in the intrinsic clearance of naproxen in the elderly as estimated by the unbound clearance. In young and elderly patients with osteoarthritis, age was associated

with an increase in the elimination half-life of naproxen and higher total naproxen concentrations despite the fact that the albumin concentrations were similar (McVerry et al, 1986). Both of these studies indicate that there is a reduction in the intrinsic clearance of naproxen with age.

The elimination half-life of total naproxen was equal in healthy individuals and patients with moderate or severe renal failure (Anttila, Haataja & Kasanen, 1980). Serum concentrations of total drug tended to be lower in patients with severe renal failure, while concentrations of DMN were considerably higher. The clearance of free drug was not determined so it was established whether renal impairment was associated with a decrease in the intrinsic clearance. There was, however a correlation between the percentage of free drug (at a total concentration of 50µg/ml) and serum creatinine.

There was a reduction in the clearance of free drug in patients with alcoholic cirrhosis compared with healthy volunteers. On the basis of total drug, however, there was no evidence of any difference in the elimination between the two groups. Again lower albumin concentration in patients with cirrhosis masked a reduction in the intrinsic clearance of free naproxen (Williams et al, 1984).

There is some evidence that glucuronides of naproxen and ketoprofen are labile in plasma, and that reduced renal function will result in the accumulation of glucuronide and subsequent liberation of the parent drug (Upton et al, 1980b; Upton et al, 1982; Verbeeck, Wallace & Loewen, 1984).

Benoxaprofen, one of the newer NSAIDs, was claimed to be an inhibitor of both cyclo-oxygenase and the lipoxygenase enzyme (Walker & Dawson, 1979). Reports of the effect of age or renal impairment were conflicting but in general they suggested that the elimination of benoxaprofen was reduced with increasing age or decreasing renal function (Arnoff et al, 1982; Hamdy et al, 1982). In addition, these studies only investigated the clearance of total benoxaprofen, and it is likely that reduced protein binding in renal impairment or age would have masked an even greater reduction in the intrinsic clearance. Later reports of fatal cholestatic jaundice often associated with nephrotoxicity in the elderly led to the withdrawal of benoxaprofen from clinical use (Taggart & Alderice, 1982).

1.5 NSAID DOSE AND CONCENTRATION-RESPONSE RELATIONSHIPS

In the past decade, measurement of plasma concentrations of a number of drugs has become an integral part of routine clinical practice. Monitoring of salicylate was widely practiced by physicians in the treatment of rheumatic fever and has also been used to guide aspirin therapy in rheumatoid arthritis. However a recent controlled study has indicated monitoring salicylate offers no improvement over the standardised procedure of systematically increasing the dose until side effects appear or a dose of 6g/day is reached (Tugwell et al, 1984).

The rationale for monitoring plasma concentrations of

drugs is based on several prerequisites, the most important of which is that there should be a better correlation between plasma concentrations and the pharmacological effect than between the administered dose and clinical response. Studies of the pharmacokinetics of NSAIDs have indeed indicated considerable inter-individual variability in the pharmacokinetics of these drugs which are eliminated almost exclusively by hepatic metabolism. Of the host of NSAIDs available, however, there is little or no information on minimum effective concentrations, therapeutic ranges or toxic concentrations.

A more rational approach to the use of NSAIDs is needed. Doses of these drugs are often increased by physicians and patients above those recommended. Although this suggests that patients achieve greater effect from the higher doses, the possibility of toxicity cannot be ignored. NSAIDs are traditionally prescribed in fixed doses to patients of all ages despite the fact that renal impairment may reduce the elimination of these drugs. A drug that is not toxic in healthy adults may cause serious toxicity in the elderly due to accumulation of the parent drug or a particular metabolite.

It has been found in practice that despite the common mode of action of all NSAIDs some patients will respond to one but not another (Scott et al, 1982; Huskisson et al, 1976). In 1976 Huskisson and colleagues suggested that from the results of a study comparing four different propionic acid derivatives (ibuprofen, naproxen, ketoprofen and

fenoprofen) there was considerable variation in individual responses to different drugs in terms of both effectiveness and the incidence of side effects. He proposed that: "Since we cannot yet predict which patients will respond to a particular drug it may be necessary to try them all to find the best."

It is possible, however, that a poor clinical response may in part be due to pharmacokinetic variability and that there is more room for dosage adjustment than is normally practiced with NSAIDs. The results of studies to answer these questions have been inconclusive often due to inappropriate study design and variability in patient response.

There was no correlation between plasma concentration and clinical effect for phenylbutazone (Brooks et al, 1975; Orme et al, 1976) indomethacin (Ekstrand et al, 1980) or ibuprofen (Grennan et al, 1983). However these results are not altogether surprising as dose response relationships within an individual have in general been very difficult to detect. The results have been disappointing for a number of reasons:

a) Clinical response is often determined over a small range of doses, usually at the upper end of the dose range (Grennan et al, 1983). There was no difference in the response to three doses of indomethacin 45, 75 and 100mg/day (Ekstrand et al, 1980) however this was not entirely unexpected as at a daily dose of 37.5mg, indomethacin was associated with a 60% reduction in the excretion of prostaglandins (Rane et al, 1978).

b) There is considerable intra-individual variability in the response measures and often too few patients have been studied to achieve significance (Orme et al, 1976).

c) The inter-subject variability in disease severity must be taken into account in the analysis of this type of data.

d) Patients should exhibit active disease, a 'flare' of symptoms when anti-inflammatory treatment is withdrawn should be demonstrated. Patients will not respond to an NSAIDs if there is no inflammation.

There was no difference in the pharmacokinetics of flurbiprofen and indomethacin at steady state in responders and non-responders (Capell, Konetshnik and Glass, 1977; Baber et al, 1979).

To date, only one study has demonstrated a concentration-response relationship (Day et al, 1982). However, in this study there was no evidence that naproxen concentration gave an improved description of clinical effect over dose.

The propionic acid derivatives exist as stereo isomers, the pharmacological activity residing in the S(+)-enantiomer. With the exception of naproxen these drugs (eg ibuprofen, flurbiprofen, and ketoprofen) are given as enantiomeric mixtures. Metabolic chiral inversion of the inactive R(-)-enantiomer to the active isomer has been identified in man in vivo (Hutt & Caldwell, 1983) for ibuprofen and benoxaprofen. On average, 63% of an administered dose of R(-)-ibuprofen is inverted to the S(+)-isomer (Lee et al, 1985). Inter-individual differences in the elimination of the respective isomers may also add to

the problems in the detection of a concentration-response relationship if only total drug is measured.

The failure to establish a plasma concentration - response relationship for a number of NSAIDs might indicate that other factors determine the pharmacological response. NSAIDs must cross the synovial barrier to reach their site of action, and clinical effect might be more closely related to the concentration of total or free drug achieved in synovial fluid or synovial tissue.

The accumulation of acidic and non-acidic NSAIDs has been compared in acute and chronic animal models of inflammation (Graf, Glatt & Brune, 1975). There was a greater accumulation of the acidic NSAIDs in inflamed tissue. This could be explained in terms of an ion trapping effect (the lower pH in inflamed tissues will lead to a greater proportion of the acidic drugs in the un-ionised form, ie the drug will be more lipophilic) in addition to the higher degree of protein binding exhibited by acidic NSAIDs. Concentrations in inflamed joints were considerably higher than in controls. This observation has been proposed as the reason why non-acidic aspirin-like drugs have little anti-inflammatory activity (Brune, Rainsford & Schweitzer, 1980). In rheumatoid arthritis, the 'levels' of oxyphenbutazone were significantly higher in patients with actively inflamed joints than in patients with little or no inflammation (Gaucher et al, 1983). It is more practical, however, to determine the concentration in synovial fluid. Studies in patients have found that synovial tissue concentrations were

either similar to or less than concentrations in synovial fluid (Franke, Manz & Glynn, 1976; Jalava et al, 1977).

Simkin (1979) has proposed that the synovium behaves as a double barrier (previously regarded as a single barrier or simple 'dialysis membrane') between plasma and synovial fluid (Figure 1.5). It is proposed that passive diffusion through the interstitial space limits the overall trans-synovial exchange of most small molecules. The microvascular endothelium determines synovial permeability to proteins. In rheumatoid arthritis, microvascular changes may increase the permeability to proteins while interstitial changes (cellular hyperplasia, infiltration of inflammatory cells and deposition of fibrinous debris) restrict the synovial permeability to smaller water soluble molecules eg glucose and urea. However, the permeability of benzoyl alcohol, a small lipophilic molecule, was not reduced in the rheumatoid synovium (Simkin,1979).

Sholkoff et al (1967), investigating aspirin, were the first workers to conduct a kinetic study of an NSAID in synovial fluid. The majority of complete profile studies since then have been conducted on drugs with short half-lives, since anti-inflammatory activity was often noted to be sustained for longer than expected from knowledge of plasma concentrations (Wallis and Simkin, 1983). However, no study has investigated the relationship between NSAID concentration in synovial fluid and clinical response.

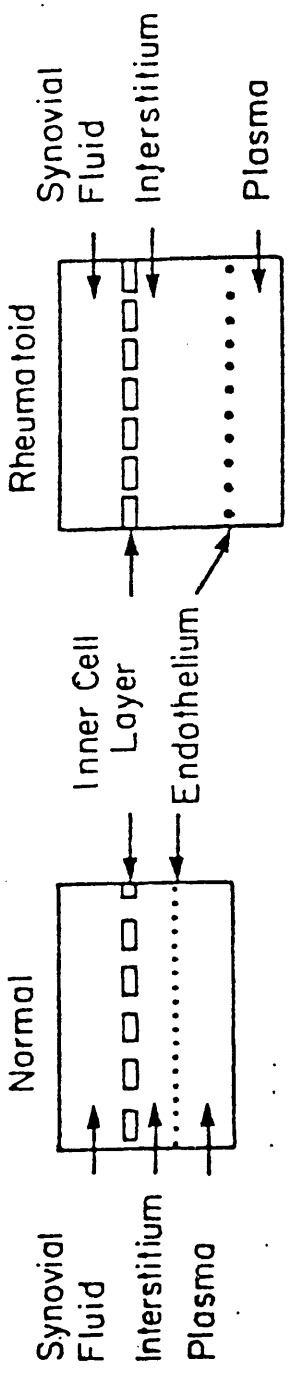


FIGURE 1.5 'Tissue' model of synovial permeability proposed by Simkin (1979)

CHAPTER 2

ANALYTICAL TECHNIQUES

2.1 MATERIALS AND EQUIPMENT

2.1.1 Materials

Potassium dihydrogen phosphate and di-ethyl ether were of HPLC grade. Acetonitrile was HPLC grade with a far UV cut-off of 210nm (90% transmission at 210nm). Bovine serum albumin was purchased from the Armour Pharmaceutical Company Ltd. All other reagents were of Analar grade. Aqueous based reagents were made up in distilled water. HPLC mobile phases were filtered through either aqueous (Type AA 0.8μ) or organic (Type FA 1.0μ) filters supplied by Millipore and degassed by bubbling with helium.

Fenclofenac, [2-(2,3,5,6-tetrachlorophenoxy)phenyl] acetic acid (TCPPA), 5-hyroxy fenclofenac and ^{14}C-fenclofenac were kindly supplied by Reckitt and Colman. Naproxen, 6-O-desmethyl naproxen (DMN) and 2-naphthylacetic acid were gifted by Syntex. Indomethacin was kindly supplied by Merck, Sharp and Dohme. Flufenamic acid was purchased from Sigma Chemical Company. Spectra/Por 2 dialysis membrane (molecular weight cut off 12,000-14,000) was purchased from Spectrum Medical Industries Inc.

2.1.2 Equipment

The HPLC system consisted of a Gilson model 302 pump and a Pye Unicam PU4020 UV variable wavelength detector. A Waters U6K manual injection system was used for the fenclofenac assay and a Waters Wisp autosampler was used for

the naproxen and indomethacin assays. The output from the detector was collected by a Gilson HPLC Data Master system (Apple 2e microcomputer and Gilson Data Master module) (Figure 2.1).

Equilibrium dialysis was carried out using a Dianorm^{R} system consisting of 20 Teflon 1ml cells contained in a rotating carrier unit (Figure 2.2). A Hewlett-Packard Liquid Scintillation Spectrometer was used to count β-emission from ^{14}C-fenclofenac. A Pye Unicam PU8600 UV/VIS Spectrophotometer was used in the estimation of total protein and albumin concentration.

2.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

2.2.1 Introduction

Reverse phase high performance liquid chromatography (HPLC) is ideally suited for the measurement of drugs and their metabolites in biological fluids. Earlier methods developed to measure NSAIDs depended on spectrofluorimetric techniques which in general are non-specific as these methods are not able to distinguish between the parent drug and metabolites or other NSAIDs. Before the advent of HPLC, gas liquid chromatography (GLC) gave specificity. However, sample preparation for GLC tends to be rather complex and laborious. The compound of interest has to be volatile, so a derivatisation step is often necessary for drugs containing highly polar substituents. Overall, HPLC is a much more

FIGURE 2.1 HPLC system

FIGURE 2.2 Dianorm^{R} dialyser

versatile system: sample preparation is shorter and simpler, an infinite number of mobile phases may be used, various types of columns and packings are available and detection can be relatively specific with the use of variable wavelength UV absorbance and fluorescence detectors.

Sample preparation should be as simple as possible and yet allow the specific assay of a drug in the presence of numerous biological components and other drugs. The extent of work-up is dependant on the specificity of the analytical technique and the relative amount of the drug present. Potentially interfering endogenous compounds need to be removed. If sample concentration is not necessary then protein precipitation using an organic solvent (usually acetonitrile) or a strong acid is a useful sample clean-up method. Sensitivity is then usually limited to the µg/ml range. Organic solvent extraction is useful for clean-up and sample concentration. The most appropriate organic solvent and aqueous phase pH can be chosen for a specific drug or metabolite depending on it's physico-chemical properties.

After standard doses of fenclofenac and naproxen, concentrations in plasma are relatively high compared to possible endogenous interference. With a specific HPLC set up, it is possible to analyse samples after a simple precipitation step. Indomethacin, however, is present in plasma in much lower concentrations (two orders of magnitude less) so it is necessary to carry out an acid extraction into an organic solvent in order to clean up the sample and

concentrate it.

In all cases the aqueous component of the mobile phase was acidic giving ion suppression of all of these drugs (pKa 3-4.5). The selectivity of each assay was determined by the use of different columns, slight alterations in the proportions of water and acetonitrile and the specific UV wavelengths for maximum absorption for the particular drug. A summary of the final HPLC conditions for the measurement of fenclofenac, naproxen and indomethacin is given in Table2.1.

2.2.2. Assay for the measurement of fenclofenac in plasma

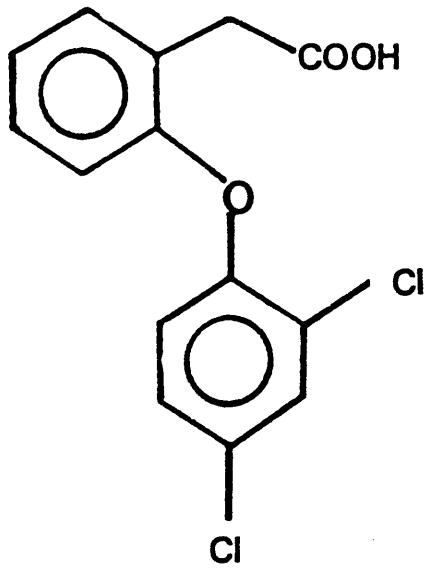
A specific, simple and rapid HPLC assay was developed for the determination of total fenclofenac in plasma. Previous methods reported for the measurement of fenclofenac included GLC (Henson et al, 1980) and a rather laborious HPLC method requiring 1ml of plasma sample and the use of a solid phase extraction procedure (Flockhart & Binns, 1979). The phase I metabolite of fenclofenac (5-hydroxy fenclofenac) could be quantified using this method. The assay described here for the determination of fenclofenac is a modification of a procedure used for other NSAIDs (Nielsen-Kudsk, 1980). The extraction of the drug is achieved by simple precipitation of plasma proteins with acetonitrile containing the internal standard, TCPPA, (a structural analogue of fenclofenac). The structures of fenclofenac and the internal standard are shown in Figure 2.3.

TABLE 2.1 Summary of HPLC conditions used for the analysis of NSAIDs.

	Non-steroidal anti-inflammatory drug		
	FENCLOFENAC	NAPROXEN	INDOMETHACIN
Column	12.5cm Hypersil 5µ ODS	25cm Spherisorb 5µ ODS	25cm Spherisorb 5µ ODS
Detector	UV 215nm	UV 230nm	UV 260nm
AUFS	0.05	0.05 0.001[a]	0.005
Internal standard	TCPPA	2-naphthylacetic acid	flufenamic acid
Mobile phase:			
A (H_2O, pH 3 with H_3PO_4)	50%	60%	55%[b]
B (acetonitrile)	50%	40%	45%
Flow rate	2ml/min	2.5ml/min	2ml/min
Pressure	1000psi	3000psi	3000psi
Retention times:			
Drug	4.6 min	6.0 min	4.9 min
Internal standard	6.7 min	4.3 min	6.6 min
Concentration range (µg/ml)	1-200	2-150 0.01-5[a]	0.025-5.0
Extraction	acetonitrile precipitate.	acetonitrile precipitate. acid extraction into ether.[a]	acid extraction into ether.

[a] modifications to measure naproxen in dialysate.
[b] H_2O acidified to pH3 with glacial acetic acid.

Fenclofenac



TCPPA

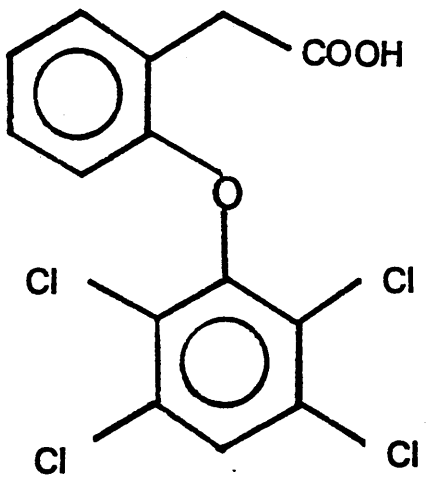


FIGURE 2.3 Chemical structures of fenclofenac and the internal standard, TCPPA

(i) Preparation of solutions

All solutions of fenclofenac and TCPPA were prepared in acetonitrile. Stock fenclofenac (1mg/ml) was prepared by dissolving 10mg of fenclofenac in 10ml acetonitrile. Working standards of 1, 10 and 100µg/ml were prepared by appropriate dilutions of this stock. Stock TCPPA (500µg/ml) was prepared by dissolving 10mg of TCPPA in 20ml of acetonitrile. Acetonitrile for precipitation was prepared by dilution of this stock to give 5µg/ml.

(ii) Preparation of plasma standard curves

Plasma standards, 2, 5, 10, 20, 50, 100 and 150µg/ml were prepared from standard fenclofenac working standard solutions. After evaporation of the acetonitrile at room temperature the residue was reconstituted in 0.1ml of plasma.

(iii) Extraction of plasma

The addition of 0.5ml of acetonitrile containing the internal standard (5µg/ml) to duplicate 0.1ml samples of plasma resulted in the formation of a precipitate. After brief centrifugation the supernatant was decanted into a clean polypropylene tube. Aliquots (10-50µl) of the supernatant were injected directly onto the column.

(iv) Chromatographic conditions

The mobile phase was a mixture of 50% acetonitrile and 50% distilled water acidified to pH3 with orthophosphoric acid. This mixture gave a good separation of fenclofenac from the TCPPA when pumped through a 12.5cm Hypersil 5µ ODS

reverse phase column at 2ml/min with a pressure of 1000psi. The retention times of fenclofenac and the TCPPA were 4.6min and 6.7min respectively. The metabolite 5-hydroxy fenclofenac had a retention time of 2 minutes, but as this coincided with plasma constituents it was not possible to detect the very low concentrations expected. The detector was set at 215nm, the wavelength of maximum UV absorbance for fenclofenac. The detector attenuation was set at 0.05 AUFS. Sample chromatograms of standard and patient samples are shown Figure 2.4.

(v) Quantitation

Quantitation of fenclofenac concentrations in patient samples was achieved by calculating the peak height ratio (PHR) of fenclofenac to the internal standard. Plots of PHR against fenclofenac concentration were linear (Figure 2.5). The lower limit of detection defined as two times baseline noise was 0.5µg/ml.

(vi) Assay precision

Low, medium and high quality control (QC) samples were run with each assay. Patient samples and QCs were analysed in duplicate. All samples from individual patients were analysed on the same day to reduce intra-subject variability.

The results of analysis of quality control samples on the same day and on different days is shown in Table 2.2.

(vii) Stability of fenclofenac

Solutions of fenclofenac in acetonitrile were stable

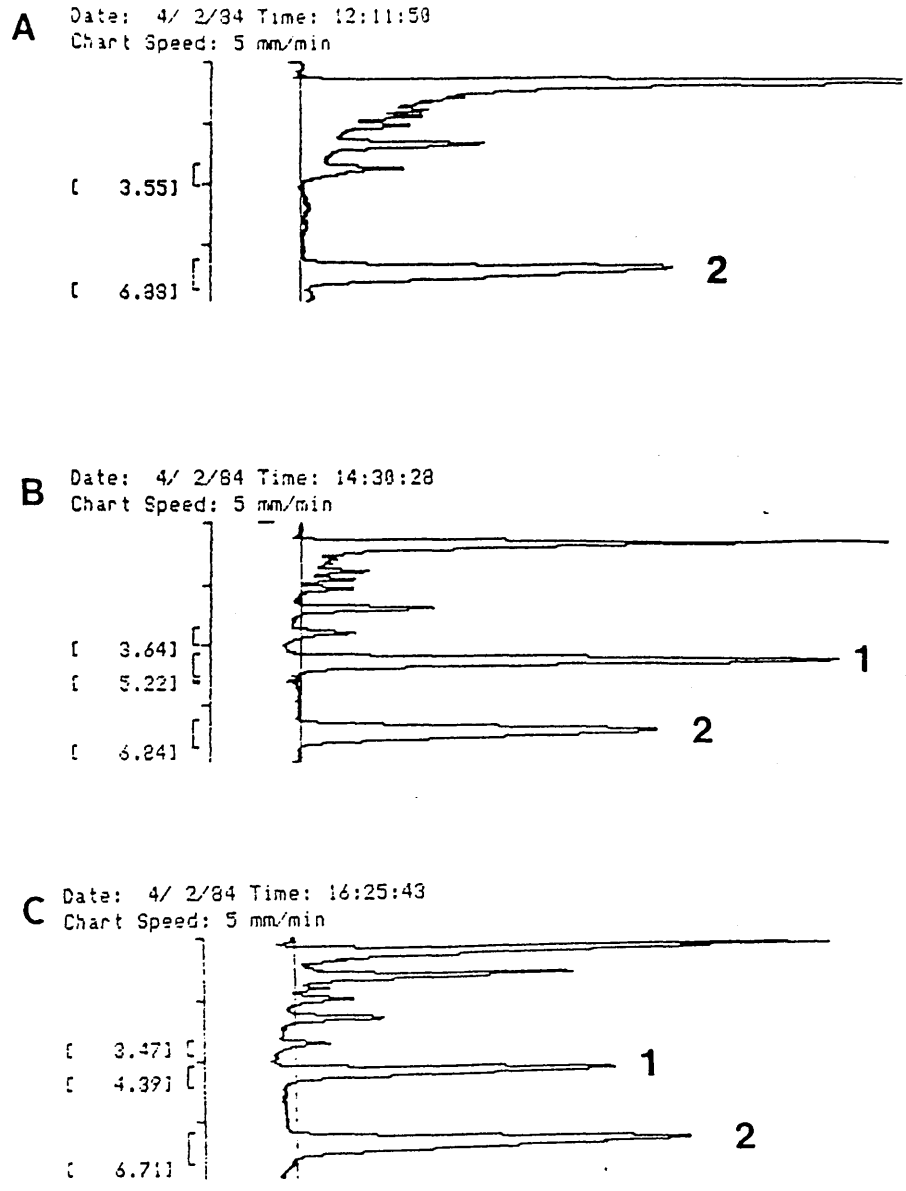


FIGURE 2.4 Typical chromatograms of fenclofenac (1) and the internal standard (2) extracted from plasma

A Blank plasma extract
B Plasma standard extract: fenclofenac 100ug/ml
C Patient plasma sample extract, fenclofenac concentration 55ug/ml

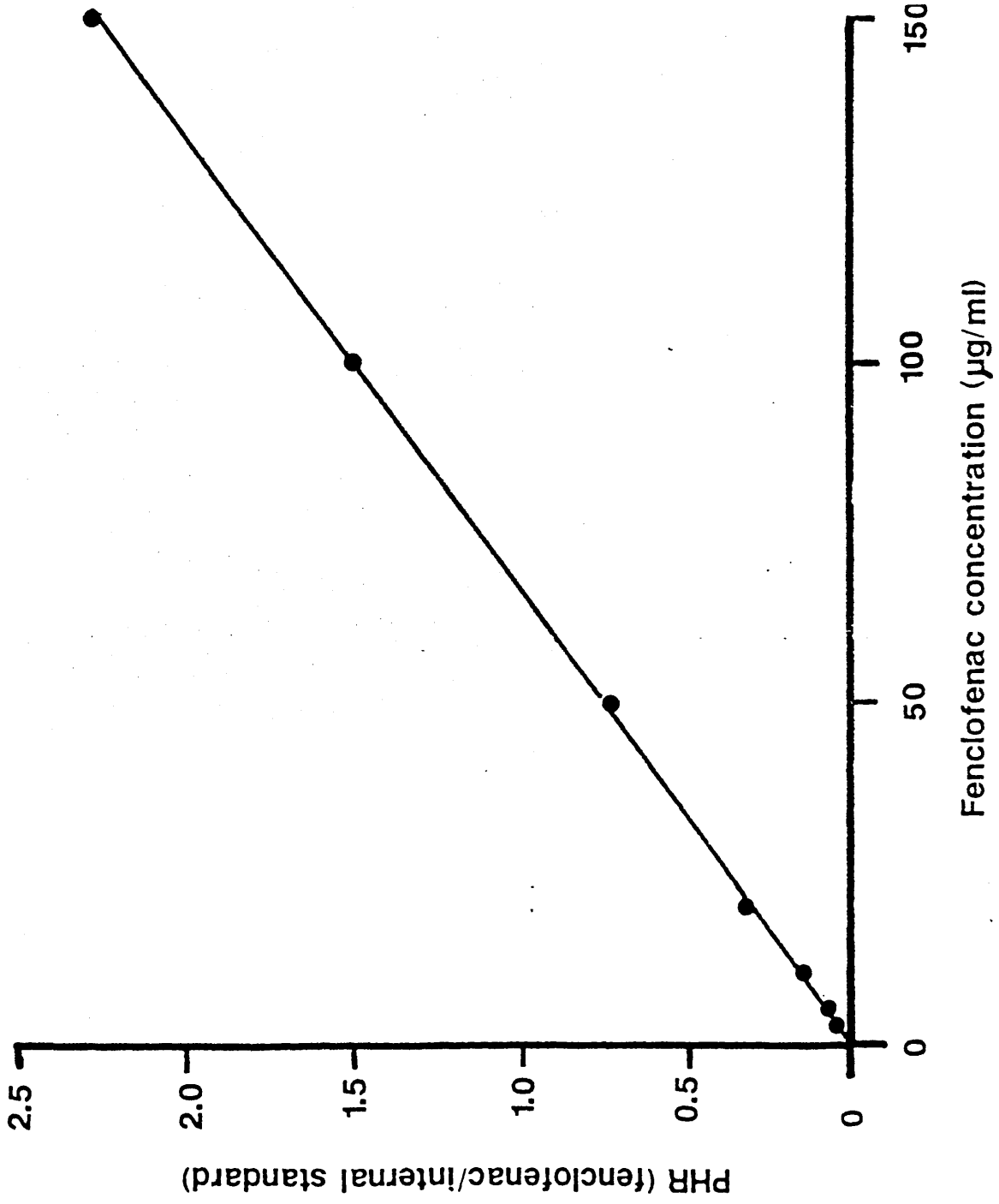


FIGURE 2.5 Typical calibration line for fenclofenac over the concentration range 2-150ug/ml

TABLE 2.2 Precision of fenclofenac HPLC assay

Quality Control	Number of samples	mean concentration (µg/ml)	SD	%CV
Intra-assay				
Low (20µg/ml)	5	20.1	0.502	2.5
Inter-assay				
Low (25µg/ml)	8	27.6	0.930	3.4
Medium (50µg/ml)	8	49.4	1.07	2.2
High (100µg/ml)	8	101	3.59	3.6

at 4°C. There was no evidence of breakdown of fenclofenac over a four month period. Long periods of storage at -20°C and freezing and thawing of plasma samples did not influence the analysis of fenclofenac.

3.2.3 Assay for the measurement of total naproxen and DMN in plasma

A number of HPLC methods have been published for the measurement of naproxen in plasma using UV detection (Upton et al, 1980a; Neilsen-Kudsk, 1980; Shimek, Rao & Wahba-Khalil, 1982) some simpler than others, and some offering greater sensitivity. Since sensitivity was not a problem for total naproxen measurements (as with fenclofenac), precipitation of plasma proteins with acetonitrile was found to be the most appropriate sample preparation method. The method developed was similar to that used to analyse fenclofenac except from the use of a more appropriate internal standard, a different reversed phase column, a slight modification of the mobile phase and a different UV wavelength. The phase I metabolite DMN was also separated from endogenous interference. The structures of naproxen, DMN and the internal standard 2-naphthylacetic acid (a structural analogue of naproxen) are shown in Figure 2.6.

(i) Preparation of solutions

All standard solutions were prepared in acetonitrile. Stock naproxen (1mg/ml) was prepared by dissolving 20mg in 20ml. Serial dilutions of the stock were prepared to give

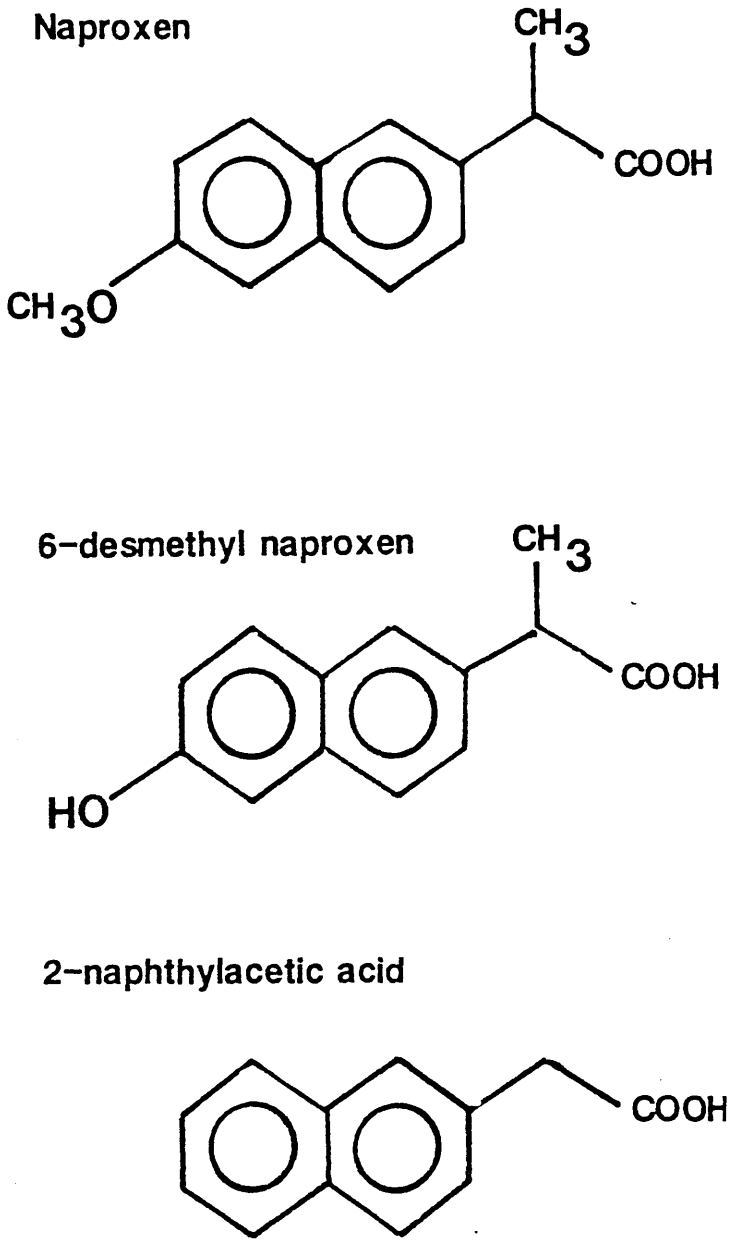


FIGURE 2.6 Chemical structures of naproxen, DMN and the internal standard, 2-naphthylacetic acid

working standards of 0.1, 1, 10 and 100µg/ml. Stock 2-naphthylacetic acid (100µg/ml), was prepared by dissolving 10mg in 100ml of acetonitrile. This stock was diluted in acetonitrile to give a concentration of 0.4µg/ml. Stock DMN (1mg/ml) was prepared by dissolving 10mg in 10ml. Working standards (1 and 10µg/ml)were prepared from dilutions of this stock in acetonitrile.

(ii) Preparation of plasma standards

Plasma standards containing naproxen, 2, 5, 10, 20, 50, 100 and 150µg/ml and DMN, 0.5, 1.0, 2, 5, 8 and 10µg/ml were prepared from naproxen working standards. After evaporation of acetonitrile at room temperature the residue was reconstituted in 0.1ml of plasma. A plasma blank was also taken through the assay.

(iii) Extraction

The addition of 0.5ml of acetonitrile containing the NAA (0.4µg/ml) to duplicate 0.1ml plasma samples resulted in the formation of a protein precipitate. After brief centrifugation the supernatant was decanted into a clean tube and 35µl aliquots were injected directly onto the column.

(iv) Chromatographic conditions

A 25cm Spherisorb 5µ ODS reverse phase column was necessary to achieve a good separation of naproxen from endogenous interference as naproxen is more polar than fenclofenac. In addition it was necessary to increase the proportion of acidified water in the mobile phase so that

naproxen was retained to some extent and separated from a small interfering peak with a similar retention time. The mobile phase was 60% water acidified to pH3 with orthophosphoric acid and 40% acetonitrile. The flow rate was 2.5ml/min giving a pressure of approximately 3000psi. The retention times of DMN, naproxen and NAA were 2.6, 4.3 and 6.0 minutes respectively. The absorbance of the eluent was monitored at 230nm and the attenuation was set at 0.05 AUFS. The metabolite was well separated from any endogenous interference, however under these conditions DMN could only just be detected in patient single dose study samples. Examples of plasma standard and patient samples are shown in Figure 2.7.

(v) Quantitation

The peak height and peak area ratios were calculated for naproxen or DMN to the internal standard. Plots of PAR or PHR against naproxen or DMN concentration were linear over the concentration range of interest (Figure 2.8). In most cases analysis yielded similar results, however if there were slight problems with the chromatography or interference, peak areas were subject to larger errors (especially at lower concentrations). A comparison of concentrations determined by the PHR and PAR methods is given in Table 2.3. The lower limit of detection was 0.5µg/ml for naproxen and 0.08µg/ml for DMN.

(vi) Assay Precision

Naproxen low, medium and high quality control samples

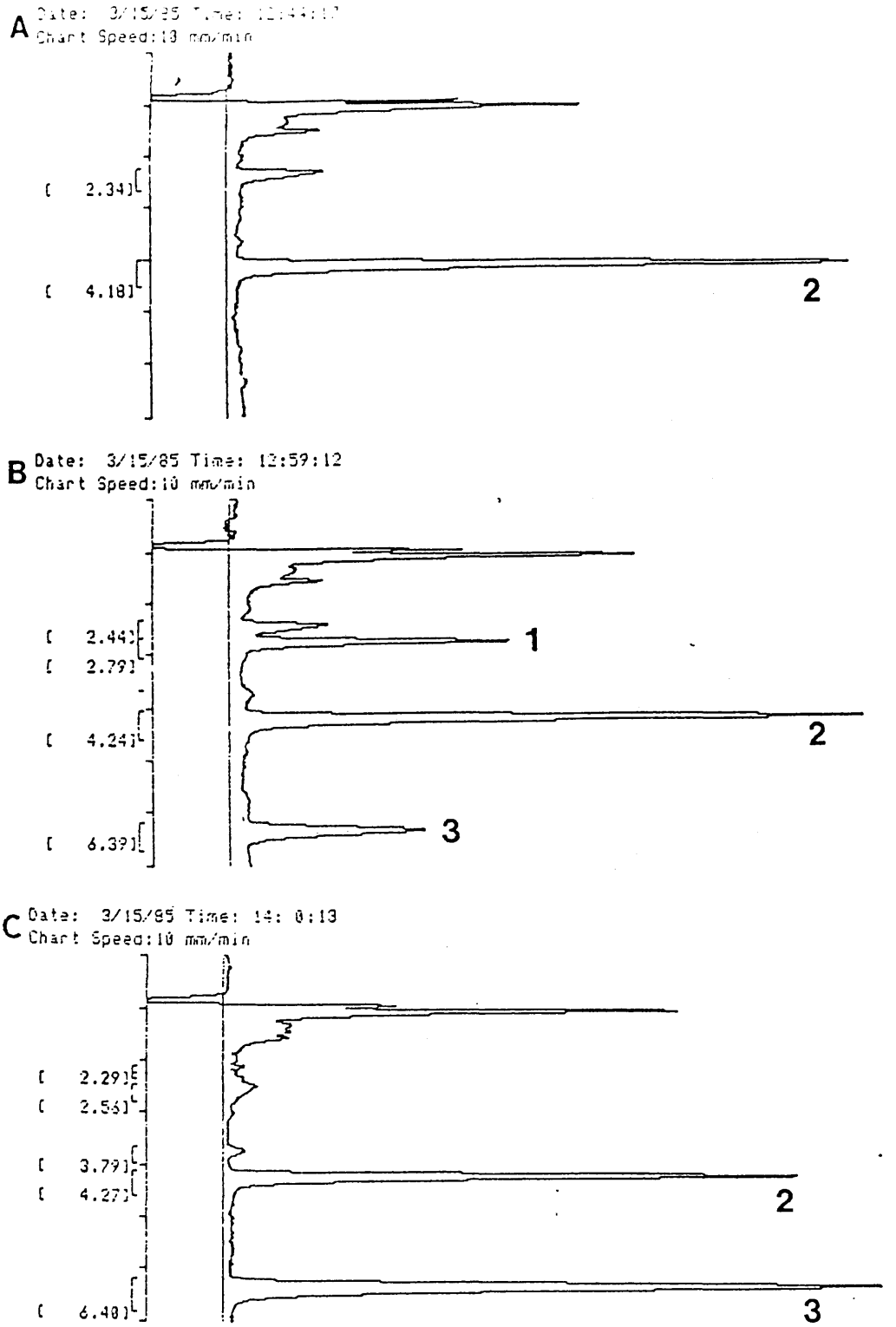


FIGURE 2.7 Typical chromatograms of DMN (1), the internal standard (2) and naproxen (3)

A Blank plasma extract
B Plasma standard extract: naproxen 10ug/ml
DMN 8ug/ml
C Patient plasma sample extract, naproxen concentration 39ug/ml

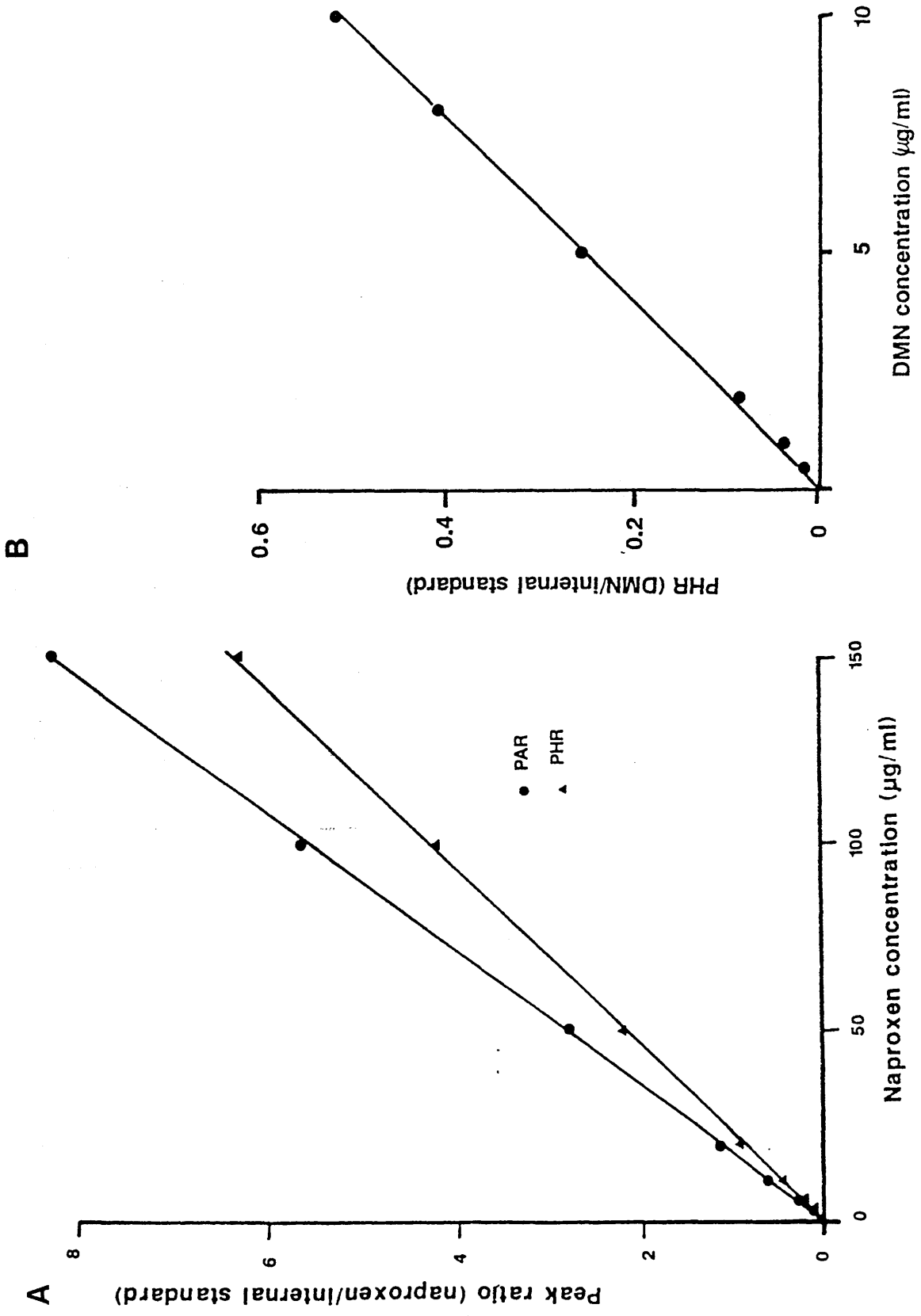


FIGURE 2.8 Typical calibration lines for naproxen and DMN

A Naproxen
B DMN

TABLE 2.3 Comparison of the use of peak height ratio (PHR) and peak area ratio (PAR) to determine naproxen concentration

Naproxen concentration (µg/ml) PAR	PHR	Difference (PAR-PHR)
42.Ø	42.5	-Ø.5
48.Ø	49.5	-1.5
6Ø.Ø	6Ø.5	-Ø.5
6Ø.6	6Ø.5	Ø.1
95.2	93.5	1.7
69.Ø	71.Ø	-2.Ø
55.Ø	54.5	Ø.5
46.2	46.5	-Ø.3
35.2	36.2	-1.Ø
12.3	12.3	Ø.Ø
6.8	6.9	-Ø.1
3.4	3.5	Ø.1
6Ø.Ø	58.Ø	2.Ø
37.8	38.5	-Ø.7
23.2	22.2	1.2
32.5	34.5	2.Ø
39.2	4Ø.Ø	Ø.8
3Ø.5	3Ø.7	-Ø.2
3.6	3.6	Ø.Ø
3.3	3.4	-Ø.1
	mean	Ø.Ø7
	SD	1.Ø6

TABLE 2.4 Precision of naproxen HPLC assay

Quality Control	Intra-assay mean conc. (µg/ml)	SD	%CV	Inter-assay mean conc. (µg/ml)	SD	%CV
Low (5.Ø)	4.4	Ø.179	4.1	4.9	Ø.36	7.2
Medium (3Ø)	27.5	Ø.793	2.9	29.2	1.2Ø3	4.1
High (13Ø)	-	-	-	129	3.97	3.1

mean of six samples at each concentration

were run with each assay. The low quality control sample also contained DMN.

The results of the analysis of naproxen in quality control samples analysed on the same day and on different days are given in Table 2.4. The inter-assay coefficient of variation for DMN at a concentration of 1.5µg/ml was 7.6%. All samples from the same patient were analysed on the same day to reduce intra-subject variability.

(vii) Stability of naproxen and DMN in acetonitrile and plasma

Naproxen was stable in stock solutions for a number of months. Concentrations in plasma samples which had been thawed and defrosted were not altered. It has been reported that the hydrolysis of the naproxen conjugates may occur in samples of naproxen stored at -20°C for two months (Upton et al, 1980b), theoretically leading to a 10% increase in the measured total naproxen concentration. In this study all samples were frozen immediately and assayed at least 1 month later. It is not known whether this breakdown of the conjugate occured in these samples.

As DMN is sensitive to light and moisture, stock solutions were protected from light and were stable for a few months. Working standards were prepared freshly.

3.2.4 Assay for the measurement of indomethacin in plasma and synovial fluid

It was necessary to develop a sensitive and specific

assay for indomethacin in order to analyse the relatively low concentrations of indomethacin in plasma and synovial fluid that were expected. Numerous methods for the analysis of indomethacin in plasma have been published (Skellern & Salole, 1975; Soldin & Gero, 1979; Astier & Renat, 1982; Mehta & Calvert, 1983). Most reported a limit of detection of 100ng/ml; the method of Astier & Renat quoted a limit of 20ng/ml. The following assay was developed after experimenting with a number of these reported methods.

(i) Development of the assay.

It was most appropriate to start with the method which claimed the lowest sensitivity (Astier & Renat, 1982). The extraction method was found to be more complex than was necessary. The initial precipitation of proteins with acetonitrile was found to be no better than a simple acid extraction into ether. In addition, smaller plasma volumes could be used (0.4ml instead of 1ml). Initially phenylbutazone was investigated as an internal standard, however there were problems with stability: it is oxidised and hydrolysed on contact with air. Even if the evaporation of the organic layer was carried out under nitrogen there was still some considerable breakdown of phenylbutazone. The reduction in the phenylbutazone peak was associated with the appearance of an additional peak in the chromatogram. Flufenamic acid was used as an internal standard instead since it was considerably more stable. The structure of indomethacin and flufenamic acid are shown in Figure 2.9.

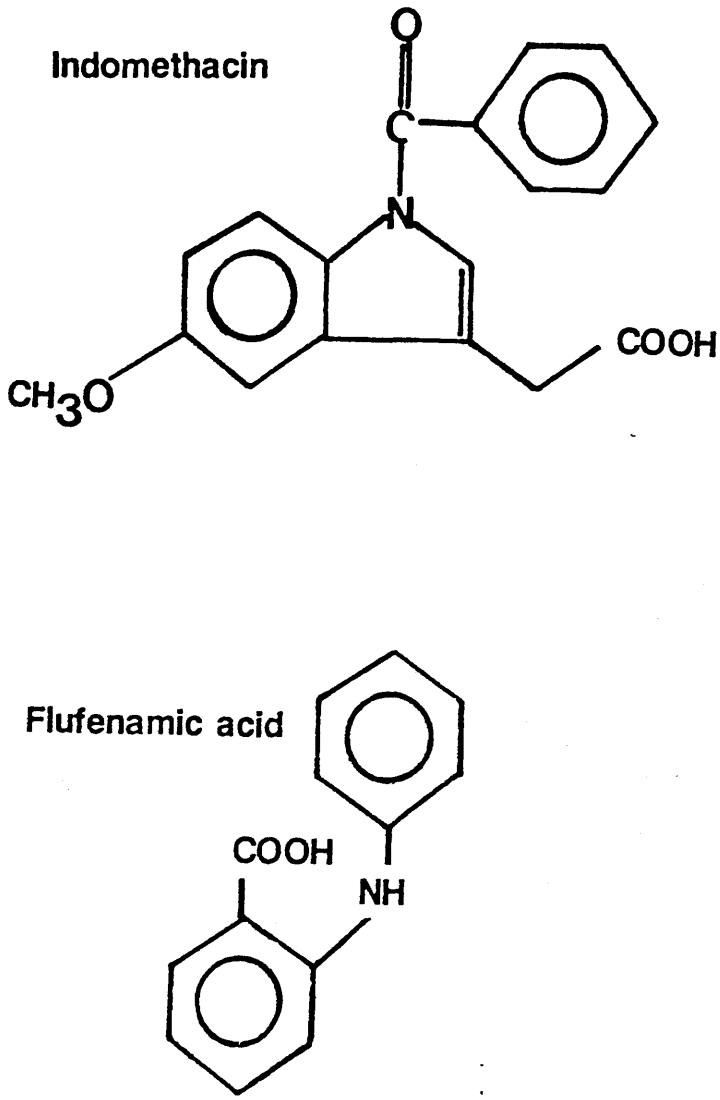


FIGURE 2.9 Chemical structures of indomethacin and the internal standard, flufenamic acid

A number of reversed phase columns were tested. Although slightly greater sensitivity could be obtained with a shorter column (12.5cm Hypersil 5μ ODS or Waters Nova pak C_{18}) better resolution from interfering endogenous materials or other NSAIDs could be obtained with a longer column (25cm Spherisorb 5μ ODS). Plasma from patients with rheumatoid arthritis contained considerably more potential interference than plasma from healthy individuals. Recovery of indomethacin and the flufenamic acid were reduced in extracts of synovial fluid compared to plasma, possibly due to the greater viscosity and stickiness of the synovial fluid. To take account of this problem separate plasma and synovial fluid standards were prepared for each assay. The patients' own plasma and synovial fluid samples taken at 'zero time' were used because slight interferences at a similar retention time to that of indomethacin varied from patient to patient. The conditions of the final assay method are given below.

(ii) Preparation of solutions

Indomethacin and flufenamic acid were made up in acetonitrile. Flufenamic acid was prepared by dissolving 20mg in 20ml of acetonitrile to give a stock solution of 1mg/ml. This stock was diluted to give a working standard of 20μg/ml. Stock indomethacin was prepared by dissolving 20mg in 20ml of acetonitrile. Working standards of 1, 10 and 100μg/ml were prepared by serial dilutions of the stock. A 0.2M solution of potassium di-hydrogen phosphate was prepared

by dissolving 6.8g in 250ml of water.

(iii) Preparation of plasma and synovial fluid standards

Plasma standards, 0.025, 0.05, 0.10, 0.20, 0.50, 1.0, 2.0 and 5µg/ml indomethacin were prepared together with synovial fluid standards up to 1µg/ml using the patients' zero time samples.

(iv) Extraction

Duplicate plasma or synovial fluid samples (0.4ml) were acidified with 0.4ml of potassium di-hydrogen phosphate 0.2M (pH 4.5) after the addition of 50µl flufenamic acid (20µg/ml). After brief vortex mixing, 5ml of di-ethyl ether was added, the tubes were capped and placed in an orbital shaker for 15 minutes. Following brief centrifugation, the organic layer was transferred to a clean conical tube and evaporated under a stream of air at 30°C. The residue was reconstituted in 120µl of mobile phase and 60µl was injected onto the column.

(v) Chromatographic conditions

The mobile phase, a mixture of water acidified to pH3 with acetic acid (45%) and acetonitrile (55%), was pumped through a 25cm Spherisorb 5µ ODS column at 2ml/min giving a pressure of 3000psi. A pre-column was used to protect the analytical column. It was repacked regularly with Lichroprep RP18 packing material. The UV detector was set at a wavelength of 260nm and the attenuation was 0.005 AUFS. Samples were introduced onto the column using a Waters Wisp autosampler. Under these conditions the retention times of

indomethacin and flufenamic acid were 4.9 and 6.6 minutes respectively. Typical chromatograms are shown in Figure 2.10.

(vi) Quantitation

Plots of peak area ratio (PAR) of indomethacin to the internal standard against the concentration of indomethacin in plasma or synovial fluid were linear over the concentration range of interest (Figure 2.11). Separate standard curves for the range 0.025 to 0.5µg/ml and 0.2 to 5.0µg/ml were used for quantitation to prevent excessive weighting of the higher concentration points. The limit of detection of the assay was 10ng/ml for both plasma and synovial fluid.

(vii) Recovery of indomethacin

For plasma the recovery of indomethacin and flufenamic acid was approximately 90% but at lower indomethacin concentrations there was a slight reduction in the recovery (Table 2.5). The recovery from synovial fluid tended to be less than that from plasma in the same patient.

(viii) Precision

Low, medium and high quality control samples were run with each assay. The results, together with the intra- and inter-assay coefficients of variation, are given in Table 2.6. Quality control samples of synovial fluid spiked with indomethacin could not be prepared due to the lack of blank samples. All plasma and synovial fluid samples from the same patient were analysed on the same day to reduce intra-subject variability.

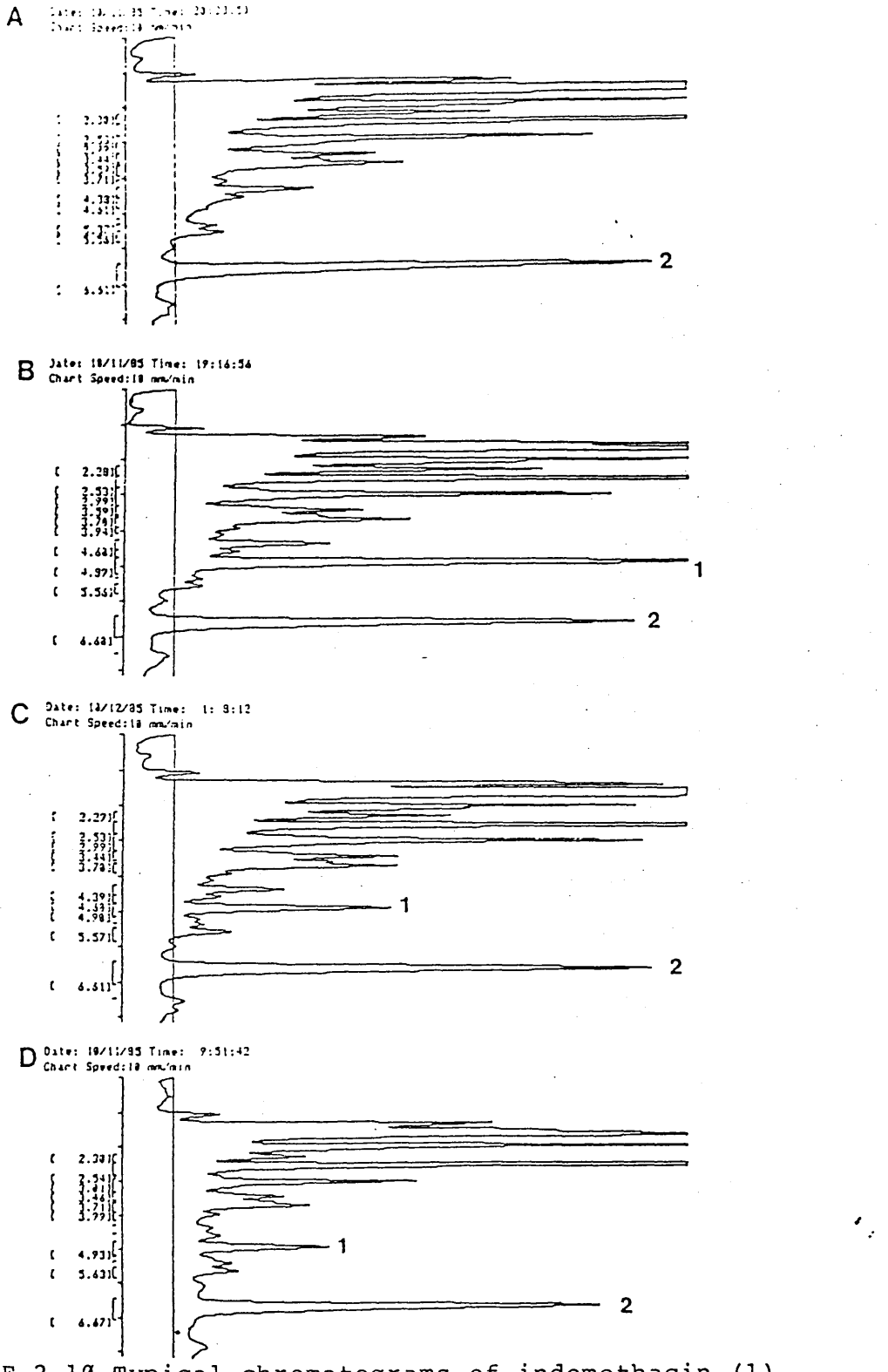


FIGURE 2.10 Typical chromatograms of indomethacin (1) and the internal standard (2)

A Blank plasma extract
B Plasma standard extract: indomethacin 0.5µg/ml
C Patient plasma sample extract, indomethacin concentration 0.150µg/ml
D Patient synovial fluid sample extract, indomethacin concentration 0.098µg/ml

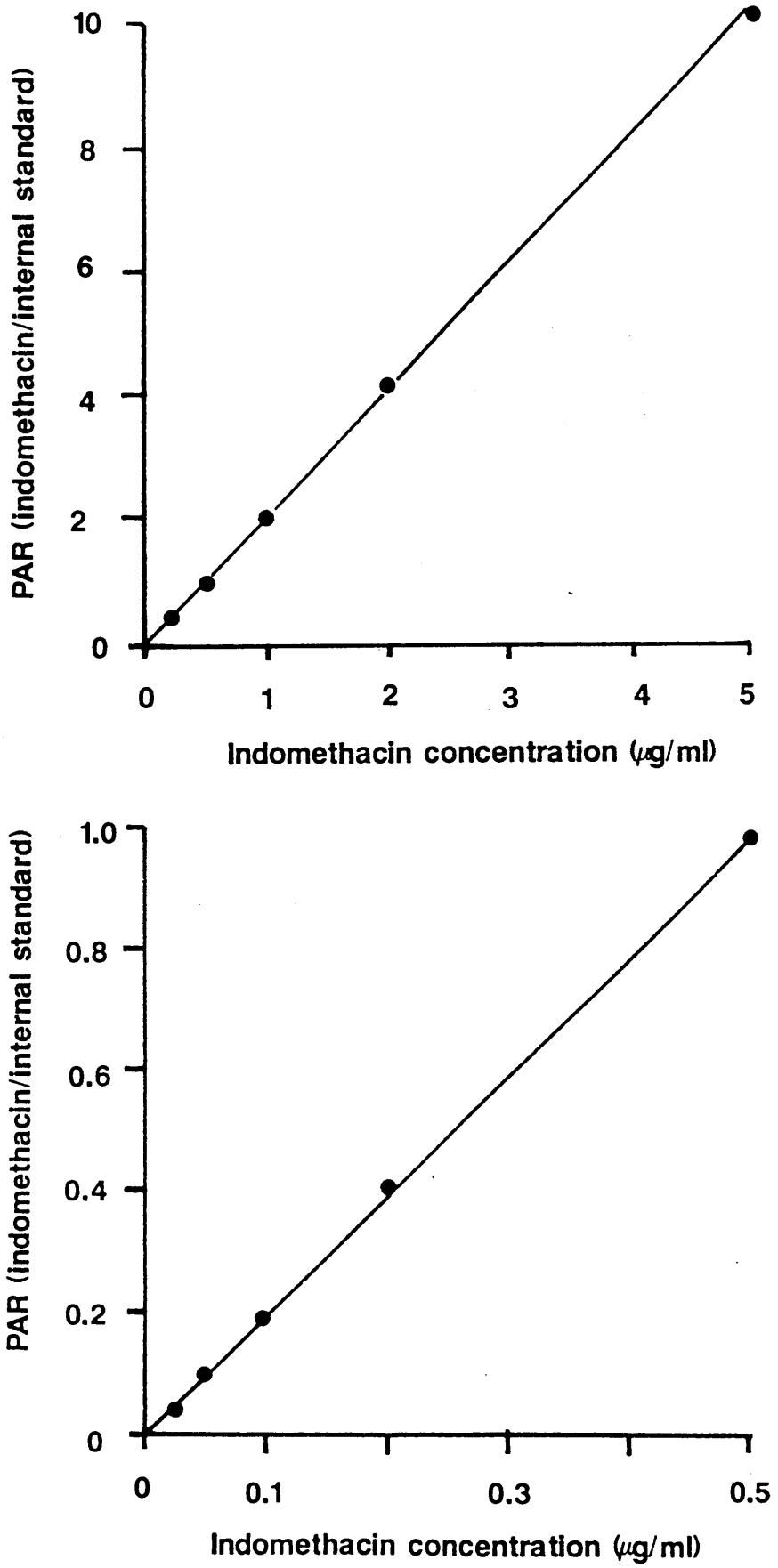


FIGURE 2.11 Typical calibration lines for indomethacin

TABLE 2.5 Percentage extraction of indomethacin and flufenamic acid from plasma

Quality Control	indomethacin % extraction	flufenamic acid (IS) % extraction	ratio I/IS
Low (Ø.Ø5µg/ml)	87.9 (6.5)	91.6 (4.1)	Ø.96 (Ø.Ø4)
Medium (Ø.4µg/ml)	86.3 (2.4)	9Ø.6 (4.Ø)	Ø.96 (Ø.Ø4)
High (4.Øµg/ml)	9Ø.3 (2.8)	9Ø.8 (4.Ø)	Ø.99 (Ø.Ø4)

SD is given in parenthesis

TABLE 2.6 Precision of indomethacin HPLC assay

Quality Control	Intra-assay mean conc. (µg/ml)	SD	%CV	Inter-assay mean conc. (µg/ml)	SD	%CV
Low	Ø.Ø49	Ø.ØØ24	4.8	Ø.Ø45	Ø.ØØ23	5.1
Medium	Ø.392	Ø.Ø142	3.6	Ø.4ØØ	Ø.Ø142	4.9
High	4.Ø1	Ø.146	3.6	4.Ø6	Ø.132	3.2

mean of eight samples at each concentration

(ix) Stability of indomethacin in stock solutions and samples

Indomethacin, protected from light, was stable in stock solutions for a number of weeks.

2.3 EQUILIBRIUM DIALYSIS

2.3.1 Introduction

A number of methods have been described to study the binding of drugs to plasma proteins. These may be divided into separation methods (ultrafiltration, ultracentrifugation, equilibrium dialysis and gel filtration) and non-separation methods (spectroscopy, optical rotatory dispersion and circular dichroism). The choice of technique depends on the type of binding information required. Separation techniques yield information on the affinities and number of binding sites, while the spectroscopic methods allow the qualitative nature of the interaction between the drug and protein molecule to be studied.

Several improvements in the technique of equilibrium dialysis have been made since it was first used in the 1940's (Davis, 1943 and Klotz, 1946). Procedures have been standardised (use of dialysis systems such as Dianorm) and equilibrium times are much shorter as a result of improved dialysis membranes. Equilibrium dialysis has often been used as a reference for other separation methods, although there are a number of problems associated with all of these methods (eg perturbations of the equilibrium between the

bound and free drug or dilution of protein concentration) (Kurz, Trunk & Weitz, 1977).

In equilibrium dialysis a protein solution (eg plasma or serum) is separated from a buffer solution by a semi-permeable membrane. A drug added to the system will equilibrate across the membrane according to the affinity of the drug-protein interaction, the concentration of drug and the amount of protein. When equilibrium has been reached the concentration of the free drug on either side of the membrane will be equal. With the Dianorm^{R} dialyser, the volume of plasma and buffer are equal.

It is normal when using radiolabelled tracer to count aliquots of both the buffer and plasma after dialysis. The fraction of drug not bound to plasma proteins is then:

f_u = CPM (buffer) / CPM (plasma) 2.1

However, during most dialyses, there is a shift of water from the buffer to plasma due to the osmotic pressure created by protein molecules. This volume shift should be taken into account if it is greater than 10% and especially if the free fraction of drug is small (Jin -Ding, 1983). Table 2.7 shows the effect of different degrees of volume shift on the free fraction determined by the above method. Hypothetical observed and actual free fractions are given for a range similar to that observed for fenclofenac. For example if there is a 10% shift in volume from buffer to plasma, and f_u is 0.01, there will be a 10% error in calculating f_u. The over-estimation of the free fraction,

TABLE 2.7 The theoretical error in the estimation of the free fraction determined from the ratio of radioactivity in buffer to that in plasma after equilibrium dialysis if volume changes are not taken into account (according to the method of Jin-Ding, 1983)

F	Free fraction (f_u) 0.0001	0.001	0.01	0.1
0.95	0.0526	0.0526	0.0521	0.0474
0.90	0.1111	0.1110	0.1100	0.1000
0.85	0.1764	0.1763	0.1747	0.1588
0.80	0.2500	0.2498	0.2475	0.2250

F is the ratio of final protein concentration to initial protein concentration.

Fractional error in calculating $f_u = (1-F)(1-f_u)/F$

will become larger the greater the volume shift and the lower the actual free fraction.

The free drug concentration measured directly in buffer is independent of any shift in water from buffer to plasma as the number of binding sites, and therefore the amount of drug bound, remains constant. Even if there is a volume shift this causes no additional error in the determination of the free fraction if the initial total drug concentration is used in the calculation.

The total concentration at the end of dialysis is not the same as the initial concentration due to the distribution of the free drug into twice the initial plasma volume. However if the free fraction is very small this has only a minute effect on the total concentration. The concentration of protein in plasma before and after dialysis may be measured to take account of the effect of volume changes on the determination of the free fraction.

Care must be taken in assembling the dialysis cells as any slight leak of protein can cause significant over-estimation of the free fraction especially for a drug which is highly protein bound. If a drug is 99% bound, a 0.5% leak of protein could lead to a 50% over-estimate of the free drug concentration and the free fraction. The absence of protein in dialysate should be confirmed by a sensitive protein assay (Lowry et al, 1951).

The extent of the volume shift can vary greatly depending on the drug, the membrane, the buffer and the

duration of dialysis. The extent of the volume shift can be reduced by the use of as short a dialysis time as possible, a relatively thick membrane or the addition of a high molecular weight compound (eg dextran) to the buffer (Lima et al, 1983). These workers also found that the volume shift was much smaller for highly bound drugs (eg clofibrate) when compared to drugs which are less extensively bound (eg lignocaine).

For a drug that is highly protein bound it is important to have a specific assay to determine the free fraction or free concentration of the drug (Yacobi & Levy, 1975). It is therefore better to measure the drug directly than to use a radiolabel which is perhaps only 98-99% pure. The free fraction will often be over-estimated when the total concentration (and the free fraction) is very small (the radiolabelled tracer is a larger percentage of the total drug concentration).

2.3.2 General methods for equilibrium dialysis

(i) Dialysis buffer

The phosphate buffered saline was prepared as follows:

Stock sodium dihydrogen phosphate (dihydrate) (0.02M)

3.12g in 1L water.....Solution A

Stock disodium hydrogen phosphate (0.02M)

5.68g in 2L water.....Solution B

1L of buffer was prepared by dissolving 7.84g of sodium chloride in a mixture of 200ml of solution A and 800ml of

solution B. The buffer was adjusted to pH7.4 using 2N sodium hydroxide.

(ii) Preparation of dialysis membrane

The following washing procedure was used to prepare Spectra/Por 2 dialysis membrane prior to each dialysis.

1. The appropriate length of dialysis tubing was soaked in distilled water for at least 15 minutes.
2. The membrane, once pliable, was cut along the entire length and opened up.
3. The membrane was rinsed 5 times with distilled water.
4. After draining off the distilled water, the membrane was soaked for 15minutes in dialysis buffer.
5. The buffer solution was renewed and the membrane was soaked overnight at 4 ^{o}C.
6. The membrane was cut into pieces of the appropriate size and the buffer was changed once again just prior to assembling the dialysis cells.

(iii) Dialysis cell assembly, filling and emptying

Each cell is made up of two halves, the lid and the base. Each half-cell has three stoppered holes, two close together are for filling, one accepts the pipette tip and the other acts as an air vent. A single hole on the opposite side of the chamber allows the cell to be emptied. The cells are assembled with stoppers inserted into the single emptying hole. The drained membrane is placed on the lid and any creases are smoothed out. The base is then placed on top, ensuring that the inlet and outlet holes on both cells

are in line (Figure 2.12). The assembled cell is inverted before stacking in the cell carrier stand. Each cell is separated by a spring loaded cell spacer. The cells should be stacked so that all the stoppers are aligned in a row. The cells are secured tightly in position before filling.

With the cell carrier unit mounted in the filling clamp, 1ml of plasma was added to the left-hand side of the cell and 1ml of buffer was added to the other side (Figure 2.13). The two sides of the cell were filled in quick succession using a Gilson pipette. Adjacent stoppers on each half cell were inserted simultaneously. The four assembled cell units once filled were mounted in the drive unit (Figure 2.2) and the unit was immersed in a water bath set at 37°C. Gentle rotation of the cells about an axis perpendicular to the membrane ensures thorough mixing (the actual total volume of each half cell is 1.36ml, if a maximum volume of 1ml is used complete mixing can occur).

At the end of the dialysis, the the cell unit was placed in the filling clamp. With the emptying hole in a horizontal position, the plug was removed and replaced by a PTFE emptying tube. With the end of the tube in a test tube, the cell stack is turned round so that one of the filling stoppers can be removed and the Gilson pipette was used to blow the fluid out of the cell and into the test tube.

(iv) Cleaning cells

After the cells had been emptied, they were dismantled and layed flat up in a drip tray containing a dilute Decon

FIGURE 2.12 Assembling a dialysis cell

FIGURE 2.13 Filling the dialysis cells

solution. The cells were soaked in this solution for 1 hour and rinsed overnight with running water. The cells were rinsed finally in distilled water before placing in a drying cupboard. The cells were completely dry before use.

(v) Measurement of total plasma concentration

Total protein concentration in plasma before and after dialysis was determined by an improved Biuret method (Yatzidis, 1977). The Biuret reagent was prepared as follows: 3.8g cupric sulphate, 6.7g disodium EDTA, 17.5g glycine and 14.0g sodium chloride were dissolved in 750ml of water. Sodium hydroxide(40g) was added slowly and the solution was finally made up to one litre. If stored at $4^{o}C$ in a plastic container the reagent was stable for at least one month. Standards of 25, 50, 75 and 100g/l were prepared from a stock solution of bovine serum albumin. 5ml of the Biuret reagent was added to 0.1ml of standard or duplicate plasma sample and to 0.1ml of water for the reagent blank. After mixing, the tubes were allowed to stand at room temperature for 5 minutes. The absorbance of standards and samples at 545nm was determined after the instrument had been zeroed using the reagent blank. With this reagent, the optical density of a 50g/ml albumin standard gave an absorbance of 0.25 absorbance units.

2.3.3 Determination of fenclofenac plasma protein binding

The free fraction of fenclofenac was determined in patient trough plasma samples. For a few patients blank

plasma obtained at the start of the study was spiked with fenclofenac up to 800µg/ml. Quantitation of the free fraction was achieved by using radiolabelled fenclofenac as a tracer.

(i) Radiochemical purity of ^{14}C-fenclofenac

Radiolabelled fenclofenac was used to quantitate the free fraction of fenclofenac in plasma samples. It was necessary to confirm the radiochemical purity before proceeding with protein binding studies. The radiochemical purity of ^{14}C-fenclofenac was determined using thin layer chromatography (TLC) with two different solvent systems.

The first 'lot' of ^{14}C-fenclofenac was dissolved in 0.1ml of 2N sodium hydroxide and then made up to 1ml with dialysis buffer (stored at 4°C). The radiochemical purity at this time was 98-99%. However during preliminary experiments there was a gradual increase in the free fraction for any total drug concentration over a two month period. The radiochemical purity was checked again and was found to be only 90%. It appears that there was some breakdown of fenclofenac or loss of label during storage in alkali solution at 4°C. The results of analysis using this label were discarded.

New ^{14}C-fenclofenac was obtained, and this time it was dissolved in organic solvent, 35:65 ratio of ethyl acetate to ethanol. The radiochemical purity was 98%. From inter-assay measurements of quality control samples a number of months apart it was obvious that the radiolabelled drug was

considerably more stable in the organic solvent. The ^{14}C-fenclofenac had a specific activity of 45.7μCi/mg.

The concentration of ^{14}C-fenclofenac in the stock solution was 2.19mg/ml (radioactivity 100μCi/ml). 15μl of this stock was diluted to 25ml with dialysis buffer immediately before each dialysis experiment (final concentration 1.3μg/ml, 0.06μCi/ml and 133200dpm/ml).

(ii) Quantitation of radioactivity

The radioactivity in samples of buffer and plasma at the end of dialysis was measured by liquid scintillation counting. 10ml of liquid scintillation fluid was added to 500μl samples in plastic scintillation vials. After mixing, the vials were counted for five minutes at the appropriate energy setting for emission of β particles from ^{14}C. Since colour or chemicals will cause quenching of emitted particles it was necessary to count the samples on the external standard channel ratio (ESCR) setting so that the plasma sample counts could be corrected to the equivalent in buffer. A quench curve was determined for each set of samples using haemolysed plasma. A constant amount of radioactivity was added to each vial and varying proportions of plasma and buffer. The percentage efficiency of counting was expressed relative to the sample containing buffer alone and the % efficiency was plotted against the ESCR to give a quench curve (Figure 2.14). Plasma caused a reduction of 1 to 5% in the counting efficiency in comparison to buffer.

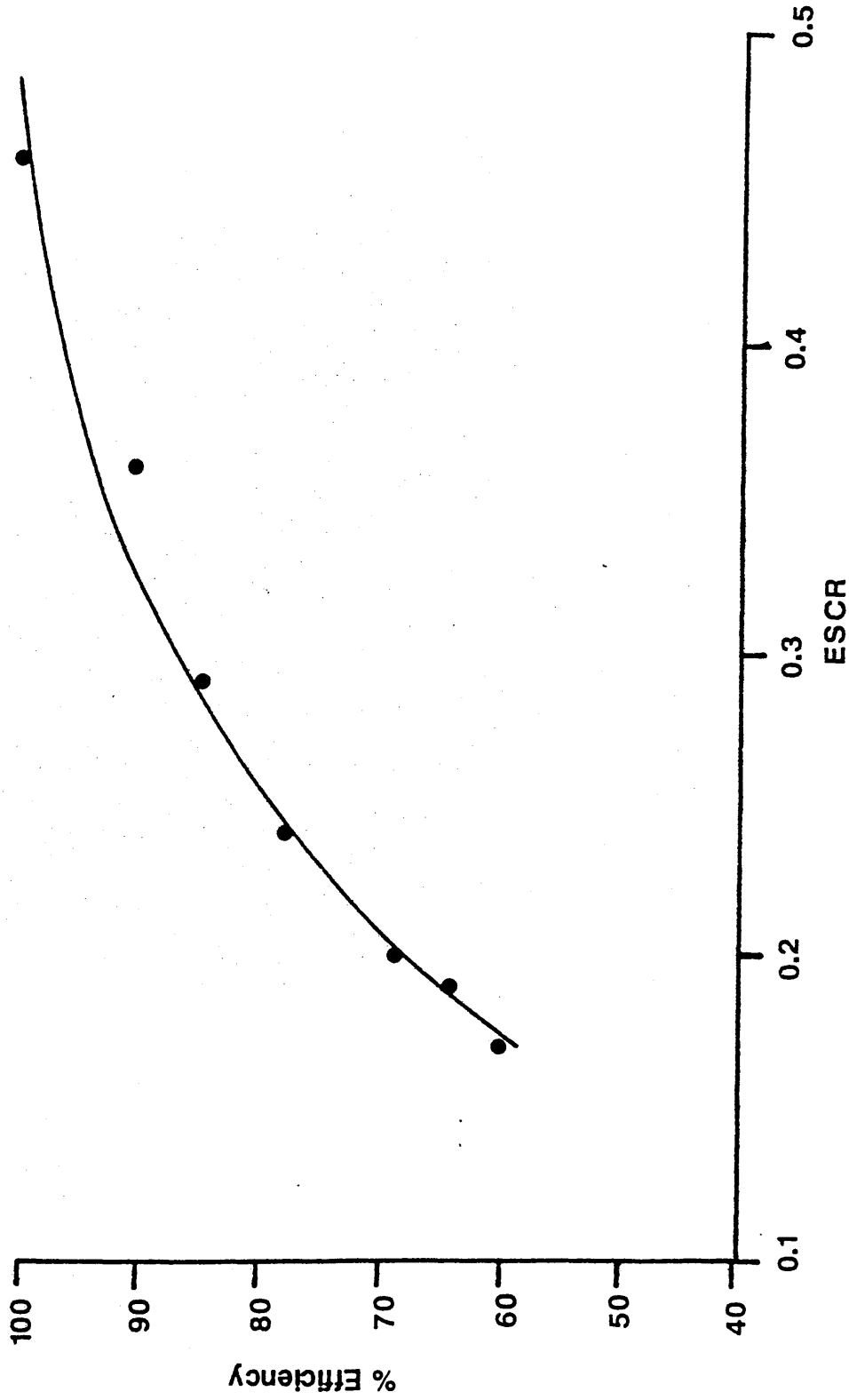


FIGURE 2.14 An example of a quench curve for ^{14}C-fenclofenac in plasma

(iii) Conservation of mass

The absence of non-specific binding of ^{14}C-fenclofenac was tested by carrying out a dialysis of buffer containing radiolabelled drug against blank buffer. At the end of dialysis the sum of the radioactivity on both sides of the cell was equivalent to the activity in the initial buffer. This demonstrated that there was no binding of ^{14}C-fenclofenac to the membranes or cells.

(iv) Time to reach equilibrium

The time to reach equilibrium was determined by carrying out dialysis experiments for 2, 3, 4 and 6 hours using a range of concentrations from 25 to 500µg/ml. The results of these experiments are given in Table 2.8. From the free fraction measurements it appeared that equilibrium had been reached by 3 hours. Thereafter the slight rises in the free fraction over the concentration range could be attributed to the gradual shift of water from the buffer to the plasma side of the membrane. Subsequent dialysis were carried out over 3 hours.

(v) Effect of pH and temperature

The binding of fenclofenac was unaltered in plasma over the pH range 6-9. The free fraction was equivalent whether determined at 37°C or at 25°C.

(v) Calculation of the free fraction and free concentration

The counts per minute (cpm) for plasma and buffer were first corrected for background radioactivity (approx. 25cpm), determined by counting buffer or plasma with no

TABLE 2.8 The free fraction of fenclofenac after various dailysis times

Total conc. (µg/ml)	Dialysis Time (hours) 2	3	4	6
25	-	Ø.3Ø	Ø.34	Ø.4Ø
5Ø	Ø.42	Ø.33	Ø.36	Ø.4Ø
1ØØ	Ø.46	Ø.4Ø	Ø.44	Ø.56
2ØØ	Ø.7Ø	Ø.65	Ø.63	Ø.76
3ØØ	Ø.96	Ø.86	Ø.9Ø	1.Ø3
4ØØ	1.Ø7	1.Ø2	1.Ø9	1.12
5ØØ	1.24	1.2Ø	1.28	1.41

TABLE 2.9 Inter-assay precision of fenclofenac free fraction determination by equilibrium dialysis

Plasma conc. (µg/ml)	Free fraction ($\times 10^2$) mean	SD	%CV
1.3	Ø.28	Ø.Ø2Ø	7.3
51.3	Ø.34	Ø.Ø15	4.5
2ØØ	Ø.66	Ø.Ø3Ø	4.5
4ØØ	1.Ø5	Ø.Ø46	4.3

six observations at each concentration

radioactivity present. After correcting the plasma cpm for quenching using the ESCR the free fraction (f_u) was calculated according to Equation 2.1.

In a number of dialysis experiments, the pre and post dialysis plasma protein concentration was measured. It was found that dilution of plasma did not exceed 10% so it was considered unnecessary to correct for this volume shift. The free concentration (Cu) was calculated from the free fraction and the total fenclofenac concentration (C) determined by HPLC:

$$Cu = f_u.C \quad \text{.....2.2}$$

(vi) Precision

At least one quality control sample was taken through each dialysis experiment. The inter-assay precision is given in Table 2.9 for a range of total fenclofenac concentrations.

2.3.4 Determination of naproxen plasma protein binding

The binding of naproxen was investigated in patient trough samples at steady state. In addition, binding data were obtained over a wider concentration range by dialysis of blank patient plasma (taken at the end of an initial wash-out period) against dialysis buffer spiked with naproxen from 25-500µg/ml.

The only radiolabelled naproxen available was ^{3}H-naproxen. Tritium has less specific activity than ^{14}C and is therefore not ideal as a tracer for binding experiments especially when the free fraction is very small. The

radiochemical purity of the compound provided was only 95% so it was considered inappropriate to use it. Instead, the concentration of naproxen in dialysate was measured directly by HPLC.

(i) Preparation of solutions

Stock naproxen (20mg/ml) for the preparation of spiked dialysis buffer was prepared by dissolving 200mg of naproxen in 10ml of acetonitrile. 0.5ml of this stock was evaporated at 30°C and the residue was reconstituted in 20ml of fresh dialysis buffer (500µg/ml). Dilutions of this solution in dialysis buffer were prepared to give naproxen concentrations of 25, 50, 75, 100, 150, 200, 300 and 400µg/ml.

For the HPLC determination of naproxen in dialysate, stock solutions of naproxen (1mg/ml), DMN (1mg/ml) and 2-naphthyl acetic acid (500mg/ml) were prepared as given in section 2.2.3. Dilutions of stock naproxen were prepared to give working standards of 0.1, 1 and 10µg/ml. The internal standard was diluted to give working standards of 0.2 and 0.05µg/ml.

(ii) HPLC determination of free drug concentration in dialysate

(a) **Extraction**

At the end of a dialysis experiment duplicate 200µl samples of dialysate were extracted into 2.5ml of di-ethyl ether after acidification with 200µl of 0.2M potassium di-hydrogen phosphate (pH 4.5) and addition 50µl of the internal

standard (0.2µg/ml for dialysis of blank plasma against spiked buffer, and 0.05µg/ml for trough samples). After mixing on an orbital shaker for 15 minutes and brief centrifugation, the organic layer was transferred to a clean tube and evaporated at 30°C under a stream of air. The residue was reconstituted in 120µl of mobile phase and 30µl aliquots were injected onto the column. In the experiments to determine binding parameters (total concentration 25-500µg/ml), post dialysis buffer from 200 and 300µg/ml total concentrations were diluted 1 in 2 with dialysis buffer and buffer from dialysis of 400 and 500µg/ml total concentration were diluted 1 in 4 before extraction.

(b) **Standards**

Standards of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0µg/ml were prepared in dialysis buffer using the working standards. For the measurement of free naproxen concentrations in trough samples the top four standards were omitted and DMN was also added at the same concentrations.

(c) **Chromatography and quantitation**

The chromatographic conditions were identical to those used to measure total naproxen concentrations (section 2.2.3.) except that the detector attenuation was set at 0.01 AUFS. Sample chromatograms are shown in Figure 2.15. Quantitation was by the peak area ratio (PAR) method. Standard curves of PAR against naproxen concentration were linear. Separate standard curves for the range 0.01 to

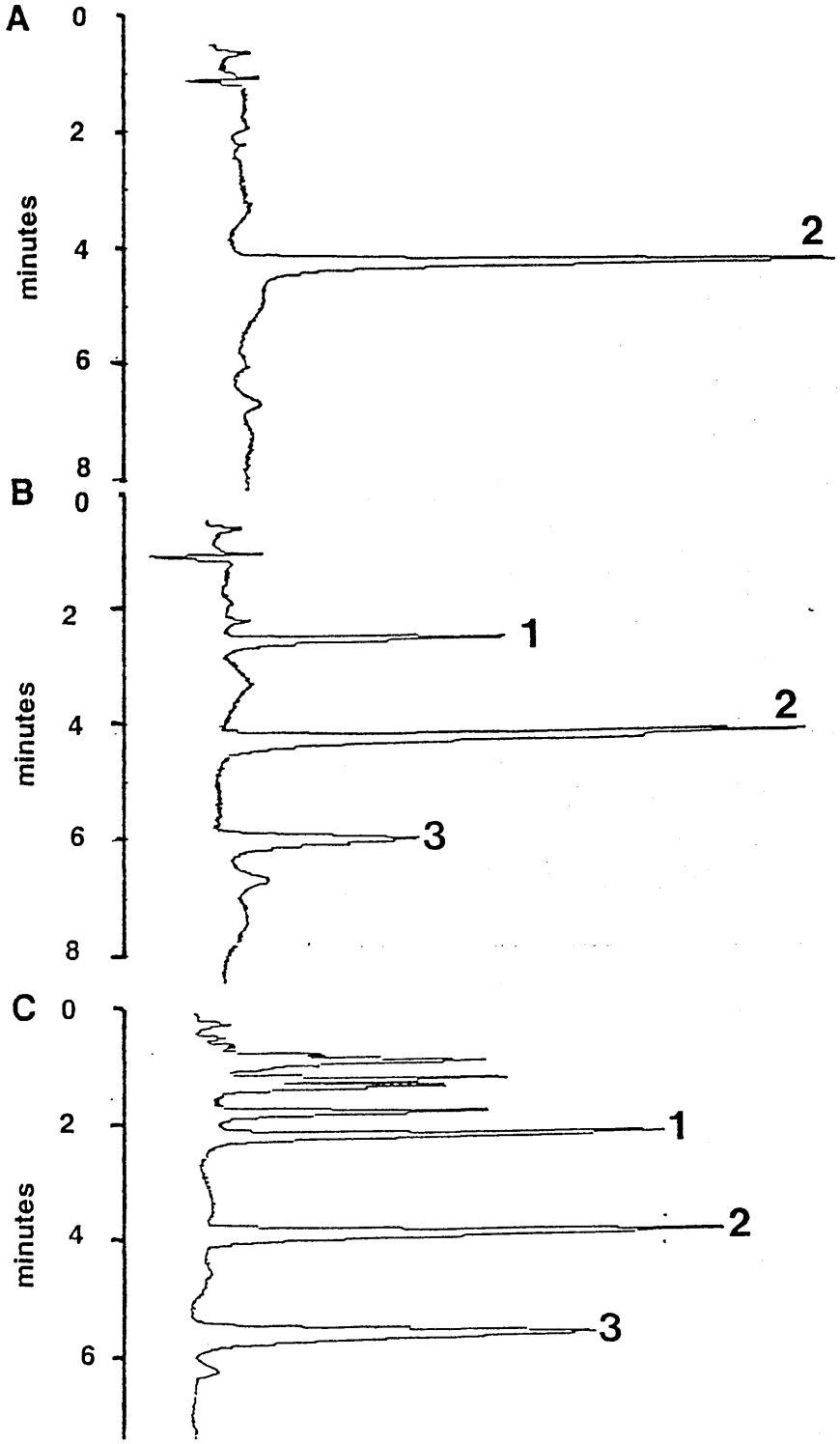


FIGURE 2.15 Typical chromatograms of DMN (1), internal standard (2) and naproxen (3) extracted from buffer after dialysis

A Blank dialysis buffer extract
B Dialysis buffer standard extract:
naproxen 50ng/ml
DMN 50ng/ml
C Buffer extract after dialysis against a patient plasma trough sample, naproxen 128ng/ml and DMN 80ng/ml

0.2µg/ml and 0.2 to 5µg/ml were used for quantitation to prevent excessive weighting of the higher concentration points.

(d) **Precision**

Buffer quality control samples were analysed with each assay, the coefficients of variation are given in Table 2.10.

(iii) Conservation of mass

Table 2.11 gives the results of an experiment to determine whether naproxen bound non-specifically to membranes or cells. Dialysis of plasma samples spiked with a range of naproxen concentrations from 25 to 800µg/ml for 3 hours indicated that there was no loss of naproxen due to non-specific binding. At the end of the dialysis naproxen concentrations in plasma and dialysis were measured by HPLC and the protein concentration before and after dialysis was determined. After correction for a 10% volume change, the total amount of naproxen was not different from the initial amount added to the dialysis cell. In all subsequent dialysis experiments only the concentration of naproxen in dialysate was determined.

(iv) Time to reach equilibrium

Dialysis of spiked plasma samples at 50 and 200ug/ml for 1, 2, 3 and 4 hours indicated that equilibrium was possibly reached by as early as 1 hour. The 3 and 4 hour results, however, were more comparable so it was considered that the 3 hour dialysis time would be ideal

TABLE 2.10 Precision of free naproxen measurements by HPLC. Spiked buffer and buffer after dialysis against spiked plasma.

Quality Control (µg/ml)	Inter-assay mean conc. (µg/ml)	SD	%CV	Intra-assay mean conc. (µg/ml)	SD	%CV
BUFFER						
0.025	0.026[1]	0.0017	6.6	-	-	-
0.250	0.242[1]	0.0094	3.9	-	-	-
0.500	0.482[1]	0.0228	4.7	-	-	-
PLASMA						
50	0.034[2]	0.0030	8.8	0.036[4]	0.0018	5.0
100	0.146[2]	0.0109	7.5	0.135[3]	0.0022	2.0

[1] mean of 9 samples
[2] mean of 8 samples
[3] mean of 7 samples
[4] mean of 4 samples

TABLE 2.11 Conservation of naproxen during dialysis

Initial total amount of naproxen (µg)	Post dialysis Buffer conc. (µg/ml)	Post dialysis Plasma conc. (µg/ml)	Final total amount of naproxen[a] (µg)	% at end of dialysis
25	Ø.ØI5	24	26.4	1.Ø6
5Ø	Ø.Ø4Ø	46	5Ø.6	1.Ø1
75	Ø.Ø77	66	73	Ø.97
1ØØ	Ø.154	88	97	Ø.97
15Ø	Ø.748	134	148	Ø.99
2ØØ	1.39	174	193	Ø.96
3ØØ	3.ØØ	271	3Ø3	1.Ø1
4ØØ	5.15	35Ø	39Ø	Ø.98
6ØØ	16.1	526	595	Ø.99
8ØØ	51.6	696	8Ø6	1.Ø1

[a] The amount of naproxen recovered at the end of dialysis is calculated taking into account a 1Ø% increase in the volume of plasma during dialysis

(Table 2.12).

(v) The effect of adding naproxen to the plasma or buffer

Since the volume of plasma collected at the end of the initial washout was limited, it was more practical to spike the dialysis buffer with naproxen than to spike the plasma. It is possible that this would affect the time to reach equilibrium so a comparison was made between the free concentration over a range of total concentrations initially in either buffer or plasma. Table 2.13 shows that after a 3 hour dialysis the free concentration was consistent whether the drug was present initially in buffer or plasma.

(vi) Calculation of the bound concentration and free fraction

The concentration of naproxen bound (Cb) to plasma proteins at the end of the dialysis was calculated as follows:

$$Cb = C - 2.Cu \quad \ldots\ldots 2.3$$

where C is the total concentration in plasma and Cu is the free concentration in dialysis buffer.

The total concentration (C') of naproxen in plasma after dialysis:

$$C' = C - Cu \quad \ldots\ldots 2.4$$

and the free free fraction (f_u) of naproxen at the end of dialysis:

$$f_u = Cu / C' \quad \ldots\ldots 2.5$$

(vii) Volume shifts during dialysis

The degree of volume shift due to the movement of water

TABLE 2.12 Naproxen concentration in plasma (P) and buffer (B) after various dialysis times

Initial plasma conc.	Dialysis Time (hours)							
	1		2		3		4	
	P	B	P	B	P	B	P	B
50	42	0.052	42	0.048	42	0.045	41	0.045
200	186	0.395	186	0.367	180	0.395	178	0.386

All concentrations are in μg/ml

TABLE 2.13 Comparison of free drug concentrations in dialysate after a three hour dialysis with the drug initially in the plasma (1) and buffer (2)

Initial total drug concentration	Naproxen concentration in dialysate	
	1	2
50	0.034	0.035
100	0.159	0.144
200	1.32	1.40
400	4.92	5.15

All concentrations are given in μg/ml

from the buffer to the plasma side of the membrane was assessed by measuring the total protein concentration before and after 3 hour dialysis experiments. The ratio of post dialysis to pre-dialysis protein concentration (F) was calculated for 200 samples (Figure 2.16). The mean value of F was 0.909+0.025 (%CV=2.8): on average, the volume shift was just under 10%.

(viii) Effect of pH

There was no change in the binding of naproxen in plasma over the pH range 5-9 consistent with a previous study of naproxen binding to bovine serum albumin (Kaneo et al, 1981)

(ix) Precision

At least one quality control plasma sample was taken through the dialysis and HPLC assay. There was a limit to the number of quality control samples included in one dialysis experiment since there were only a total of 20 cells.The inter and intra-assay precision for plasma concentrations of 50 and 100µg/ml are shown in Table 2.10. In addition the inter-assay precision of buffer taken through the HPLC assay is given.

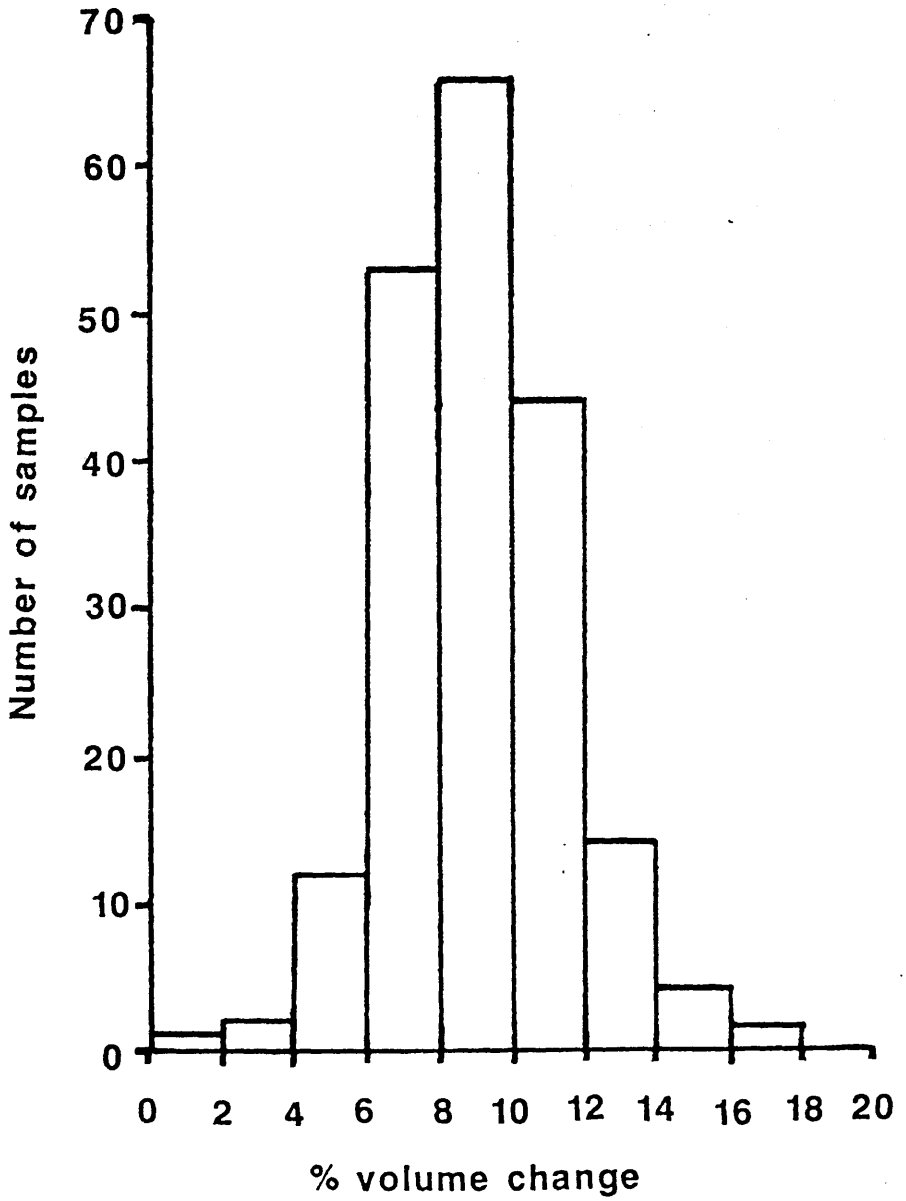


FIGURE 2.16 The distribution of the volume shift during dialysis of 200 samples

CHAPTER 3

RHEUMATOLOGICAL ASSESSMENTS

3.1 INTRODUCTION

In general the clinical assessment of antirheumatic drugs is largely subjective, or at best, semi-objective. The number of different measures available reflect the relative inadequacy of any one particular measure.

The more objective assessments of antirheumatic drug effect are based on relatively crude measurements of the degree of inflammation. They have not changed dramatically for a long time and consist of measures of joint tenderness, the time to walk a set distance, digital joint circumference and grip strength. These measurements tend to be variable and therefore lack sensitivity. In previous studies investigating dose or concentration relationships, digital joint size and walking time have proved to be the least useful of these semi-objective measurements (Orme et al, 1976; Baber et al, 1979; Ekstrand et al 1980 & Day et al, 1982). In addition they have often been shown to be no better than purely subjective measures such as the patients' own assessment of pain or the duration of morning stiffness.

If a response to NSAIDs is to be used to measure the effect produced by different doses or concentrations, it should be fairly sensitive and subject to as little measurement error as possible to allow comparisons of small changes. Unfortunately, the degree of variability associated with rheumatological assessments results not only from the crude and rather subjective nature of the measures

but also a result of the variable nature of the disease, differences in individual perceptions of pain and changes in mood which may affect attitudes towards disease.

Newer approaches which provide a more objective measure of the degree of inflammation, such as ^{99}Technetium pertechnetate (^{99}Tc) uptake and thermography have not been used widely because they are time consuming, require special equipment and rarely provide better results than the older more subjective methods. These techniques however can only be applied to specific joints. Recently, De Silva et al (1986) compared two of these more objective techniques with subjective measurements of pain and inflammation in the knee. They showed that there was some correlation between objective and subjective methods, but in most cases the correlation coefficients were less than Ø.5. Correlations were much better for ^{99}Tc uptake than for the 'heat distribution index' (HDI) which has previously been shown to correlate better with clinical assessment than with the usual thermographic index (Salisbury et al, 1983). Grennan et al, 1983 found that infrared thermography was less sensitive than an articular index or analogue pain score when one week of ibuprofen treatment was compared to placebo. However, it had previously been suggested that the clinical indices of disease activity achieve their maximum improvement more rapidly than changes in the thermographic indice (Bacon et al, 1976) with NSAID treatment. The usefulness of these types of assessments in studies

investigating dose or concentration-response relationships has yet to be established.

Biochemical measurements have proved to be unsatisfactory in the assessment of NSAID effects. Reduction in the ESR, C-reactive protein, globulin and rheumatoid factor and increases in albumin, haemoglobin and iron have only been observed during long-term treatment with second-line antirheumatic drugs (Amos & McConkey, 1981).

A further development in objective assessment in rheumatoid arthritis is the use of an ambulatory monitoring technique (MacGregor , 1981). A 'physiological cost index' (PCI) which relates the walking (RHI(w)) and resting (RHI(r)) heart rates to the walking speed, thus

$$PCI(beats/m) = \frac{RHI(w) - RHI(r)\ (beats/min)}{Walking\ speed\ (m/min)} \quad .....3.1$$

In a study comparing a NSAID with placebo, there was a reduction in the PCI in 8 out of 1Ø patients. Thus the patients expended less energy in walking the same distance when they were receiving the NSAID. This type of monitoring device is useful as it is objective and can be worn by the patient at home.

The more traditional and commonly used rheumatological measures were used in the assessment of the disease in the subsequent clinical studies. These methods are described and discussed here in some detail.

3.2 RITCHIE ARTICULAR INDEX

A measure of joint tenderness should give a good indication of the degree of joint inflammation. However, no totally satisfactory method has yet been described. One of the most commonly used methods was introduced by Ritchie et al in 1968. It is simple and quick to perform.

3.2.1 Scoring procedure

The tenderness of each joint or group of joints is scaled from Ø-3 to give the index a degree of discrimination. If there is no pain the score is zero. A score of one is given if the patient complains of pain, two if the patient also winces and three if the patient withdraws. The tenderness of the cervical spine, hip joint, talo-calcaneal and midtarsal joints are elicited by passive movement. The joints treated as a single unit are the temporo-mandibular joints, the joints of the cervical spine, the sterno and acromio-clavicular joints, the metacarpal-phalangeal and proximal interphalangeal joints of each hand, and the metatarsal-phalangeal joints of each foot. A number of joints are omitted either because they are rarely involved or because they may be painful for some other reason. These are the distal interphalangeal joints of the hand and foot, joints of the lumbar spine, sacro-iliac joints and the proximal interphalangeal joints of the toes. The total possible score is 78. An example of an articular tenderness score is shown in Table 3.1.

TABLE 3.1 Example of a Ritchie Articular Index score from Ritchie et al, 1968

Joints examined		Result of joint examination: Not tender (Ø)	Tender (+1)	Tender and winced (+2)	Tender, winced and withdrew (+3)	Joint score
Temporomandibular			+			1
Cervical spine			+			1
Sternoclavicular			+			1
Acromioclavicular		+				Ø
Shoulders	(left)				+	3
	(right)		+			1
Elbows	(left)				+	3
	(right)				+	3
Wrists	(left)				+	3
	(right)				+	3
M.C.P	(left)				+	3
	(right)		+			1
P.I.P	(left)	+				Ø
	(right)	+				Ø
Hips	(left)				+	3
	(right)				+	3
Knees	(left)		+			1
	(right)			+		2
Ankles	(left)		+			1
	(right)		+			1
Talocalcaneal	(left)				+	3
	(right)				+	3
Midtarsal	(left)				+	3
	(right)				+	3
Metatarsal	(left)			+		2
	(right)			+		2
					Total	+5Ø

3.2.2 Intra and inter-observer variability

The Ritchie Articlar Index is associated with a fairly small degree of intra-observer variability, but a large degree of inter-observer variability. Index differences as much as 20 (total possible score of 78) between two observers assessing the same patient may not be taken as significant (Ritchie et al, 1968). This is a result of the difference in the amount or position of pressure exerted on the joint by different assessors and may also be related to the attitude of the patient towards the assessor. Thus it is important that measurements of joint tenderness should be made by the same observer throughout an entire study.

3.2.3 Comparison with other articular indices

The Ritchie Articular Index correlates well with the articular index of the Co-operating Clinics Committee of the American Rheumatism Association (1965) ($r = 0.89$) which scores the number of active joints according to tenderness on pressure, pain on passive movement and swelling. The Lansbury index records the number of active joints and is weighted for joint size, so that the hip is given greater weight than a joint in the finger (Lansbury & Haut, 1956; Lansbury, 1968). This gives a measure of joint involvement but there is no grading of tenderness, so it is unlikely to discriminate between different doses or concentrations of NSAIDs.

The Ritchie Articular Index is often modified by

allowing the proximal interphalangeal and metacarpal-phalangeal joints to be scored individually rather than as a unit. This obviously weights the index to some extent if patients have disease mainly limited to the hands (Day et al, 1982; Palmer et al 1981). It is likely that the variability in the measurement will increase in parallel with the increase in the total possible score.

The use of an instrument which applies a standard pressure might reduce inter-observer variability. The spring gauge dolorimeter can be used to determine the subjective pain threshold in an inflamed joint. The degree of tenderness is scored on a 10-point scale (McCarty, Gatter & Phelps, 1965). More recently, a simpler dolorimeter has been described which was more sensitive than a modified Ritchie Articular Index in measuring the degree of joint tenderness as the tenderness is scored on a continuous scale (Langley et al, 1983). These instruments, however, cannot be applied to all joints. It appears that the best approach is to have the same observer throughout a study and use simple digit pressure.

3.3 GRIP STRENGTH

Although grip strength appears to be a more objective measure of inflammation and pain, it is also affected by the patient/observer interaction and by the patients degree of motivation. In addition patient grip will be dependent on

the degree of muscularity or loss of function due to joint or tendon damage.

3.3.1 Measurement of grip strength

The patient is asked to grip a small bag which is usually inflated to 30mmHg. The pressure corresponding to the maximum sustained grip is recorded on a pressure gauge. The mean of at least two observations of each hand is determined.

3.3.2 Intra and inter-observer variability

A study by Lee et al (1974) indicated there was a large degree of inter-observer variability in the measurement of grip strength. Mean differences of up to 20mmHg occured with different observers. The mean intra-observer variability was of the order of 9mmHg.

3.3.3 Diurnal variation

Grip strength showed a dramatic diurnal variation in patients and also in healthy volunteers (Wright, 1959). Grip strength was weakest in the early hours of the morning, gradually improved during the morning, was maintained for a few hours and then fell off during the evening. Lee et al (1974) found a significant improvement in grip strength at midday and in the evening when compared to the morning, but the improvement was small and of the same order of magnitude as the intra-observer error. It is probable that the

difference in these two studies in terms of the magnitude of the diurnal variation was due to differences in the severity of the disease in the two patient groups. More recently a study of flurbiprofen in rheumatoid arthritis has also indicated a significant circadian rhythm associated with grip strength (Kowanko et al, 1981). In this study, patients carried out their own assessments at home throughout the day during treatment periods. Analysis of variance indicated that there was a significant diurnal variation in both grip strength and finger joint size. It is therefore important to determine grip strength at the same time of day throughout a study.

3.3.4 Newer approaches

A group of workers in New Zealand has developed a grip strength analyser which gives a dynamic measurement of grip strength function. Pressure-time recordings allow the determination of several aspects of grip. These include the power (related to the rate of grip development), work done (the area under the pressure-time curve) and maximum grip strength (Myers, Grennan & Palmer, 1980; Palmer et al, 1981). The measurement of power and rates of grip release and grip development showed greater percentage changes than maximum grip strength and power in a study of sodium meclofenamate compared with placebo (Palmer et al, 1981). They suggest that the dynamic parameters are likely to be affected by joint stiffness and swelling whereas the static

parameters (eg maximum grip strength) probably more closely reflect muscle power.

3.4 PAIN RATING SCALES

There are numerous types of scales which can be used to determine either pain levels or the degree of relief from pain (Figure 3.1). They range from 'simple descriptive scales' to visual analogue scales. Numerical scales fall somewhere in between the two extremes.

3.4.1 Simple descriptive scale

The simple descriptive scale, to which numerical values can be given, uses 4 or 5 points eg nil, mild, moderate, severe, very severe. This type of scale is easily understood by the patient but there are not many categories available and it is likely that this approach will lack sensitivity in detecting small changes. It is therefore unlikely to be of use in determining dose or concentration-effect relationships. An improvement in discrimination can be achieved, however, by using a numerical rating scale marked from Ø-1Ø or Ø-2Ø.

3.4.2 Visual analogue scales

A visual analogue scale should theoretically allow for even greater discrimination. A 1Øcm line represents a continuum of pain from no pain to the worst pain ever experienced. The patient is asked to make a mark on the line

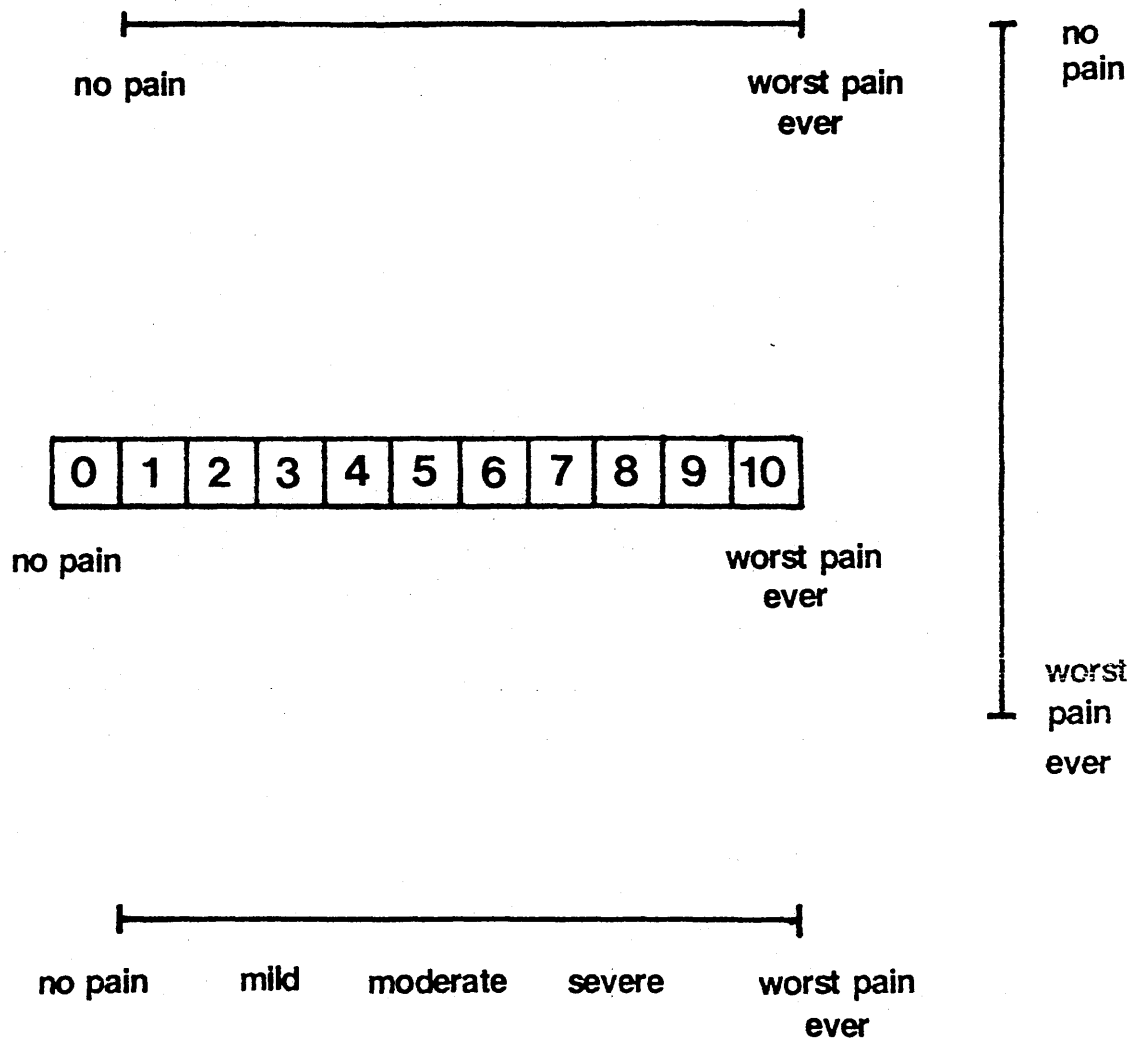


FIGURE 3.1 Various pain rating scales

at a position between the two extremes which represents the perceived level of pain. Joyce et al (1975) found that the visual analogue scale was more sensitive than a four point scale in discriminating between the analgesic effect of two doses of dihydrocodiene in patients with rheumatic disorders.

The design of the visual analogue scale, however, has been shown to affect the final result. Scott & Huskisson (1979a) investigated the performance of horizontal and vertical analogue pain scales. They found a uniform distribution of results on a horizontal scale whereas there was a clustering of results if a vertical scale was used. These scales were associated with descriptions. However similar results have been obtained with scales without descriptions. In contrast, Downie et al (1978) found that there was no appreciable difference between a horizontal or vertical scale. On balance, however, the uniformity of results across a horizontal scale gives the method greater sensitivity.

Other workers investigating pain scales have suggested that a numerical rating scale may be used more accurately than an analogue scale (Downie et al, 1978). They suggest that this may be because it provides a compromise between the simple descriptive scales in terms of discrimination and the analogue scale where the freedom of choice may be confusing to the patient.

Another factor which has to be considered if these

measurements are repeated over a period of time is whether the patient should be allowed to see their previous scores. Joyce et al (1975) found little difference in visual analogue pain scores whether or not patients were allowed to see their previous score. Another study has suggested that it is important for patients to observe their previous score as patients tend to overestimate their pain with the passage of time (Scott & Huskisson, 1979b). They are able to correct their scores when shown their initial starting point.

3.4.3 Comparison of pain scales

A number of studies have investigated the degree of correlation between various pain rating scales. Downie et al (1978) found there was good correlation between four different scales, 4 point descriptive scale, Ø-1Ø numerical scale rating and the visual analogue scale used both horizontally and vertically. However the 11 point scale and the horizontal analogue pain scale appeared more precise. Another study compared the performance of three different scales; a 4-5 point pain scale, a horizontal analogue pain scale and a 6 point pain relief scale (Littman, Walker and Schneider, 1985). These were used in the assessment of various analgesic drugs. This study again showed that there was a good correlation between the various pain rating scales. In this case the descriptive pain relief scale appeared to be more sensitive than the analogue pain scale which in turn was more sensitive than the descriptive pain

intensity scale. It is not surprising that 4 point pain intensity scales are not very sensitive since if a patient starts with moderate pain there is only one step available between the baseline pain category and no pain. The pain relief scale allowed for a greater degree of flexibility.

3.5 DURATION OF MORNING STIFFNESS

Often the major problem facing patients with rheumatoid arthritis is morning stiffness. The duration of morning stiffness or the time taken to 'limber up' is a useful measure to test the effect of antirheumatic drugs. It is, however, important that the patient can distinguish between stiffness and joint pain (Steinberg, 1978). The recording of morning stiffness, however, has rarely been found to be a sensitive measure of disease activity.

More objective measures of the degree of morning stiffness may provide improved sensitivity in this assessment than is available by simply asking 'how long does it takes you to get going in the morning?'. Using an improved hand grip assessment, Myers and collegues have been able to demonstrate that stiffness is reflected in the power developed during the establishment of hand grip (Myers, Wilson & Palmer, 1981).

3.6 COMPOSITE SCORES AND PATIENT PREFERENCE

It is popular in trials of anti-inflammatory drugs to sum a number of effect parameters to obtain a composite index. This allows an overall view of the success or failure of a treatment when there is possibly improvement in some parameters but not in others. These composite scores may also increase the statistical efficiency of the study as this type of composite score will tend to normalise individual patients' clinical effect. However the clinical significance of a statistically significant effect may be difficult to determine if the relative weighting of each component in the composite score is not taken into account. A concentration-effect relationship has been demonstrated for naproxen using a composite score of several response indices (Day et al, 1982).

Patient preference or order of preference for a particular treatment is often a useful measure as it is related to the efficacy of the treatment and to the severity of side-effects.

3.7. SUMMARY OF ASSESSMENT METHODS USED IN THIS THESIS.

An example of the assessment forms used in the studies presented in this thesis is given in Appendix I. The assessments were almost identical for the studies of fenclofenac and naproxen. One clinical metrologist carried

out the assessments for the fenclofenac study and another did the assessments for the naproxen study. The assessments used in dose and concentrations response analyses were carried out at the same time of day throughout each study.

1. The Ritchie Articular Index was determined as described in section 3.2.1.
2. The duration of morning stiffness was stated by the patient and recorded in minutes.
3. Mean grip strength was determined from the mean of two observations of each hand. The patient was asked to grip a small bag inflated to 30mmHg. The pressure was recorded on a gauge scaled in 2mmHg increments.
4. Global pain was determined using:
    a) 10cm horizontal visual analogue scale.
    b) 4 point descriptive scale. The four categories were none, mild, moderate and severe.
5. 4 point descriptive scale of the therapeutic effect as assessed by the patient and by the clinical metrologist. The categories were none, fair, good and very good.

CHAPTER 4

DATA ANALYSIS

4.1 INTRODUCTION

This chapter describes the general approaches used in the analysis of data generated from the studies described later in this thesis. There were several models used to describe the data:

1. Pharmacokinetic models, to describe the time course of drug concentrations.
2. Pharmacodynamic models, to determine the relationship between drug concentration and response.
3. Models to describe the binding of drugs to plasma proteins.

The analysis of data in terms of a model allows the relationship between at least two variables to be quantitated and in some cases the parameters of a model may be used in a predictive manner. In this thesis, model parameters were determined by the method of 'least squares'. Individual patient data sets were analysed to obtain parameter values for the relevant model. In some cases, however, it was more appropriate to analyse all data simultaneously to determine the average parameter values and their variability within the patient population. In this situation the programs GLIM (Baker & Nelder, 1978) and NONMEM (Beal & Sheiner, 1980) were used.

Standard statistical tests such as simple linear regression, Students't-test and analysis of variance were

applied where appropriate.

4.2 PHARMACOKINETICS

Pharmacokinetics is the study of the time course of drugs in the body. In this thesis, emphasis was placed on the investigation of inter-individual differences in the processes of of absorption, distribution, metabolism and excretion which help in the understanding of the pharmacological effect of a drug assuming that the clinical response is in some way related to the plasma concentration. Factors such as age and disease can have considerable effects on the pharmacokinetics of some drugs. This in turn may be reflected in differences in clinical response or toxicity.

4.2.1 Compartmental models

The concentration-time profile of a drug in plasma is commonly represented by a system of compartments. These compartments do not necessarily have any physiological or anatomical meaning. It is imagined that a drug is distributed throughout one or more compartment 'spaces' and that the drug concentration in any one compartment is homogeneous. The rates of transfer between compartments are assumed to obey first order kinetics. The parameters determined using this type of analysis may be used subsequently to predict the plasma concentration of a drug

at any time after multiple doses assuming that the kinetics are linear.

The one compartment model describes the concentration of drug in plasma (C) at any time (t) in terms of a single exponential:

$$C = C_{\emptyset} e^{-k_e t} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.1)$$

where k_e is the elimination rate constant which can be expressed as a half-life:

$$t_{1/2} = \ln 2 / k_e \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.2)$$

and $C_{\emptyset}$ is the initial concentration of the drug after intravenous administration. The volume of distribution is:

$$V = \text{Dose}/C_{\emptyset} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.3)$$

and the clearance (defined as the volume of plasma which is cleared per unit time) is:

$$Cl = V.k_e \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.4)$$

In all studies described in this thesis, however, the drug was given orally. Values of clearance and volume of distribution are therefore approximations as absorption is uncertain, hence the terms apparent clearance (Cl/F) and apparent volume of distribution (V/F) are used. If the distribution of the drug from plasma and highly perfused tissues is rapid in comparison to the rate of absorption, the profile in plasma will approximate to a one compartment model (Figure 4.1a). The equation describing the concentration-time profile is:

$$C = A\ (e^{-k_e t} - e^{-k_e t}) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.5)$$

which can be expressed in terms of the Bateman function:

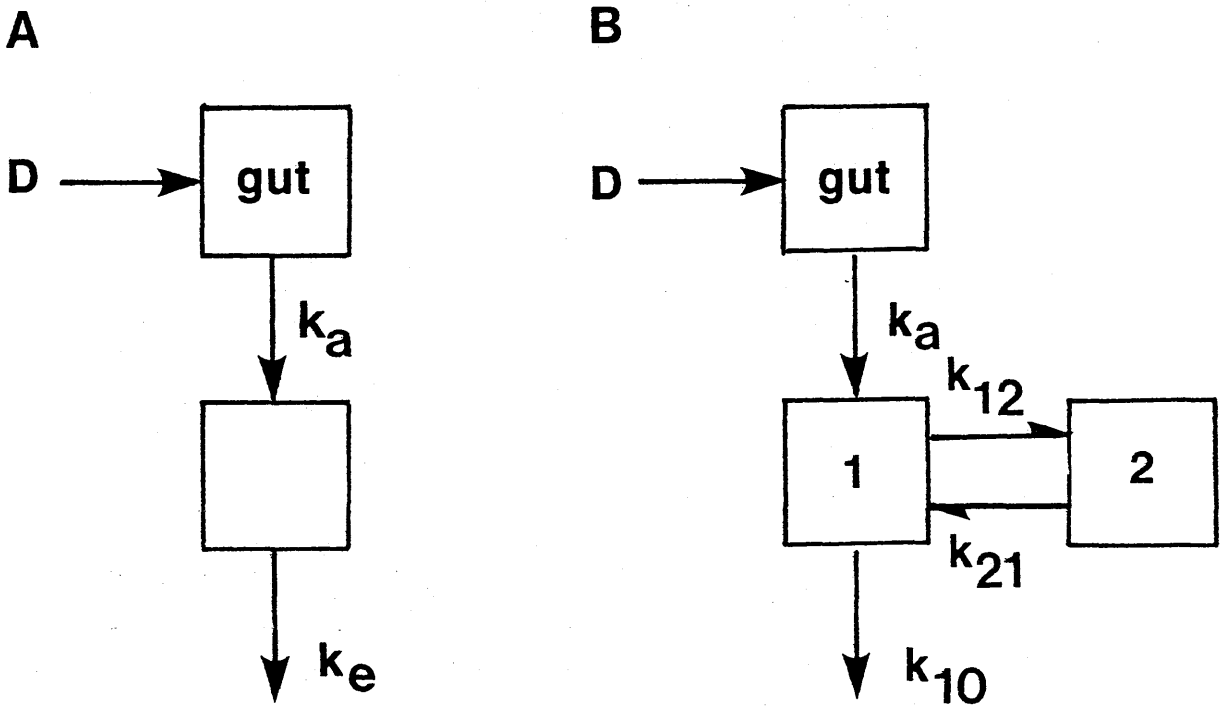


FIGURE 4.1 One and two compartment pharmacokinetic models

A One compartment model
B Two compartment model

$$C = \frac{k_a FD}{V(k_a - k_e)} (e^{-k_e t} - e^{-k_a t}) \quad \text{.....(4.6)}$$

where D is the dose and k_a is the absorption rate constant. If the distribution phase is more prolonged then the kinetics of the drug after oral administration may be described better by a two compartment model (Figure 4.1b). The equation describing the concentration-time profile is given in Appendix II.

The absorption of a drug after oral administration has generally been described by a first order rate constant despite the fact that gastrointestinal absorption of drugs involves several processes which may or may not be first order (eg. dissolution of the tablet formulation, different rates of absorption from different parts of the gastrointestinal tract and gastric emptying). Some investigators have found that the absorption of certain drugs after oral administration may be better described as a zero order process (analogous to a short constant rate infusion of the drug) (McNamara, Coburn & Gibaldi, 1978; Whitfield, Kaul & Clark, 1978). A comparison of the type of profile obtained using zero order or first order input is given in Figure 4.2.

The pharmacokinetic models used in the analysis of NSAID plasma concentration-time data were either one or two compartment models with first or zero order absorption. The equations for the models (Models 1-4) are given in Appendix

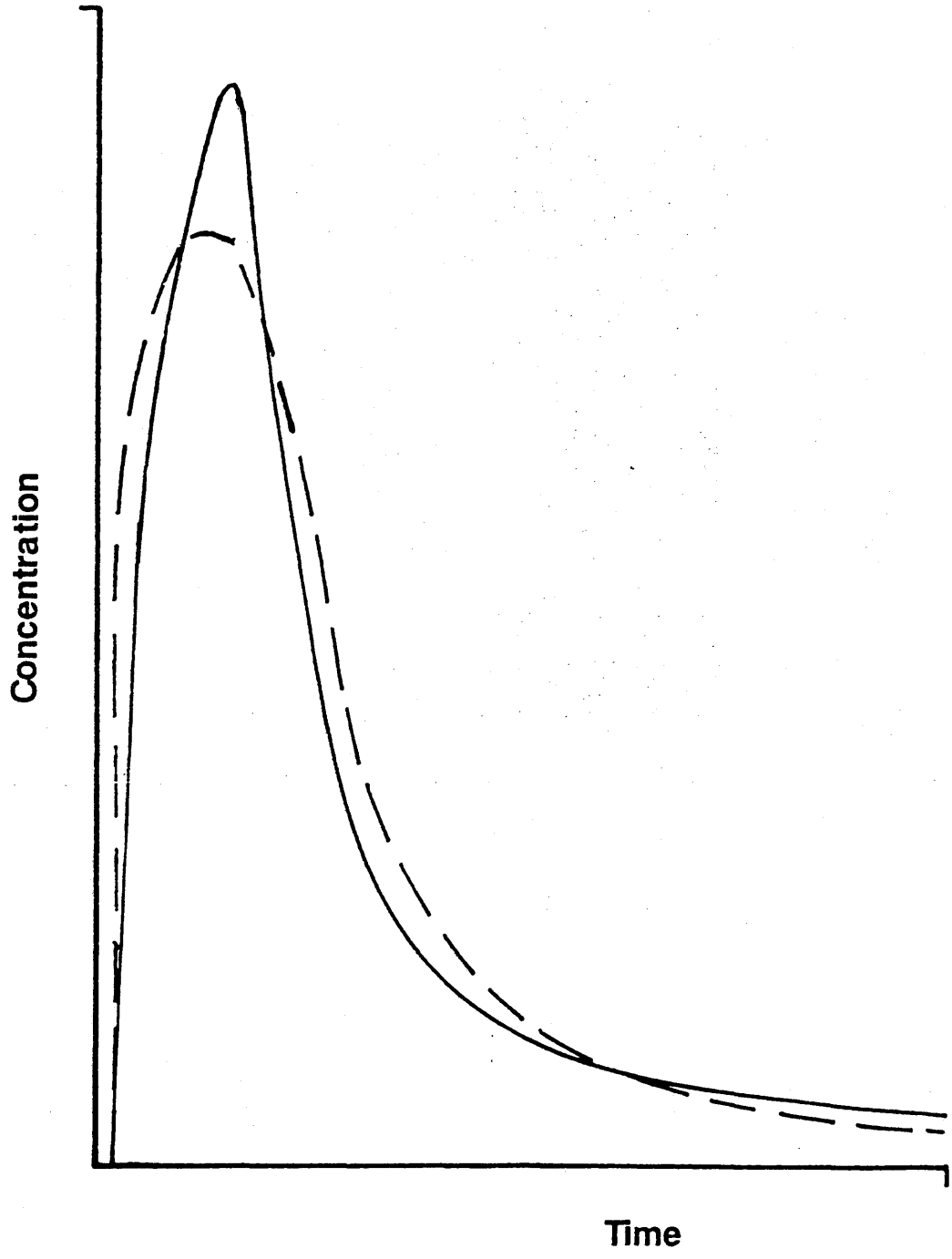


FIGURE 4.2 Comparison of the effect of a first (— — —) or zero (————) order input function on a two compartment model concentration-time profile

II. In all cases a time lag (time after administration when the drug is first detected in plasma) was incorporated in the pharmacokinetic model. In order to fit concentrations in synovial fluid, two models were proposed (Models 5 and 6 in Appendix II, Figure 4.3). Model 5 assumed that concentrations in synovial fluid could be described in terms of the kinetics of the peripheral compartment of a two compartment model. Model 6 assumed that the synovial fluid represented a distinct, relatively small compartment which did not affect the kinetics of the drug in plasma.

4.2.2 Physiological Models

The clearance of drug from the blood can be expressed as the product of blood flow to the eliminating organ (Q) and the extraction ratio (E) of the drug across the organ:

$$Cl = Q\ E \qquad \text{.....(4.7)}$$

The extraction ratio is dependent on three physiological variables; blood flow, the ability of the organ to remove the drug and the degree of plasma protein binding. The most commonly used model is the 'well stirred' model (Wilkinson & Shand, 1975). When applied to drugs which are eliminated entirely by hepatic metabolism the clearance of total drug is:

$$Cl = Q\left[\frac{f_u\ Cl_{int'}}{Q + f_u\ Cl_{int'}}\right] \qquad \text{.....(4.8)}$$

where f_u is the free fraction of the drug in blood and $Cl_{int'}$ is the intrinsic clearance. The intrinsic clearance

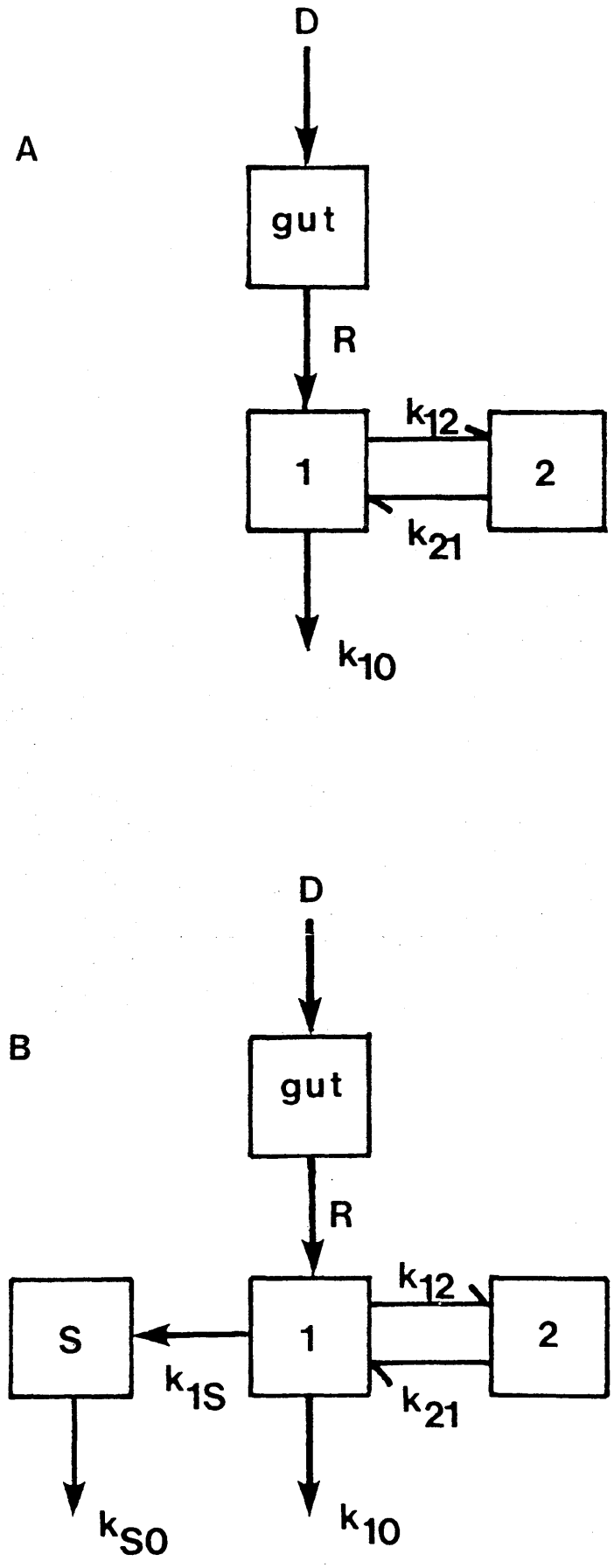


FIGURE 4.3 Pharmacokinetic models proposed to describe the concentration of drug in plasma and synovial fluid

A Model 5
B Model 6

is a measure of hepatic drug metabolising activity and is related to the enzymatic parameters, K_m and V_{max}

$$Cl_{int'} = \frac{V_{max,i}}{K_{m,i}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (4.9)$$

when the system is operating under linear conditions, ie when the unbound concentration of the drug in liver is less than $K_{m,i}$ (Pang, Rowland & Tozer, 1978). Using this model there are two extremes. The model predicts that the clearance of drugs with a low extraction ratio will be sensitive to changes in the binding and intrinsic clearance. However, the clearance of drugs with a high extraction ratio will be dependent on the liver blood flow. For low extraction drugs the clearance of total drug ($Cl_{(T)}$) and free drug ($CL_{(F)}$) are:

$$Cl_{(T)} = f_u\, Cl_{int'} \quad \ldots\ldots\ldots\ldots\ldots\ldots (4.10)$$

$$Cl_{(F)} = Cl_{int'} \quad \ldots\ldots\ldots\ldots\ldots\ldots (4.11)$$

The other parameter which can be considered in the apparent volume of distribution, in physiological terms the volume of distribution given by:

$$V = V_B + V_T\, (f_B/f_T) \quad \ldots\ldots\ldots\ldots\ldots\ldots (4.12)$$

where V_B is the volume of blood, V_T is the volume of tissues, f_B is the free fraction in blood and f_T is the free fraction in tissues.

4.3 ANALYSIS OF PLASMA PROTEIN BINDING DATA

The binding of drugs to plasma proteins is usually assumed to obey the law of mass action. The interaction

between a drug molecule and a protein molecule can therfore be described in terms of a Langmuir isotherm:

$$Cb = \sum_{i=1}^{n} \frac{nP_i.Cu}{K_{di} + Cu} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.13)$$

where Cb is the concentration of drug bound, Cu is the free concentration of drug, n is the number of classes of binding sites, and K_{di} and nP_i are, respectively the dissociation constant and the number of equivalent binding sites of the ith class of sites.

Examination of the literature of the binding of a particular drug to plasma proteins will provide a range of quite diverse parameter values for affinities and number of binding sites (Kragh-Hansen, 1981). Some examples of binding of NSAIDs are given in Table 4.1. Although this may in part be due to differences in the analytical technique it is also a result of errors in the analysis of the data (Vallner, Perrin & Wold, 1976). In the past, binding parameters were obtained by graphical analysis after linearisation of the Langmuir equation eg Klotz and Scatchard Plots. In all cases both independent and dependent variables are subject to error. If there is one high affinity site and one or more classes with lower affinity, the graphical representations are curved, and separation of the various binding parameters is more difficult. Often the intercepts and slopes obtained from graphical methods are quoted as the parameter values, this will result in errors if the affinity of the high affinity site are not much larger than that for the low

TABLE 4.1 Range of binding parameters quoted for some NSAIDs (taken from Kragh-Hansen, 1981)

Drug	n_1	K M^{-1}	n_2	K M^{-1}	Method	Temp (°C)
Salicylate	1	7.1×10^4	4	3.3×10^3	gel frontal analysis chromatography	25
	1	2.2×10^5	5	1.6×10^3	dynamic dialysis	37
	2	1.3×10^5	2	2.9×10^3	equilibrium dialysis	20
Phenylbutazone	1	2.4×10^5	2	4.6×10^4	circular dichroism	20
	1	1.0×10^5	2	4.0×10^4	equilibrium dialysis	20
	3	2.5×10^5	4	1.3×10^3	dynamic dialysis	37
	1	2.3×10^5	4	5.6×10^3	equilibrium dialysis	37
Indomethacin	1	1.0×10^6	4	1.0×10^5	equilibrium dialysis	20
	1	3.0×10^5	7	1.4×10^4	ultrafiltration	37

affinity site (Vallner et al, 1976).

The use of computer procedures should have improved the quantitation of binding parameter values. However most procedures determine the parameters through a least squares fit of the data based on the Scatchard equation. Alternatively the data are fitted simply in the form of the Langmuir equation so that the error in the free concentration (independent variable) is assumed to be small and independent of the error in the bound concentration. Free and bound concentrations are determined in general from the total concentration (which is known fairly accurately) and will therefore be correlated.

Other statistically correct least squares procedures have been proposed such that the free or bound concentration is analysed in terms of the total concentration (Perrin, Vallner & Wold, 1974; Priore & Rosenthal, 1976).

Despite the fact that these mass action models have some physiological basis, the parameter values reported may have no relevance if the data analysis was inappropriate. Often there are too few data points to be able to get a good estimate of the parameters. In addition some workers suggest that the value of 'n' should be fixed in order to reduce the number of parameters to be estimated.

Simpler mathematical functions have been fitted to binding data. These methods of analysis do not assume any specific molecular behavior but merely describe the observed data so that predictions of free concentrations or free

fractions may then be determined (Behm & Wagner, 1981; Monot et al, 1983).

In this thesis, binding data were fitted to the Langmuir isotherm for two independent binding sites:

$$Cb = \frac{nP_1.Cu}{K_{d1} + Cu} + \frac{nP_2.Cu}{K_{d2} + Cu} \quad \text{.....(4.14)}$$

where Cb and Cu are the bound and free drug concentrations and nP_1 and nP_2 are the binding capacities of two classes of binding sites with equilibrium dissociation constants of K_{d1} and K_{d2} respectively.

Rearrangement of this equation in terms of total concentration results in a cubic equation which cannot be solved easily. As a compromise the free concentration was considered as the dependent variable. When the Langmuir equation is rearranged, the free concentration is given by the positive root of a quadratic equation (Appendix III). This treatment is more appropriate than fitting bound in terms of free for drugs which are highly bound. The percentage error in the determination of the free fraction is much greater than for the bound fraction for a drug like naproxen or fenclofenac. For naproxen the coefficient of variation for free drug concentration ranged from 7.5 to 8.8, however expressed in terms of bound drug the coefficient of variation ranged from Ø.ØØ6 to Ø.Ø11. The possibility of correlation between bound and free concentration is only likely when the free fraction exceeds 1Ø%.

4.4 ANALYSIS OF CLINICAL RESPONSE DATA

The classical models to explain dose or concentration-effect relationships were based on the Langmuir isotherm assuming a reversible drug receptor complex:

$$\text{Effect} = \frac{E_{max} \cdot C}{EC_{50\%} + C} \quad \ldots\ldots\ldots\ldots\ldots\ldots (4.15)$$

where C is the concentration or dose, E_{max} is the maximum effect and $EC_{50\%}$ is the concentration or dose producing 50% of E_{max}. This model, often referred to as the E_{max} model, has been used widely to describe drug effects in isolated tissues.

A number of models have been proposed for the analysis concentration-effect relationships in vivo (Holford & Sheiner, 1981). The simplest model which can be used to describe clinical response in terms of concentration is a linear model:

$$\text{Effect} = A + B.\ C \quad \ldots\ldots\ldots\ldots\ldots\ldots (4.16)$$

where A is the baseline measurement and B is the slope of the line relating the effect to concentration. This model can be derived from the the E_{max} or hyperbolic model if concentrations are assumed to be low in relation to $EC_{50\%}$. The E_{max} model is able to describe drug effect over a wide concentration range and can be modified to allow for a baseline effect:

$$\text{Effect} = \frac{E_{max} \cdot C}{EC_{50\%} + C} + E_{\emptyset} \quad \ldots\ldots\ldots\ldots (4.17)$$

where $E_{\emptyset}$ is the baseline value, assuming that baseline measurements have the same error as the other measurements.

The log-linear model is an approximation to the E_{max} model in the range 20 to 80% of the maximum response:

$$Effect = B.\log(C) + I \quad \ldots\ldots\ldots\ldots\ldots\ldots (4.18)$$

where I is an arbitary constant with no physical meaning. The model is unable to predict the absence of an effect when there is no drug present.

If the drug effect is examined over a dosage interval, the clinical response may be described in terms of concentration using an integrated pharmacokinetic / pharmacodynamic model (Sheiner et al, 1979). In this thesis, however, the response was compared with a single steady state concentration obtained on different doses so that this type of integrated model was not possible.

4.5 PARAMETER ESTIMATION

This section outlines the general principles of least squares regression analysis together with the details of the specific computer programs used to determine the parameter values of the particular model. Non-linear regression was used to estimate individual parameters of the particular pharmacokinetic or binding model and the programs GLIM (linear models) and NONMEM (non-linear models) were used to simultaneously analyse data from a large number of individuals.

4.5.1 Least squares regression analysis

In both linear and nonlinear least squares regression analysis, the total variation in the dependent variable may be partitioned into that due to the model (the explained variation) and the remaining residual error (the unexplained variation). The assumptions are:

a) the error in the independent variable is negligible

b) the values of the dependent variable are sampled from a normal distribution

c) the variance of the dependent variable is constant.

The best estimates of the model parameters are those which minimise the residual sum of squares or the objective (Obj) value:

$$Obj = \sum (y_i - \hat{y}_i)^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots (4.19)$$

where y is the observed value and $\hat{y}$ is the fitted value of the dependent variable. This is the objective for ordinary least squares regression. If the error in the independent variable is known (eg the error in the measurement of drug concentration) an appropriate weighting scheme may be applied. In general the dependent variable is weighted by the reciprocal of the fitted value itself or the fitted value squared (Boxenbaum, Riegelman & Elashoff, 1974). This is able to cope with the experimentally observed error in the measurement of drug concentrations over a wide range, since the absolute magnitude of the error tends to increase as the concentration increases. The objective value will

take account of the weighting scheme used:

$$Obj = \sum \frac{(y_i - \hat{y}_i)}{wt_i} \quad \ldots\ldots\ldots\ldots\ldots\ldots (4.20)$$

4.5.2 General Linear Interactive Modelling (GLIM)

The program GLIM (Baker & Nelder, 1978) was used for the simultaneous analysis of dose or concentration-response data from all patients (Chapters 5 & 6).

Simple linear regression assumes that all values of y are mutually independent. It is inappropriate for the analysis of data which contains more than one observation from a single individual. GLIM is able to handle this type of data. The linear model may involve one or more independent factors or variables and account is taken of the fact that some of the observations are associated (ie from the same individual). The parameters of the linear model are those which minimise the residual sum of squares and as for simple linear regression the parameters are unique for any given set of data. In it's simplest form the program can be used for analysis of variance.

A hierarchical series of linear models were proposed to test the effect of dose or concentration (total or free) on a particular clinical response measurement. The models tested are illustrated in Figure 4.4. The full model describes the response in a individual($effect_i$) in terms of a unique intercept (a_i) and slope (b_i):

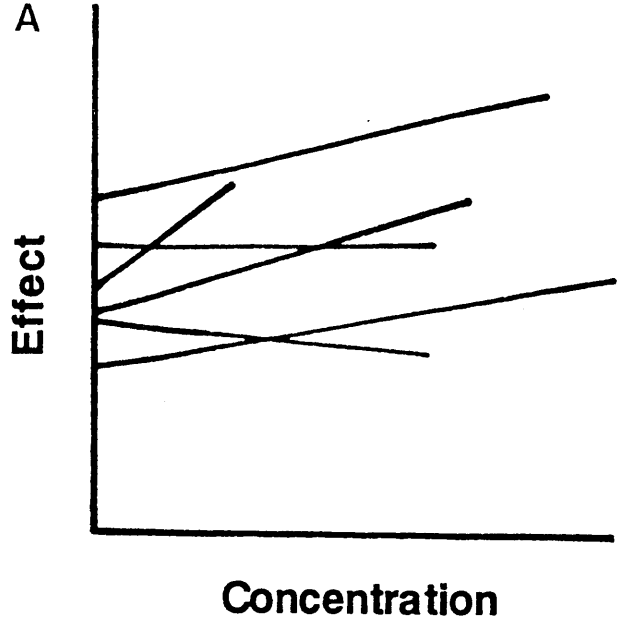
$$1.\ Effect_i = a_i + b_i.C \quad \ldots\ldots\ldots\ldots\ldots\ldots (4.21)$$

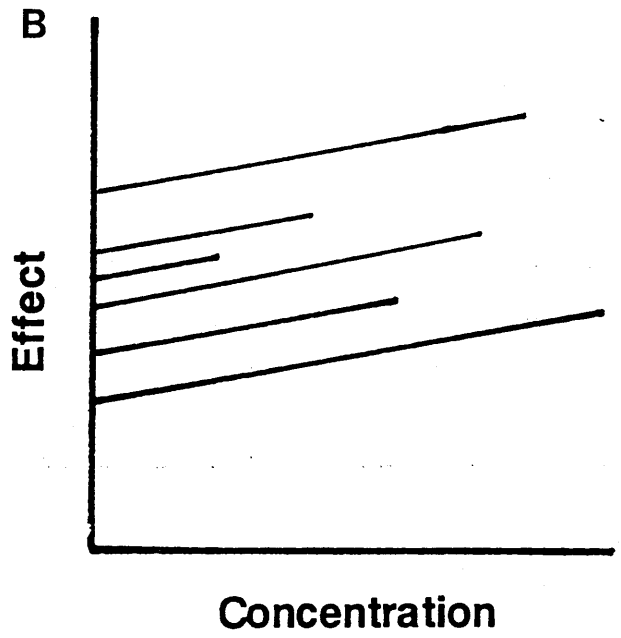
FIGURE 4.4 Linear models investigated to describe dose or concentration-response data

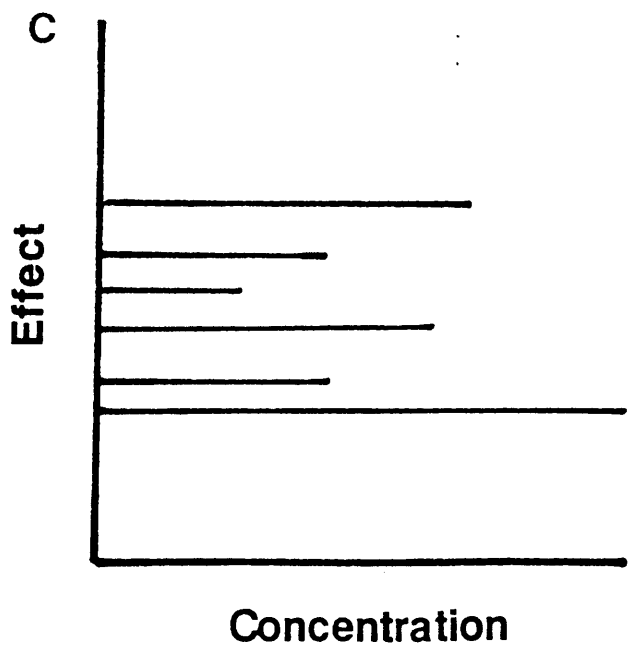
A $\text{Effect}_i = a_i + b_i.C$

B $\text{Effect}_i = a_i + B.C$

C $\text{Effect}_i = a_i$


A
Effect
Concentration


B
Effect
Concentration


C
Effect
Concentration

The reduced models tested were:

$$2.\ \text{Effect}_i = a_i + B.C \quad \ldots\ldots\ldots\ldots\ldots\ldots (4.22)$$

where the slope (B) is common for all individuals, and

$$3.\ \text{Effect}_i = a_i \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.23)$$

where the slope is zero.

GLIM was also used to test the factors which affect the free fraction of fenclofenac (Chapter 5).

Selection of the most appropriate model

The addition of parameters to the model will lead to a reduction in the residual sum of squares and an apparent improvement in the fit. However, the most appropriate model is the simplest model (ie the model which keeps the number of parameters as small as possible) that still gives a good description of the data. Statistically the best model is selected on the basis of the F-ratio test. The reduction in the residual sum of squares is tested in relation to the decrease in the number of degrees of freedom (ie increase in the number of parameters). This is often referred to as the F-to-enter statistic or the F-to-remove statistic, depending on whether the simplest or the most complex model is used as the starting point, and is based on the General Linear Test (Netter & Wasserman, 1974). The F value is calculated:

$$F = \frac{SSQ(R)-SSQ(F)}{df(R)-df(F)} \Big/ \frac{SSQ(F)}{df(F)} \quad \ldots\ldots\ldots\ldots (4.24)$$

where SSQ(R) and SSQ(F) are the residual sum of squares for the reduced and full model. df(R) and df(F) are the degrees of freedom for the reduced and full model. The significance

of F is determined for df(R)-df(F), df(F) degrees of freedom. If F is not significant the full model is rejected in favour of the reduced model. The 'goodness of fit' can be assessed by calculating the coefficient of determination (C_{det}):

$$C_{det} = \frac{\text{explained variation}}{\text{total variation}} \quad \ldots\ldots\ldots\ldots (4.25)$$

4.5.3 Nonlinear regression

Nonlinear least squares regression analysis was used to fit individual patient data sets (concentration-time data or protein binding data) to the models described in the previous section. Unlike linear regression, there is no unique solution for nonlinear regression. The nonlinear fitting procedure used in the analysis of data was a modification of the Marquardt algorithm (Marquardt, 1963) and was implemented on a Nodecrest mini computer. The non-linear model, in the form of a Fortran subroutine and initial estimates of the parameters of the model were provided.

(i)' Goodness of fit'

Examination of residual values, the difference between the observed and fitted value of the dependent variable ($y_i - \hat{y}_i$), can give an indication of the 'goodness of fit'. Plots of the residual values against the fitted values of y can be very useful and may indicate that a weighting scheme is necessary. In addition plots of the residuals against the

independent variable can provide information on how well the model appears to fit the observed data. If there are systematic patterns in the residuals then it is possible that the model is inappropriate. The coefficient of determination gives an indication of the overall 'goodness of fit',the value of C_{det} should be as large as possible. However a high C_{det} value should always be considered in the context of any trends in the residuals.

(ii) Selection of the most appropriate model

There are various methods that can be used to determine the best model if different models are to be compared. If one model is a submodel of another within an ordered hierarchy (eg comparison of a one and two compartment pharmacokinetic model) the General Linear Test should be applied and the F ratio is calculated according to equation 4.24 (Netter & Wassweman, 1974). If there are not sufficient data points in relation to the number of parameters, the full model will often have to be rejected even if examination of the residuals suggests that the full model gives a better description of the data.

If the models to be compared have the same number of parameter values (or if one model is not a submodel of the the other) the General Linear test cannot be applied. In this situation other criteria may be considered (Akaike, Schwartz etc). In this thesis the Akaike Information Criterion (AIC) was used (Akaike, 1973). The AIC is derived from information theory:

$$AIC = N.\ \ln(SSQres) + 2.P \quad \text{.....(4.26)}$$

where N is the number of data points, SSQres is the residual sum of squares and P is the number of parameters. The lowest value of the AIC indicates the best fit. There is however no statistical test for the difference in the AIC value.

4.5.4 NONlinear Mixed Effects Model (NONMEM)

NONMEM is a computer program which can be used to fit data from a large number of individuals to any non-linear model (Beal & Sheiner, 1980). As with GLIM account is taken of the fact that all data points are not mutually independent. The program has generally been used to determine population pharmacokinetic parameters of certain drugs using data collected during routine clinical monitoring (small number of samples from a large number of patients) (Sheiner, Rosenberg & Marathe, 1977). In addition the relationship between patient specific factors and the parameters of the model can be investigated.

NONMEM was used to analyse dose/concentration - effect relationships and to determine parameter values for binding of naproxen to plasma proteins. The program provides estimates not only of the mean parameters of the structural model (θ's) ie. the population mean parameter values of the binding model or effect model, but also of the inter-subject variability of each of these parameters (η's). and the intra-subject variability (measurement error or model misspecification) (ϵ). There are different types of error

models; additive, proportional or log (Beal, Boeckmann & Sheiner, 1985). The program also provides the approximate error in the estimate of the structural and variance parameters. The best estimates of the structural and variance model parameters are those which minimise the objective value for a given set of data. It is possible to test the influence of patient factors on the parameters of the structural model.

In the analysis of dose and concentration - response relationships the E_{max} and Linear models described in Section 4.4 were tested. The log or proportional error model was used for the inter-individual variance in the structural parameters:

$$\ln\theta_{ki} = \ln\theta_k + \eta_{ki} \quad \text{.....(4.27)}$$

where θ_{ki} is the value of θ_k in the individual i. This assumes a log normal distribution of the structural model parameters. The constant (additive) error model was used for the intra-individual error:

$$y_i = \hat{y}_i + \epsilon_i \quad \text{.....(4.28)}$$

An example of a 'PRED' and control file are given in Appendix IV for the E_{max} model.

Naproxen plasma protein binding data were fitted to the Langmuir isotherm for two independent binding sites given in Section 4.3 rearranged in terms of the free concentration (Appendix III). In addition the data were also analysed taking account of patient specific factors which might be expected to affect the binding. The constant error model was

used for the inter-individual variance in the structural model parameters:

$$\Theta_{ki} = \Theta_k + \eta_{ki} \quad \text{.....(4.29)}$$

This assumes that the structural model parameters are normally distributed within the population. A log or proportional model was used for the intra-individual error (error in the measurement of free concentration):

$$\ln y_i = \ln \hat{y}_i + \epsilon_i \quad \text{.....(4.30)}$$

which assumes that the coefficient in the measurement of free concentration is constant over the concentration range. An example of the a 'PRED' and control file are given in Appendix IV.

Selection of the most appropriate model

Comparison of different models is based on the objective value. If one model is a submodel of another the difference in the objective value is χ^2 distributed with degrees of freedom equal to the difference in the number of parameters (structural and variance model parameters). If the models do not conform to a hierarchy the best model is chosen on the basis of the objective value, the error in the estimate of the parameters and on the examination of the residual plots against the dependent (observed or predicted) and independent variable. If there is any trend in the residuals, the model may be inappropriate.

CHAPTER 5

FENCLOFENAC, PHARMACOKINETICS AND CLINICAL RESPONSE

5.1 INTRODUCTION

This chapter presents a dose ranging controlled study of fenclofenac in patients with rheumatoid arthritis. Attention was directed towards the determination of the variability in the pharmacokinetics of fenclofenac and the general aim was to evaluate whether knowledge of total or free drug concentrations could contribute to the explanation of clinical response or toxicity.

There has been no properly controlled trial of fenclofenac over the recommended dosage range (600 to 1800mg daily in two divided doses) and little attention has been directed towards the measurement of plasma concentrations and the relationship between concentration and clinical response. In juvenile arthritis, it appeared that a concentration of at least 100µg/ml (at steady state) was necessary for a satisfactory response (Makela et al, 1983). While there appeared to be a relationship between dose and clinical response, a more confident prediction of response could be obtained with additional information provided by a drug concentration measurement. Clinical assessments, however, were not blinded.

The specific aims of this study were to determine the following:

a) The relationship between the dose of fenclofenac and plasma concentration (free and total).

b) The relationship between fenclofenac clearance and any specific patient factor such as age, sex or severity of disease.

c) The relationship between fenclofenac dose and/or plasma concentration (free and total) and clinical response and/or toxicity.

5.2 PATIENTS AND METHODS

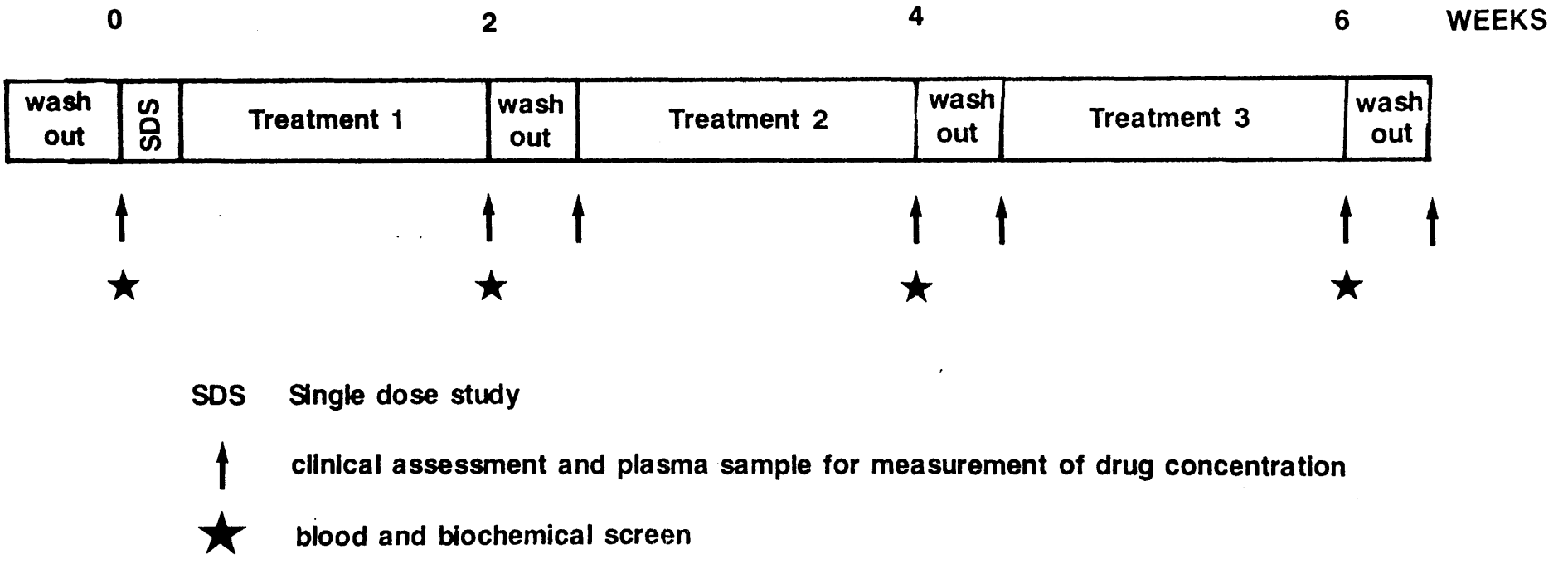
5.2.1 Study design

The overall study design is outlined in Figure 5.1. After the initial washout period, patients were given a single dose of fenclofenac (600mg) and blood samples were taken over the subsequent 48 hours. From this point the study was 'double blind'; each patient was given three doses of fenclofenac, 600, 1200 and 1800mg daily, for 12 days at a time. Doses were randomised according to a a Latin Square design. The standard rheumatological assessments given in Chapter 3 were carried out by the same observer throughout the study.

5.2.2 Patients

Eighteen outpatients with 'definite' or 'classical' rheumatoid arthritis (Ropes et al, 1959) complied with the protocol and completed the study (three 3x6 randomised treatment blocks). Twelve patients were female and six patients were male. Their disease duration ranged from 4

FIGURE 5.1 Study outline



months to 20 years (median 3.5 years) and their ages ranged from 22 to 74 years (median 56 years). All individual patient demographic features and previous NSAID treatment are given in Table 5.1. None of the patients was receiving corticosteroids or any other second line antirheumatic drug and patients were only included in the study if the withdrawal of anti-inflammatory therapy for at least 3 days resulted in a symptomatic 'flare'. Table 5.2 gives an indication of the disease severity after this initial washout period.

5.2.3 Single dose study

Patients were allowed a light breakfast (at least 2 hours before the dose) on the morning of the single dose study. Lunch was allowed 3 hours after the dose. Two 300mg tablets of fenclofenac were taken with 100ml of water at approximately 10am. Blood samples were taken from an indwelling intravenous cannula before the dose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36 and 48 hours thereafter.

All blood samples were collected into heparinised tubes and after centrifugation at 2000rpm for 5 minutes, the plasma fraction was separated and stored at -20°C. Fenclofenac concentration was determined as outlined in Chapter 2.

TABLE 5.1 Patient characteristics (fenclofenac study)

Patient number	Sex	Age (yrs)	Weight (kg)	Height (cm)	Disease duration (yrs)	ESR (mm/hr)	Alb (g/l)	Glob (g/l)	Creat (μmol/l)	Alk phos. (IU/l)	Smoking	Previous NSAID
1	M	67	64.9	163	10	72	38	30	154	119	N	naproxen
2	F	22	59.5	160	1.5	7	42	28	67	95	Y	naproxen+indomethacin
3	F	64	48.5	167	1	114	30	44	64	102	?	flurbiprofen
4	M	65	78.0	166	20	3	39	24	103	113	?	fenclofenac
5	M	40	90.7	180	3	10	46	28	78	86	Y	azapropazone
6	F	53	76.2	152	1	31	40	27	70	110	?	naproxen+fenclofenac
7	F	57	73.0	158	4	73	44	40	106	86	Y	indomethacin+fenclofenac
8	F	63	61.7	158	1	54	41	42	80	125	N	naproxen+fenclofenac
9	M	62	59.9	173	3	12	45	24	82	98	Y	indomethacin+ibuprofen
10	F	54	90.3	170	5	85	43	34	89	85	Y	mefenamic acid
11	M	59	85.7	176	0.3	65	39	32	108	123	?	indomethacin
12	F	41	45.9	155	2	17	40	32	55	98	Y	naproxen
13	F	23	50.8	155	0.5	24	40	34	76	60	?	indomethacin
14	F	45	68.0	165	4	30	42	47	60	197	?	flurbiprofen
15	F	51	63.5	158	4	40	42	34	77	165	N	flurbiprofen
16	F	52	90.3	157	20	25	42	35	102	168	Y	indomethacin
17	M	62	96.6	188	4.5	57	35	29	98	75	?	fenclofenac+indomethacin
18	F	74	82.0	158	19	26	43	30	71	182	?	none

Key: alk. phos. = alkaline phosphatase

Normal ranges: creatinine 16-120μmol/l
alk. phos. 40-115IU/l

TABLE 5.2 Disease severity assessed by rheumatological measures at the end of the initial washout period (fenclofenac study).

Patient number	Disease duration (yrs)	Ritchie Articular Index (0-78)	Duration of morning stiffness (min)	Grip strength (mmHg) LH	Grip strength (mmHg) RH	Analogue pain score (0-10cm)
1	10	30	15	130	149	8.2
2	1.5	12	90	135	149	6.4
3	1	22	60	107	114	5.1
4	20	13	60	105	131	4.8
5	3	12	120	195	226	6.5
6	1	24	60	81	129	5.6
7	4	25	120	73	64	7.7
8	1	11	240	90	95	5.0
9	3	26	180	159	99	7.8
10	5	24	420	65	77	7.4
11	0.3	30	420	75	51	8.8
12	2	21	60	141	111	5.2
13	0.5	32	420	80	87	6.5
14	4	40	180	60	50	7.4
15	4	30	180	58	78	7.4
16	20	49	420	63	59	8.3
17	4.5	15	420	61	44	7.2
18	19	6	420	91	105	7.7

5.2.4 Randomised treatment period

The randomised treatment period continued for 6 weeks (Figure 5.1). Fenclofenac (300mg) and placebo were identical in appearance. On each dose two tablets were taken three times a day, at 10.00, 15.00 and 22.00 hours, thus:

Total daily dose (mg)	Morning	Afternoon	Evening
600	F + P	P + P	F + P
1200	F + F	P + P	F + F
1800	F + F	F + F	F + F

where F is 300mg fenclofenac and P is matching placebo. Thus the 600 and 1200mg doses were given in two divided doses every 12 hours, while the 1800mg dose was given in three divided doses at unequal intervals. A three day wash-out period was included after each dose.

Rheumatologial assessments were carried out and blood samples were taken for the measurement of fenclofenac concentrations and for standard biochemical and haematological screens. These assessments were carried out at the end of the initial washout period and at the end of each treatment period as close to 10am as possible. Trough samples (10am) were taken after 5 and 14 days of each treatment period. Additional assessments and were carried out after the washout periods between treatments and at the end of the study at 3pm. Corresponding blood samples for the measurement of fenclofenac were obtained. Blood samples for drug analysis were handled as above for the single dose study.

5.2.5 Data analysis

(i) Single dose study

Individual single dose concentration-time data were fitted to one and two compartment models with either a first or zero order input function using ordinary nonlinear least squares regression analysis (Chapter 4).

The relationship between the clearance of fenclofenac and any specific patient factor was investigated using general linear regression and correlation techniques.

(ii) Dose and concentration - response analysis

The clinical response measures used in these analyses were the Ritchie Articular Index, duration of morning stiffness, mean grip strength and analogue pain score. The simple 4-point verbal pain scale and the patients' and physicians assessment of the therapeutic effect were too insensitive to show any change from dose to dose, and were not used in this analysis.

Two-way analysis of variance was used to test for time or treatment order effects. A summed efficacy score was obtained by ranking the rheumatological measures across baseline and dose from 1 to 4 and taking the sum of the ranks for all measures. Friedman two-way analysis of variance was used to test for dose related changes in each individual rheumatological measure and in the summed efficacy score.

Various linear and non-linear models were investigated to describe the relationship between dose or concentration

and clinical response. The models and the statistical analysis are presented in Chapter 4.

Changes in biochemical or haematological indices with dose were investigated using Friedman two-way analysis of variance.

(iii) Protein binding studies

It was possible to investigate the binding of fenclofenac to plasma proteins over a wide range of plasma concentrations in five patients (14-18). Plasma, taken at the end of the initial washout period was spiked with cold fenclofenac to give concentrations over the range 1.3-800µg/ml. The free fraction was determined by carrying out equilibrium dialysis against buffer containing radiolabelled drug as described in Chapter 2.

Free and bound concentrations were fitted to the classical binding isotherm with two classes of binding sites using weighted non-linear least squares regression analysis (Chapter 4). The free concentration as the independent variable was weighted proportional to the reciprocal of the fitted concentration ($1/\hat{c}_i$). Initial estimates of the parameters were obtained by plotting the data in the form of a modified Scatchard plot (bound/free vs bound).

5.3 RESULTS

5.3.1 Pharmacokinetics

(i) Single dose study

An example of a representative patient's concentration-time data (patient 4) fitted to 4 possible pharmacokinetic models (Models 1-4, Appendix II) is given in Figure 5.2. For this patient the one compartment model is obviously inappropriate as terminal concentrations are not fitted well. The effect of using either zero order or first order input are clearly shown. The AIC values for all individual patient data fitted to the four models are given in Table 5.3. Using this fitting criterion, in general the two compartment model with a zero order input was on balance the best model to describe the data. The zero order input allowed a better fit of both the peak and the terminal concentrations. Table 5.3 also indicates that in most cases, the AIC was smaller or equal for fits to the one or two compartment models with zero order input (Models 2 and 4).

It is more appropriate to compare hierarchical models using the F ratio test. Table 5.4 gives various 'goodness of fit' criteria for Models 2 and 4. The F ratio test indicated that for only 6 out of the 18 patients, the fit to the two compartment model was significantly better than the one compartment model. The residuals plots, however, showed that the one compartment model failed to fit the terminal concentration points and often the peak

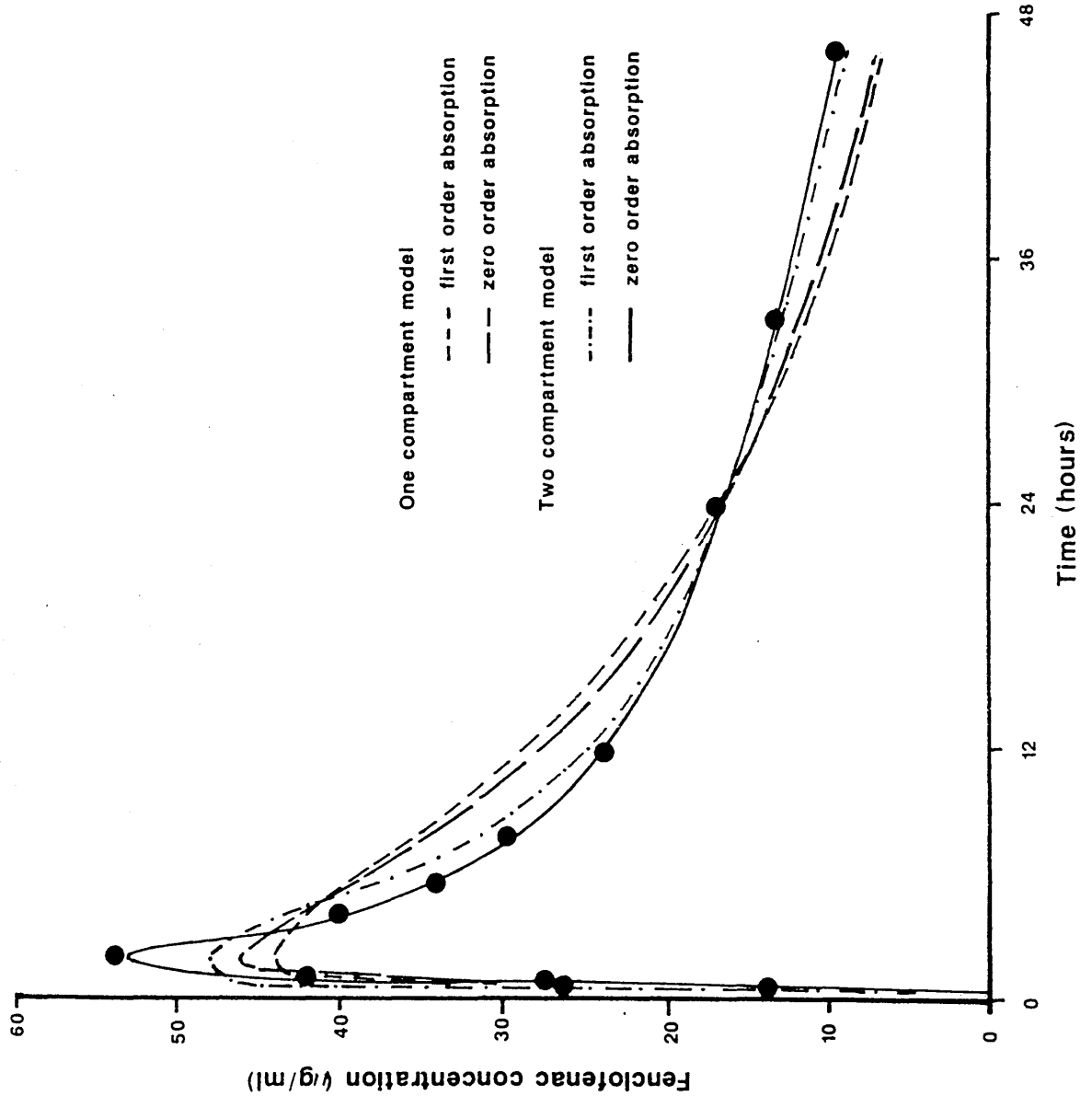


FIGURE 5.2 Representative patient (4) fenclofenac concentration-time profile fitted to one and two compartment models with first order or zero order absorption

TABLE 5.3 Comparison of AIC values for fenclofenac concentration-time data fitted to one and two compartment models with first order and zero order absorption (Models 1-4, Appendix II)

Patient number	One compartment model		Two compartment model	
	first order MODEL 1	zero order MODEL 2	first order MODEL 3	zero order MODEL 4
1	74	68	75	65*
2	100	97	94	78*
3	73*	74	75	76
4	100	93	90	67*
5	45	47	46	46
6	84	80	86	81
7	63	62	57	56*
8	63*	77	64	80
9	73	59	70	48*
10	68	67	68	62*
11	63	62*	75	66
12	97	106	97	75*
13	65	55	65	54*
14	82	79	82	80
15	77	77	53	52*
16	88	81	90	79*
17	74*	80	80	81
18	70	68	45	38*

AIC is the Akaiki Information Criterion
* the lowest AIC for the comparison of the four models

TABLE 5.4 Comparison of 'goodness of fit' for individual fenclofenac concentration-time data fitted to one and two compartment models with zero order absorption.

Patient	One compartment model MODEL 2			Two compartment model MODEL 4			
	C_{det}	SSQres	df	C_{det}	SSQres	df	
1	Ø.946	72	1Ø	Ø.967	44	8	
2	Ø.933	563	1Ø	Ø.986	114	8	**
3	Ø.937	154	9	Ø.941	144	7	
4	Ø.941	2Ø5	12	Ø.991	31	1Ø	**
5	Ø.992	2Ø	9	Ø.994	14	7	
6	Ø.941	26Ø	9	Ø.955	198	7	
7	Ø.975	66	9	Ø.987	34	7	
8	Ø.957	2Ø3	9	Ø.959	193	7	
9	Ø.986	51	9	Ø.996	16	7	*
1Ø	Ø.989	53	11	Ø.994	29	9	
11	Ø.981	46	1Ø	Ø.996	47	8	
12	Ø.877	69Ø	11	Ø.899	67	9	**
13	Ø.984	28	1Ø	Ø.988	2Ø	8	
14	Ø.958	161	1Ø	Ø.965	132	8	
15	Ø.988	99	11	Ø.998	14	9	**
16	Ø.972	133	11	Ø.98Ø	9Ø	9	
17	Ø.951	173	1Ø	Ø.916	136	8	
18	Ø.986	75	1Ø	Ø.999	6	8	**

Key: df = degrees of freedom
SSQres = the residual sum of squares
C_{det} = coefficient of determination

* $p<Ø.Ø5$
** $p<Ø.Ø1$ two compartment model significantly better than the one compartment model (F ratio test).

concentrations were not fitted well.

Parameter values determined from fits to one and two compartment models with zero order input are given in Table 5.5 and 5.6. Examination of the parameter values for the data fitted to either model suggests that there is considerable inter-subject variability in the kinetics of fenclofenac. The variability in T_{lag} and T may reflect the fact that patients were not fasted before the single dose study. For the two compartment model the wide range of values for α and k_{21} may in part be a result of too few data points to give a good estimate of these parameters: the SE of the estimate of these parameters was often large. The variability in apparent clearance was approximately 50%. Closer examination of clearance values indicated that the majority of patients had a clearance in the Ø.33-Ø.74 1/hr range: 3 patients had a clearance in the 1.23-1.49 1/hr range (patients 3,5 and 13).

Correlations between fenclofenac clearance and age, creatinine, ESR or alkaline phosphatase were tested using simple linear regression. No significant relationships were found, but there was a possible decrease in clearance with increasing age ($p<Ø.Ø83$) and with an increase in alkaline phosphatase ($p<Ø.Ø91$) (Figure 5.3). There did not appear to be any sex related differences in clearance.

(ii) Steady State

There was a proportional increase in mean trough concentrations from 6ØØ to 12ØØmg/day, the mean trough on the

TABLE 5.5 Individual parameters (SE) for fenclofenac concentration-time data fitted to a one compartment model with a zero order input.

Patient	T_{lag} (h)	T (h)	V (l)	Cl (l/h)	k_e (h-1)
1	-	1.0 (0.1)	19.2 (0.7)	0.801 (0.011)	0.042
2	-	0.7 (0.2)	7.8 (0.4)	0.768 (0.012)	0.098
3	-	0.8 (0.3)	15.0 (1.0)	1.535 (0.239)	0.102
4	-	1.4 (0.2)	12.3 (0.7)	0.565 (0.066)	0.046
5	0.67 (0.47)	0.5 (0.1)	13.7 (0.5)	1.766 (0.093)	0.129
6	0.12 (0.13)	1.4 (0.3)	14.0 (0.3)	0.481 (0.078)	0.034
7	0.08 (0.07)	1.2 (0.1)	12.0 (0.6)	0.950 (0.086)	0.079
8	0.10 (0.04)	1.0 (0.1)	9.6 (0.5)	1.016 (0.011)	0.106
9	0.20 (0.07)	1.6 (0.2)	11.4 (0.4)	0.646 (0.050)	0.057
10	0.72 (0.05)	0.7 (0.1)	12.6 (0.4)	0.753 (0.055)	0.060
11	0.32 (0.01)	0.7 (0.1)	15.9 (0.1)	0.726 (0.018)	0.046
12	-	1.8 (0.1)	10.1 (0.2)	0.598 (0.034)	0.059
13	0.98 (0.12)	3.3 (0.3)	14.2 (1.2)	1.611 (0.127)	0.113
14	0.60 (0.08)	1.2 (0.4)	10.8 (1.1)	0.978 (0.138)	0.091
15	0.83 (0.05)	0.7 (0.1)	9.2 (0.3)	0.698 (0.051)	0.076
16	1.00 (0.15)	2.4 (0.4)	12.0 (0.6)	0.387 (0.042)	0.032
17	0.33 (0.02)	1.4 (0.2)	13.6 (0.2)	0.384 (0.019)	0.028
18	0.44 (0.12)	1.6 (0.3)	11.3 (0.4)	0.402 (0.032)	0.036
median	0.26	1.2	12.2	0.740	0.060
range	0-1.0	0.5-3.3	7.8-19.2	0.384-1.77	0.028-0.129

TABLE 5.6 Individual parameters (SE) for fenclofenac concentration-time data fitted to a two compartment model with a zero order input.

Patient	T_{lag} (h)	T (h)	α (h^{-1})	β (h^{-1})	k_{21} (h^{-1})	V_1 (l)	Cl (l/h)	V_{ss} (l)
1	0.05 (0.07)	1.1 (0.1)	1.72 (0.43)	0.033 (0.006)	1.05 (0.22)	12.4 (2.1)	0.700	20.0
2	0.06 (0.02)	0.7 (0.1)	1.59 (0.04)	0.057 (0.004)	0.92 (0.02)	5.7 (0.1)	0.560	9.6
3	-	0.8 (0.2)	0.19 (0.37)	0.050 (0.106)	0.11 (0.39)	14.4 (1.6)	1.230	20.1
4	-	1.8 (0.1)	0.32 (0.10)	0.026 (0.004)	0.17 (0.06)	9.2 (0.6)	0.460	16.1
5	0.67 (0.02)	0.5 (0.1)	0.20 (0.13)	0.060 (0.058)	0.11 (0.15)	13.1 (0.7)	1.492	18.0
6	0.15 (0.02)	1.8 (0.1)	1.67 (0.23)	0.040 (0.002)	0.99 (0.01)	6.4 (0.1)	0.431	10.6
7	0.08 (0.06)	1.2 (0.1)	0.16 (0.17)	0.027 (0.014)	0.07 (0.13)	11.1 (0.8)	0.675	19.8
8	0.16 (0.04)	0.7 (0.1)	0.11 (0.01)	0.021 (0.008)	0.03 (0.01)	10.2 (0.8)	0.698	18.3
9	0.19 (0.16)	1.8 (0.2)	0.26 (0.21)	0.038 (0.008)	0.19 (0.16)	10.4 (0.8)	0.546	12.9
10	0.71 (0.06)	0.8 (0.2)	0.11 (0.01)	0.025 (0.007)	0.06 (0.01)	12.0 (0.6)	0.611	18.0
11	0.16 (0.04)	1.0 (0.1)	0.87 (0.04)	0.042 (0.005)	0.72 (0.04)	13.5 (0.5)	0.687	16.1
12	-	3.0 (0.3)	0.74 (0.05)	0.060 (0.011)	0.45 (0.04)	6.3 (0.4)	0.625	9.9
13	0.97 (0.02)	3.6 (0.1)	0.20 (0.01)	0.043 (0.001)	0.08 (0.01)	12.2 (0.1)	1.380	20.9
14	0.32 (0.11)	2.0 (0.3)	0.16 (0.17)	0.034 (0.063)	0.07 (0.18)	10.4 (1.2)	0.744	16.9
15	0.83 (0.02)	0.8 (0.1)	0.15 (0.03)	0.023 (0.010)	0.06 (0.03)	8.6 (0.2)	0.490	16.3
16	1.20 (0.08)	1.8 (0.2)	0.70 (0.02)	0.034 (0.003)	0.84 (0.03)	14.0 (0.4)	0.395	-
17	-	4.1 (0.6)	0.44 (0.63)	0.027 (0.008)	0.29 (0.35)	9.4 (3.4)	0.385	13.8
18	1.45 (0.02)	1.8 (0.1)	0.25 (0.02)	0.023 (0.002)	0.16 (0.02)	9.1 (0.2)	0.327	13.5
median	0.16	1.5	0.26	0.034	0.16	10.4	0.618	16.3
range	0-1.45	0.5-4.1	0.11-1.72	0.021-0.060	0.03-1.05	5.7-14.4	0.327-1.49	9.6-21

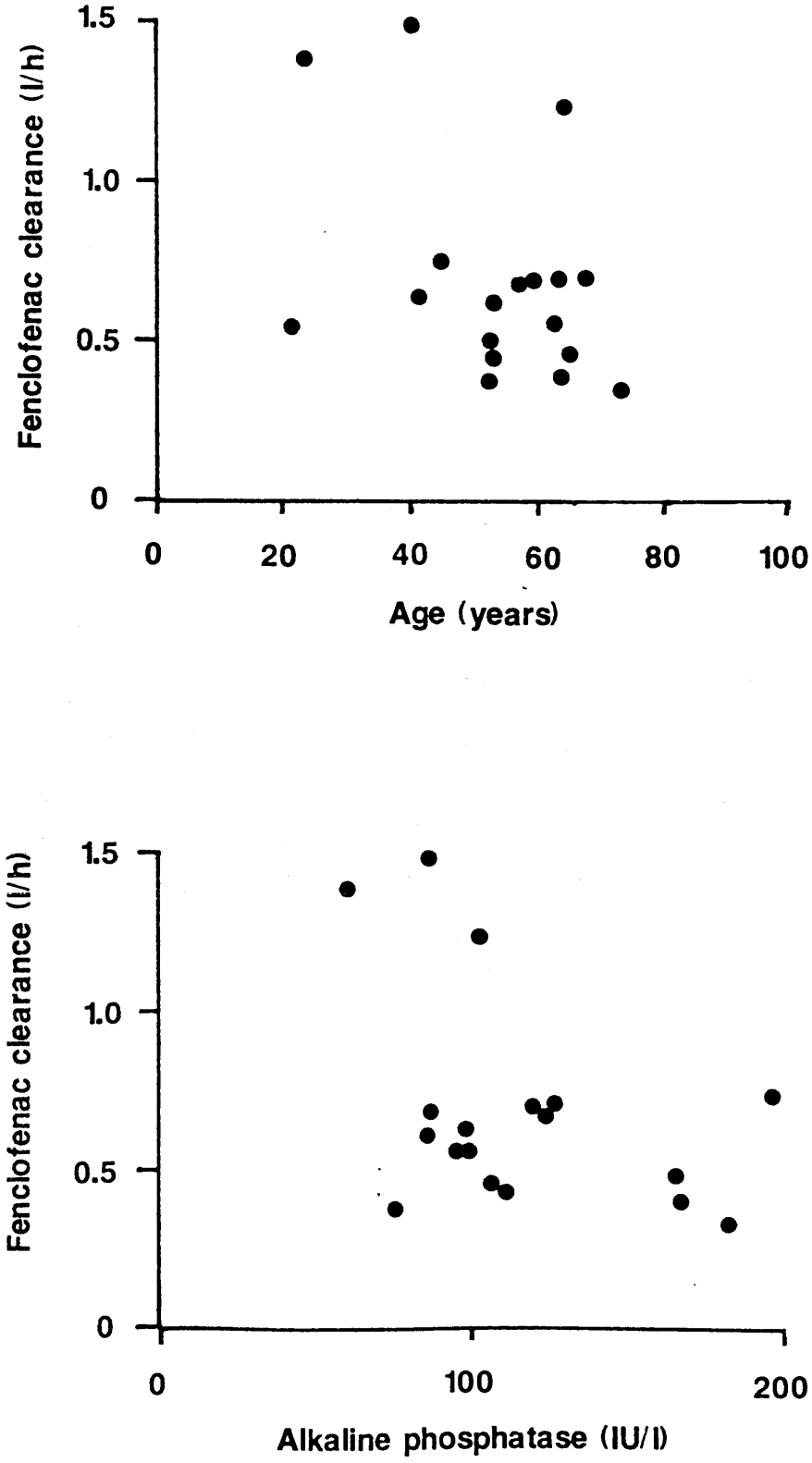


FIGURE 5.3 Correlation between fenclofenac apparent clearance and patient factors, age and alkaline phosphatase

highest dose being slightly lower due to the different dosing regimen. Mean total trough concentrations are shown in Figure 5.4a. There was considerable inter-individual variability in 12 hour trough concentrations at steady state (Figure 5.4b), with an overlap in concentrations achieved between individuals over the dosage range. For some patients, trough concentrations at steady state on the highest dose were lower than those achieved by other patients on the lowest dose.

The validity of the two compartment model to describe the pharmacokinetics of fenclofenac was further tested by examining the difference between trough concentrations predicted from the individual pharmacokinetic parameters and observed trough concentrations at steady state on each dose. The steady state equations for the one and two compartment models with zero order absorption are given in Appendix II. Allowance was made for the unequal dosing intervals on the highest dose. Predicted and observed trough concentrations were compared using a paired t-test. Figure 5.5 presents the mean prediction errors ($\pm$SD) using the one or two compartment model parameter values. At all dose levels the one compartment model gave significantly biased (underpredicted) estimates of trough concentrations. The two compartment model was less biased; only trough concentrations predicted for the lowest dose were significantly underpredicted.

The Friedman two-way analysis of variance was used to

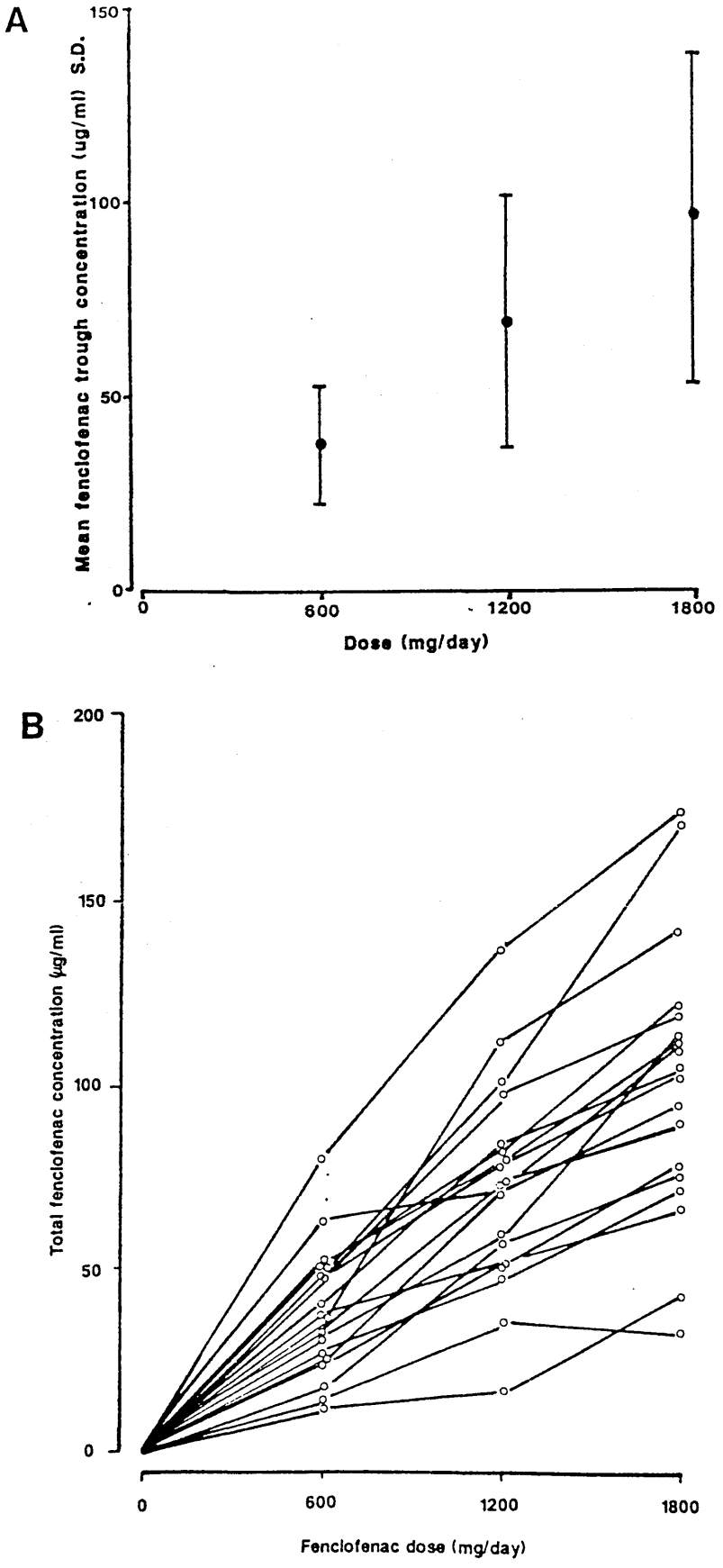


FIGURE 5.4 Total fenclofenac trough concentrations at steady state on each dose

A Mean concentrations
B Individual concentrations

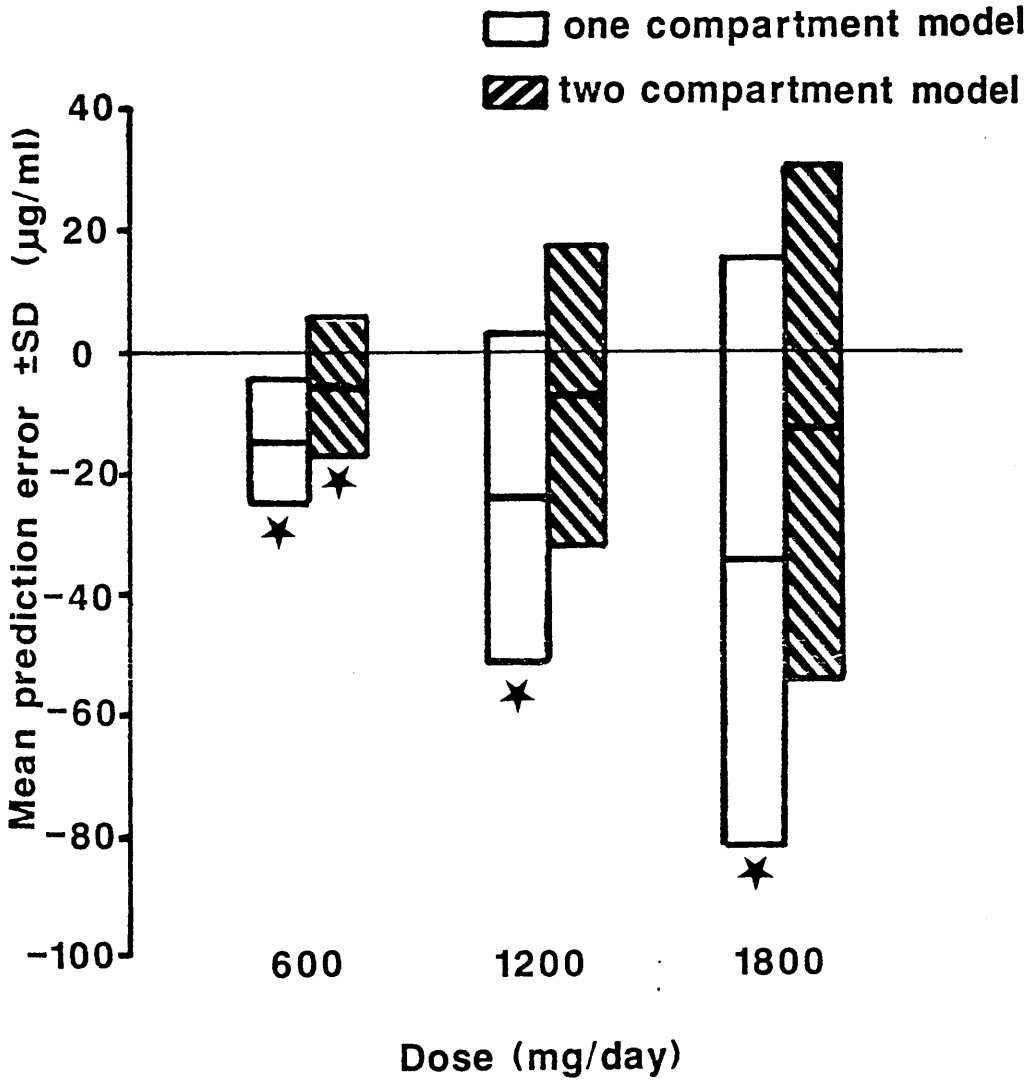


FIGURE 5.5 Mean error (±SD) in the prediction (predicted-observed) of individual total fenclofenac trough concentration at steady state using either one or two compartment model parameter estimates

test for any trends in the prediction errors (using the two compartment model) over the dose range to determine whether the kinetics of fenclofenac were linear. The analysis showed that there was no significant trend in the prediction errors over the dose range, indicating that total fenclofenac kinetics were indeed consistent with linearity.

This suggests that the binding of fenclofenac to plasma proteins is constant over the concentration range encountered in this study. One would therefore expect to observe a linear increase in the free concentration. Median free trough concentrations were 180, 406 and 565ng/ml on 600, 1200 and 1800mg respectively. Indicating that on average there was a linear increase. However from the plot of individual free concentrations against dose (Figure 5.6), the increase in free concentration was far from linear in 3 patients (14, 15 and 18) indicating perhaps, saturation of hepatic metabolic pathways.

(iii) Binding Studies

Figure 5.7 shows the free fraction of fenclofenac in trough samples plotted against total concentration. In most patients the free fraction remained relatively constant across the dose range but the free fraction increased with increasing total concentration in one or two patients.

The relationship between the free fraction of fenclofenac, total fenclofenac and albumin was investigated by multiple linear regression using GLIM (Chapter 4). The models tested to describe the free fraction (f_u) of

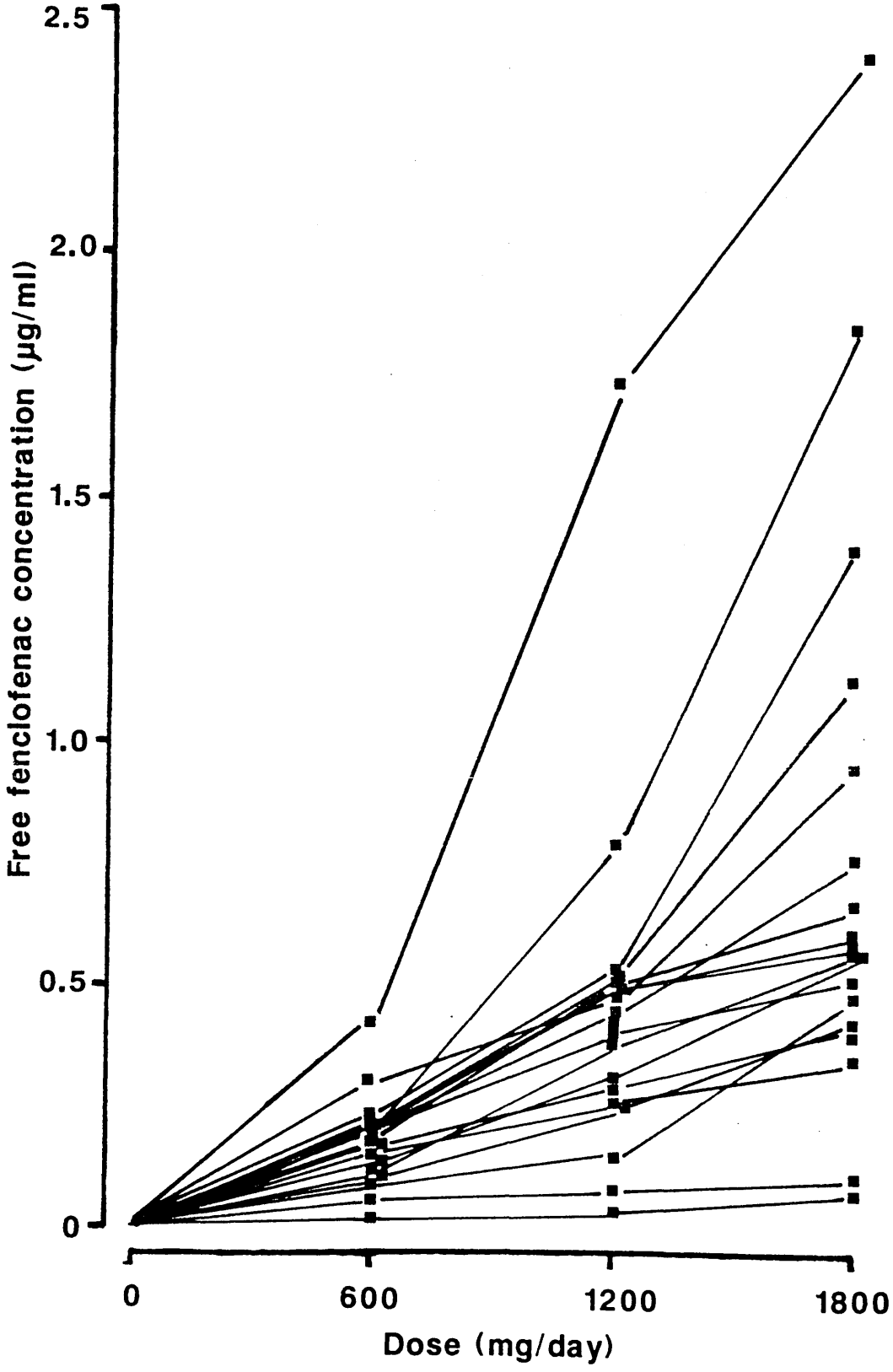


FIGURE 5.6 Individual free fenclofenac trough concentrations at steady state on each dose

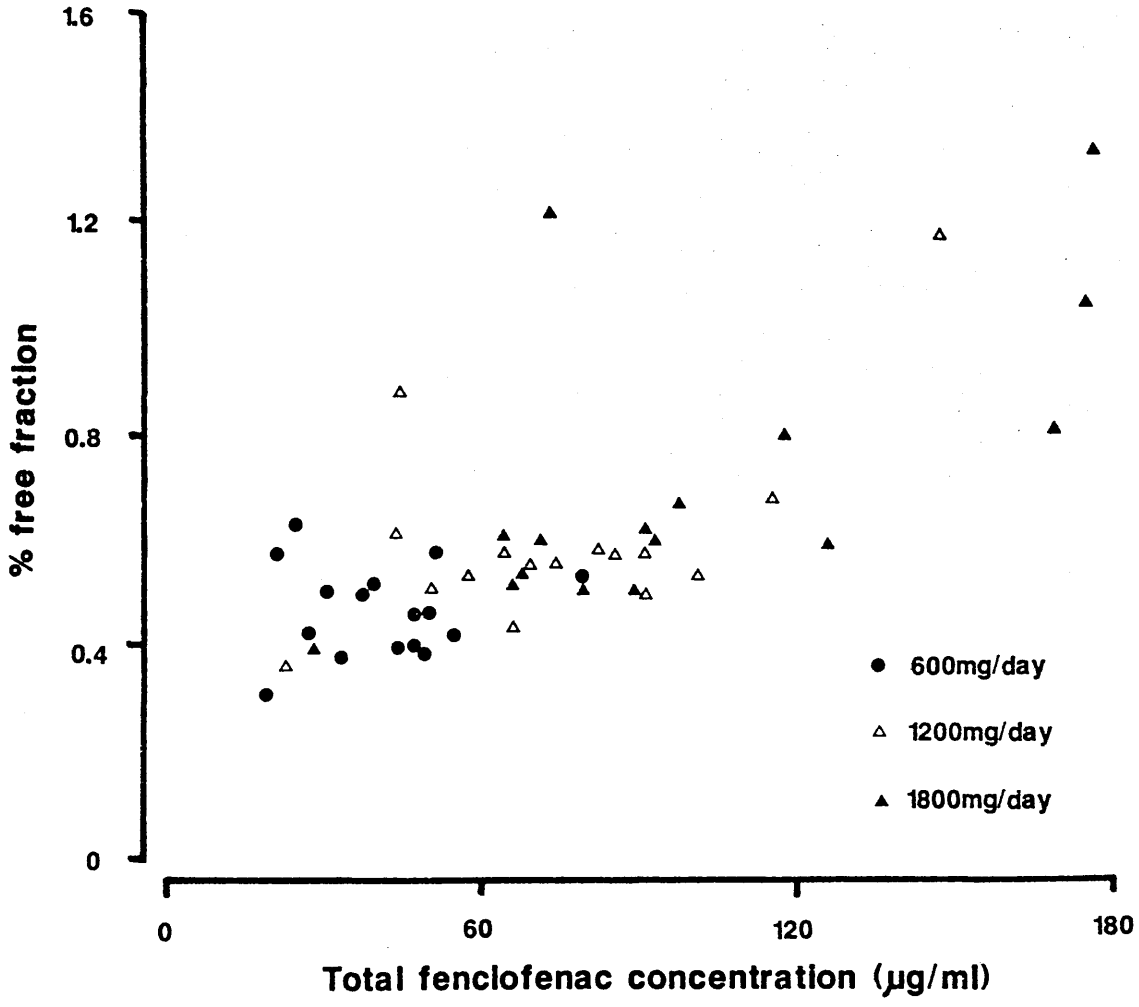


FIGURE 5.7 Free fraction in trough samples at steady state on each dose plotted against total fenclofenac concentration.

fenclofenac using GLIM are given in Table 5.7. The best model on the basis of the F ratio test was:

$$f_u = A + b_i.Ctot \quad \text{.....(5.1)}$$

The coefficient of determination for this model was Ø.9Ø5. This indicates that each subject had an individual slope (b_i) for the change in f_u with increasing C_{tot} and a common intercept (A). The regression equation for the average patient was:

$$f_u \ (x1Ø^{-3}) = 3.74 + Ø.Ø24.Ctot \quad \text{.....(5.2)}$$

and ranged from:

$$f_u \ (x1Ø^{-3}) = 3.74 - Ø.Ø59.Ctot \quad \text{.....(5.3)}$$

to:

$$f_u \ (x1Ø^{-3}) = 3.74 + Ø.256.Ctot \quad \text{.....(5.4)}$$

This relationship indicates that in general there was a slight increase in the free fraction with increments in concentration; in some patients, the increase was more dramatic. It may have been expected that albumin would explain this difference but it did not.

Fenclofenac bound and free concentrations over the total concentration range of 1.3-5ØØµg/ml were fitted well to the Langmuir isotherm for two independent classes of binding sites. An example of an individual set of data plotted in the form of Scatchard and also fitted to the double Langmuir isotherm with free concentration as the dependent variable is shown in Figure 5.8. The individual parameters and the coefficient of determination are given in Table 5.8. The mean parameters for patients were: the

TABLE 5.7 Analysis of the factors which influence fenclofenac free fraction ($x10^{-3}$).

No.	Model	SSQres	df_1, df_2	F value	p	C_{det}
1	total SSQ	479	53			
2	A + B.Ctot	391	1,52 (1)	11.7	<Ø.Ø1	Ø.184
3	A + B.alb	262	1,52 (1)	43.1	<Ø.Ø1	Ø.453
4	a_i	126	17,36 (1)	5.93	<Ø.Ø1	Ø.737
5	A + B.Ctot + C.alb	177	1,51 (3)	24.5	<Ø.Ø1	Ø.63Ø
6	a_i + B.Ctot	72	1,35 (4)	26.2	<Ø.Ø1	Ø.85Ø
			17,35 (2)	9.12	<Ø.Ø1	
7	a_i + B.alb	125	1,35 (4)	Ø.28	NS	Ø.739
			17,35 (3)	2.26	<Ø.Ø5	
8	A + b_i.Ctot	46	1,35 (4)	6Ø.9	<Ø.Ø1	Ø.9Ø4*
			17,35 (2)	15.4	<Ø.Ø1	
9	A + b_i.alb	123	1,35 (4)	Ø.85	NS	Ø.743
			17,35 (3)	2.33	<Ø.Ø5	
1Ø	a_i + B.Ctot + C.alb	72	1,34 (6)	-		Ø.85Ø
11	b_i.Ctot + C.alb	44	1,34 (8)	1.54	NS	Ø.9Ø8
12	a_i + b_i.Ctot	2Ø	17,18 (8)	1.38	NS	Ø.958
13	a_i + b_i.Ctot + C.alb	2Ø	1,17 (13)	-		Ø.958

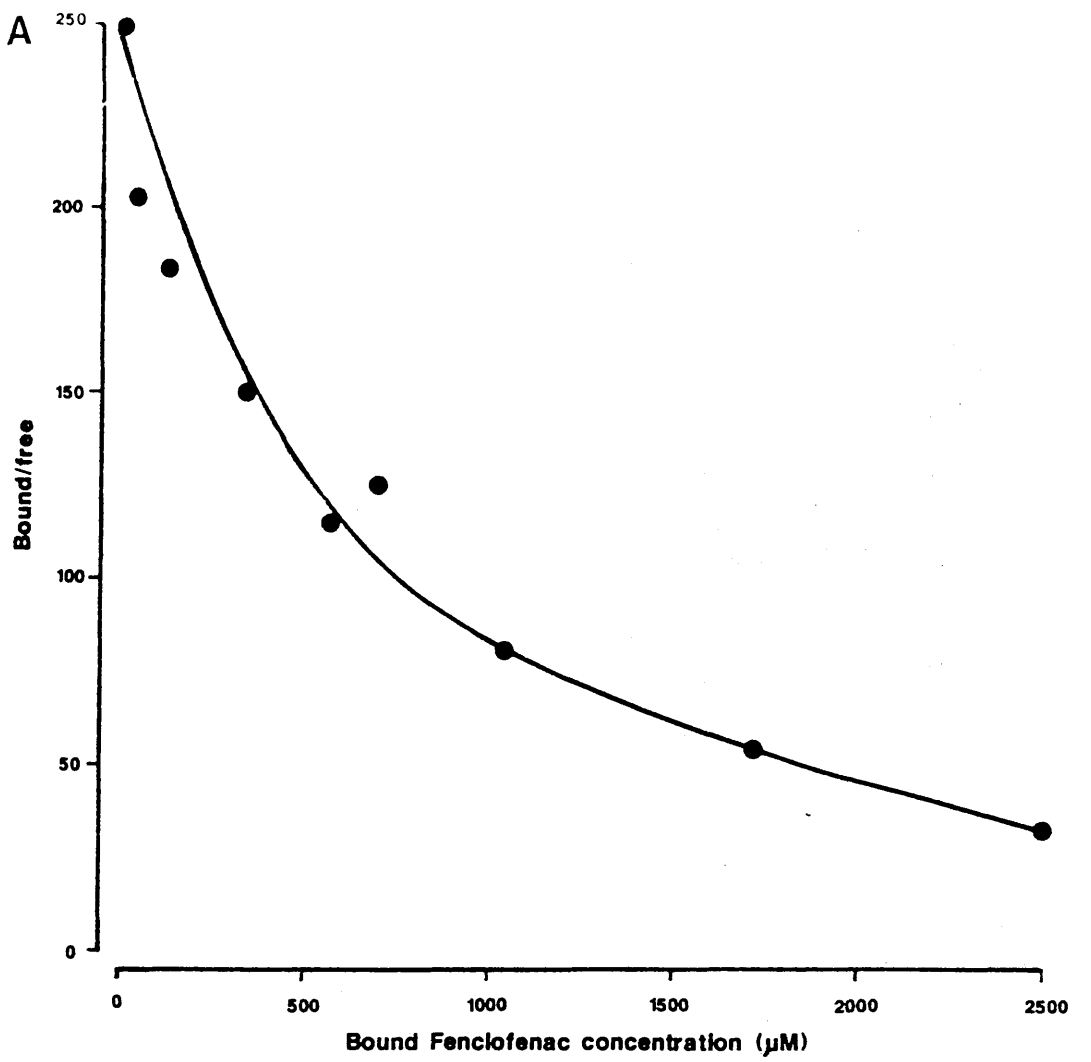
Key: Ctot = total concentration
alb = albumin concentration
sub = subject

A, B & C are constant for all individuals, a_i and b_i are different for each individual

The model compared with is given in parenthesis, * denotes the most appropriate model

FIGURE 5.8 Fenclofenac binding data for a representative patient (14)

A Scatchard plot
B Data fitted to the Langmuir isotherm with free concentration as the dependent variable


A
250
200
150
100
50
0
Bound/free
0
500
1000
1500
2000
2500
Bound Fenclofenac concentration (μM)

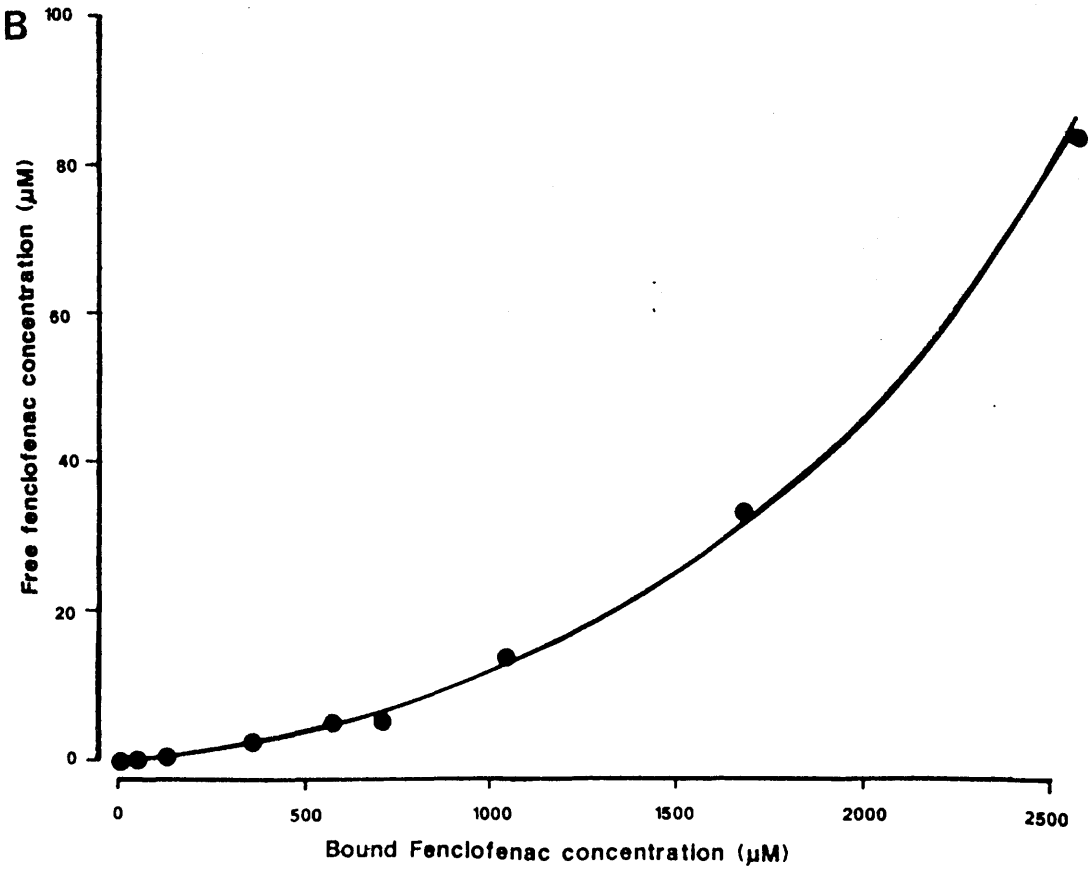

B
100
80
60
40
20
0
Free fenclofenac concentration (μM)
0
500
1000
1500
2000
2500
Bound Fenclofenac concentration (μM)

TABLE 5.8 Fenclofenac protein binding parameter estimates (SE)

Patient number	alb (g/l)	nP_1 (μM)		n_1	K_{d1} (μM)		nP_2 (μM)		n_2	K_{d2} (μM)		C_{det}	df
14	42	458	(73)	Ø.75	2.4Ø	(Ø.44)	3832	(285)	7.3	79.2	(16.1)	Ø.999	5
15	42	378	(142)	Ø.62	2.18	(Ø.76)	38Ø6	(394)	8.2	54.8	(17.9)	Ø.998	5
16	42	2Ø8	(76)	Ø.34	1.17	(Ø.46)	33Ø9	(143)	7.2	37.3	(7.2)	Ø.999	5
17	35	45Ø	(5Ø)	Ø.89	1.68	(Ø.22)	2921	(179)	6.3	72.3	(14.5)	Ø.999	5
18	43	338	(118)	Ø.56	1.94	(Ø.51)	33Ø	(291)	7.2	37.6	(11.2)	Ø.989	5
Mean		366		Ø.63	1.87		3434		7.4	56.Ø			

n_1 and n_2 are determined assuming that albumin is the only binding protein

maximum number of binding sites, nP_1 and nP_2, 366 and 3434μM respectively and the dissociation constants, K_{d1} and K_{d2}, 1.87 and 56μM respectively for the high and low affinity sites. Assuming albumin is the only binding protein, the number of each type of binding site on each albumin molecule can be determined. The molecular weight of albumin was taken as 69,000 and the mean values of n_1 and n_2 were calculated as 0.63 and 7.4 respectively. These parameters indicate that there is concentration dependent binding below 100μg/ml. However, the non-linearity becomes more apparent above 100μg/ml with saturation of the primary binding site. The change in the free fraction with total concentration predicted from the median binding parameters is shown in Figure 5.9 together with observed free fraction in the 18 patients at steady state.

5.3.2 Dose and concentration-response relationships

There were no significant time or treatment order effects. Samples taken at the end of each wash-out period indicated that fenclofenac was still present in plasma at significant concentrations, mean concentrations (SD) were 12.5(7.6), 20.6(19.1) and 33.6(23.5)μg/ml at the end of the wash-out period after 600, 1200 and 1800mg respectively. This was not surprising considering the long terminal elimination half-life determined from the single dose study. However, it meant that these assessments could not be used in the analysis to check for any week to week variability

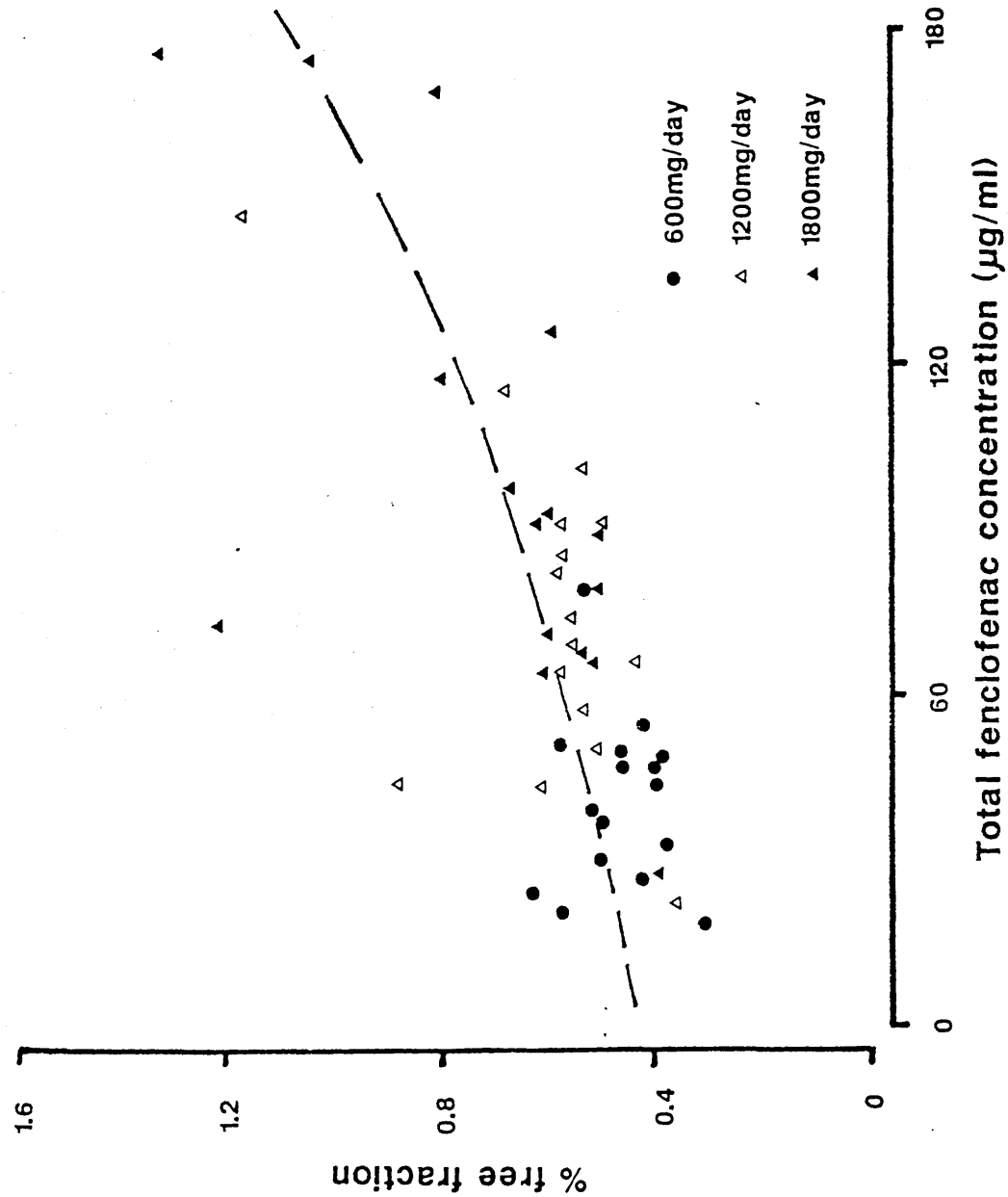
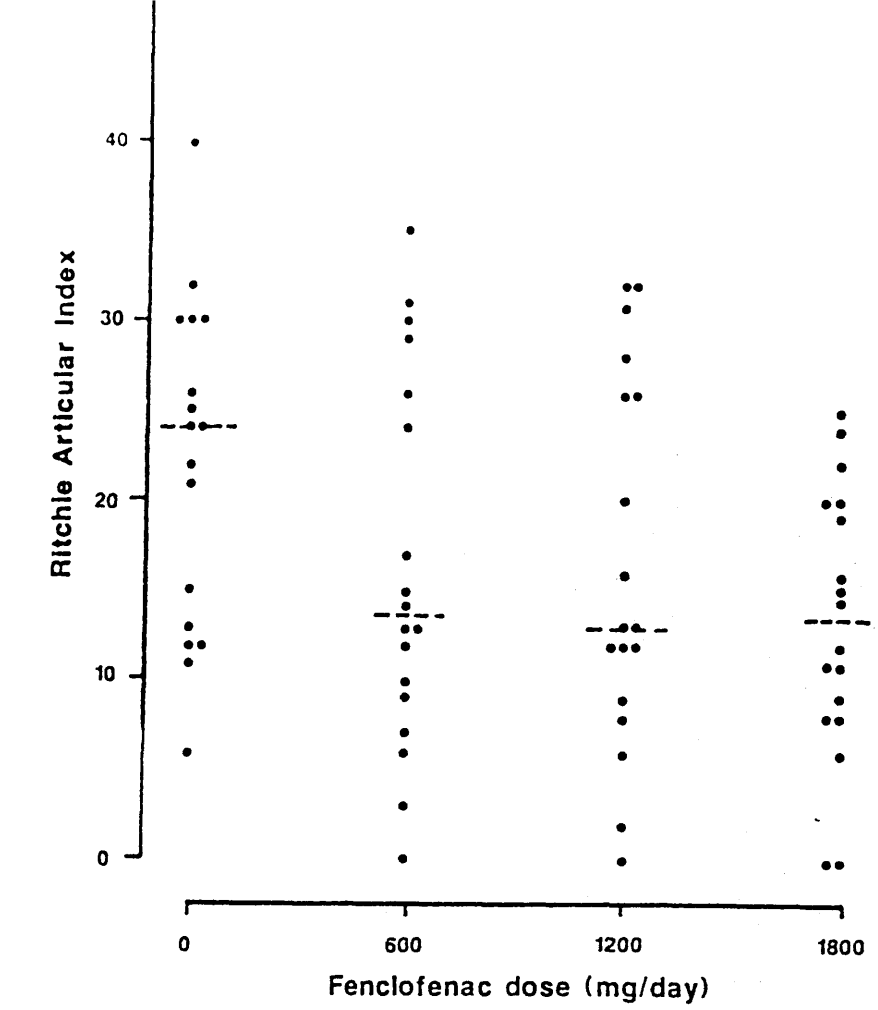


FIGURE 5.9 The change in the free fraction of fenclofenac with increasing total concentration predicted from the mean binding parameters is given together with the free fraction determined in patient samples at steady state

in the disease severity. In addition these assessments were carried out in the afternoon and therefore could not really be compared with those carried out in the morning. Since the doses were continued for 12 days it is unlikely that these residual concentrations would have affected the attainment of steady state in terms of concentration and clinical response.

Four patients showed little, if any, improvement in symptoms at any dose. Corresponding trough concentrations were 52, 82, 79 and 100µg/ml on the highest dose. All other patients showed an improvement in at least three of the effect measurements when receiving 1800mg/day. All patients with trough concentrations above 100µg/ml on 1200 or 1800 mg/day showed an improvement in all effect measurements when compared to baseline values.

A summary of the clinical effect data is given in Table 5.9 and Figure 5.10 giving an indication of the considerable variability in the response measurements. A result of both inter and intra-subject variability. Friedman two-way analysis of variance indicated that there were no significant differences from dose to dose for any of the four response measurements. The 600mg dose was not significantly different from baseline. When the data were taken as a whole the analogue pain score was the only assessment which appeared to show a dose related effect. As patients sometimes showed an improvement in one response measure but not in others, a summed efficacy score was



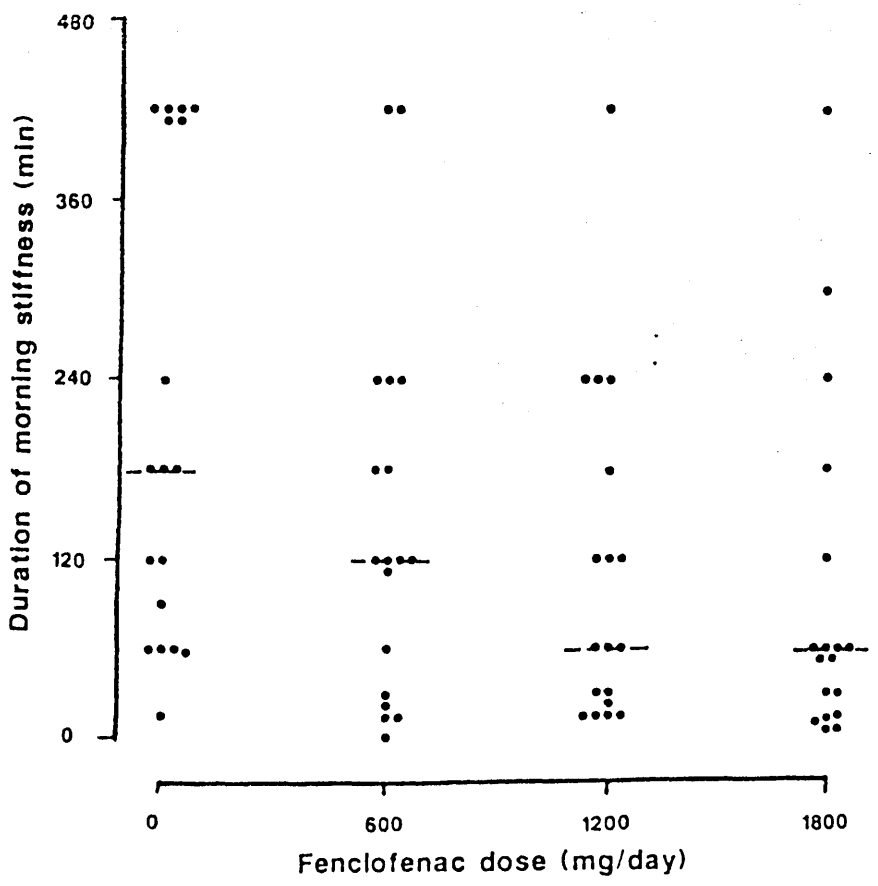
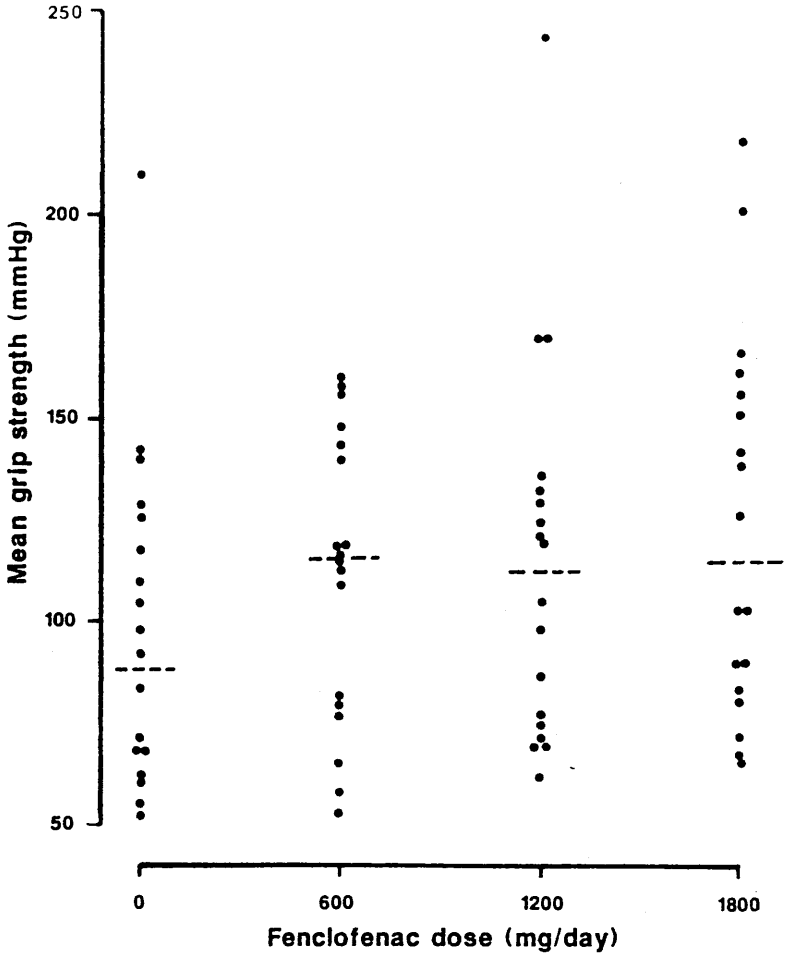


FIGURE 5.10 Summary of clinical measures at baseline and on each dose of fenclofenac



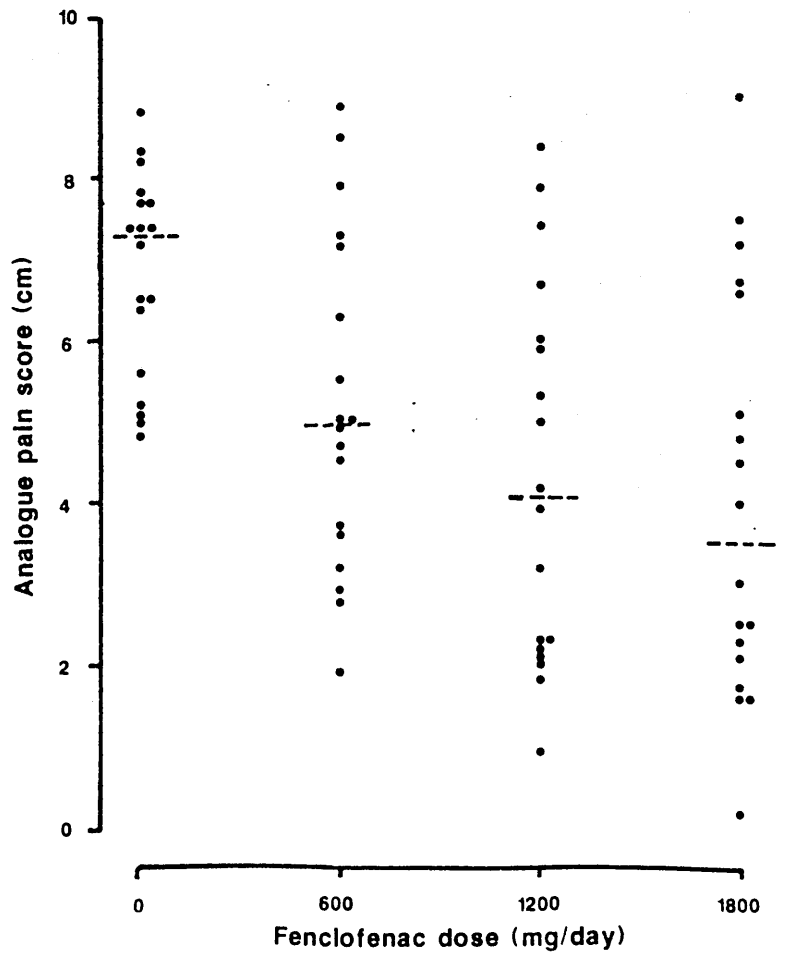


FIGURE 5.10 Summary of clinical measures at baseline and on each dose of fenclofenac

TABLE 5.9 Summary of clinical response measurements (fenclofenac study). Data are expressed as medians and the range is given in parenthesis.

Clinical measurement	Baseline	Dose (mg/day) 600	1200	1800	Freidman two-way analysis of variance	
Ritchie Articular Index	24 (6-49)	14 (0-35)	13 (0-32)	12 (0-25)	1800<baseline	p<0.01
Duration of morning stiffness(hr)	180 (15-420)	120 (0-420)	60 (15-240)	60 (5-420)	1200<baseline 1800<baseline	p<0.05 p<0.01
Mean grip strength (mmHg)	95 (52-210)	115 (53-160)	113 (62-244)	115 (72-219)	1200>baseline 1800>baseline	p<0.05 p<0.01
Analogue pain score (cm)	7.3 (4.8-8.8)	5.0 (1.9-8.9)	4.2 (0.9-8.4)	3.5 (0.2-9.0)	1200<baseline 1800<baseline	p<0.05 p<0.01

calculated. This however did not reveal a dose response relationship.

Corresponding dose or 12 hour trough fenclofenac concentrations (total and free) and clinical response data (Ritchie Articular Index, mean grip strength, duration of morning stiffness and analogue pain score) were analysed using the linear modelling program GLIM (Baker & Nelder, 1978). The data were fitted to the three possible linear models described in Chapter 4 (Equations 4.21-4.23), referred to as Models 1-3, and compared using the F ratio test. Three or four data points per individual (depending on whether or not baseline measurements were included) for eighteen patients were analysed simultaneously for each response index.

Table 5.10 shows the effect of fitting the response data in terms of total concentration to Models 1-3. Despite the large range of values for the individual slope parameter obtained by fitting the data to the full model (Model 1), this model was rejected in favour of the simpler linear model (Model 2). This was due to the large amount of 'noise' or intra-subject variability in the response measurements. The reduced model, however, took account of inter-subject variability by allowing an individual intercept (severity of disease before treatment) and a common improvement slope for all individuals. This model was tested against Model 3, to determine the significance of the slope: the subject effect accounted for a large percentage of the total sum of

TABLE 5.10 Comparison of different linear models to describe fenclofenac total concentration-response data (baseline data omitted)

RITCHIE ARTICULAR INDEX

Linear model	SSQres	df_1, df_2	F value	p value	C_{det}
Total SS	4747				
Model 1	647				Ø.864
Model 2	1Ø83	17,18 (1)	Ø.71	NS	Ø.772
Model 3	1199	1,35 (2)	3.75	NS	Ø.747*

DURATION OF MORNING STIFFNESS

Linear model	SSQres	df_1, df_2	F value	p value	C_{det}
Total SS	753ØØ				
Model 1	1Ø48ØØ				Ø.861
Model 2	144ØØ	17,18 (1)	Ø.4Ø	NS	Ø.8Ø9*
Model 3	164ØØ	1,35 (2)	4.86	<Ø.Ø5	Ø.782

Linear models:

1. $Effect_i = a_i + b_i.C$

2. $Effect_i = a_i + B.C$

3. $Effect_i = a_i$

model for comparison is given in parenthesis

* denotes the most appropriate model

TABLE 5.10 Comparison of different linear models to describe fenclofenac total concentration-response data (baseline data omitted)

MEAN GRIP STRENGTH

Linear model	SSQres	df_1, df_2	F value	p value	C_{det}
Total SS	96720				
Model 1	6079				0.937
Model 2	11690	17,18 (1)	0.98	NS	0.879
Model 3	12540	1,35 (2)	2.54	NS	0.870*

ANALOGUE PAIN SCORE

Linear model	SSQres	df_1, df_2	F value	p value	C_{det}
Total SS	287				
Model 1	61				0.787
Model 2	81	17,18 (1)	0.35	NS	0.719*
Model 3	94	1,35 (2)	6.05	<0.05	0.671

Linear models:

1. $Effect_i = a_i + b_i.C$
2. $Effect_i = a_i + B.C$
3. $Effect_i = a_i$

model for comparison is given in parenthesis

* denotes the most appropriate model

squares, particularly for grip strength.

With baseline measurements included, the slope of improvement was significant for all response indices when analysed against dose, total or free concentration (Table 5.11). The coefficient of determination was slightly higher for the fit in terms of total concentration than for dose or free concentration, especially when the dependent variable was the duration of morning stiffness.

When baseline values were removed the results were slightly different (Table 5.12). The slopes were considerably flatter and the median intercepts were different from those observed, especially for free concentration. As an example, when analogue pain score was analysed in terms of total concentration, the slope was -2.7 and $-1.7 cm/\mu g/ml x 10^{-2}$ when baseline data was included and excluded respectively. The slope was only significant for the duration of morning stiffness in terms of dose and total concentration and for the pain score in terms of dose, total and free concentration. There was a trend towards an improvement in the articular index with increasing total concentration but this was not significant. The data for total concentration are presented in Figure 5.11 together with the average slope of improvement determined with baseline measurements excluded.

The SSQres are presented in Table 5.13 for each response index fitted to Models 1 and 2 with dose, total and free concentration as the independent variable. Dose

TABLE 5.11 Summary of the results of fenclofenac dose and concentration-response data fitted to Model 2 using GLIM (Baseline data included)

Clinical effect measurement	DOSE Slope (SE) (units/mg/day x10^{-3})	DOSE Median intercept (range)	DOSE C_{det}	TOTAL CONCENTRATION Slope (SE) (units/µg/ml x10^{-2})	TOTAL CONCENTRATION Median intercept (range)	TOTAL CONCENTRATION C_{det}	FREE CONCENTRATION Slope (SE) (units/µg/ml)	FREE CONCENTRATION Median intercept (range)	FREE CONCENTRATION C_{det}
Ritchie Articular Index	-4.9* (1.1)	20 (6-35)	0.672	-8.9* (2.2)	20 (5-36)	0.689	-7.6* (2.3)	18 (10-34)	0.643
Duration of morning stiffness (min)	-65* (17)	180 (84-480)	0.631	-125* (26)	204 (62-480)	0.666	-119* (30)	190 (49-450)	0.633
Mean grip strength (mmHg	11.4* (3.6)	105 (56-198)	0.852	20.0* (5.5)	108 (50-205)	0.854	15.9* (6.2)	100 (55-208)	0.838
Pain score (cm)	-1.6* (0.3)	6.3 (4.2-9.6)	0.613	-2.7* (0.5)	6.4 (3.6-9.6)	0.636	-2.6* (0.5)	6.0 (3.6-9.0)	0.590

SE is the standard error in the estimate of the slope

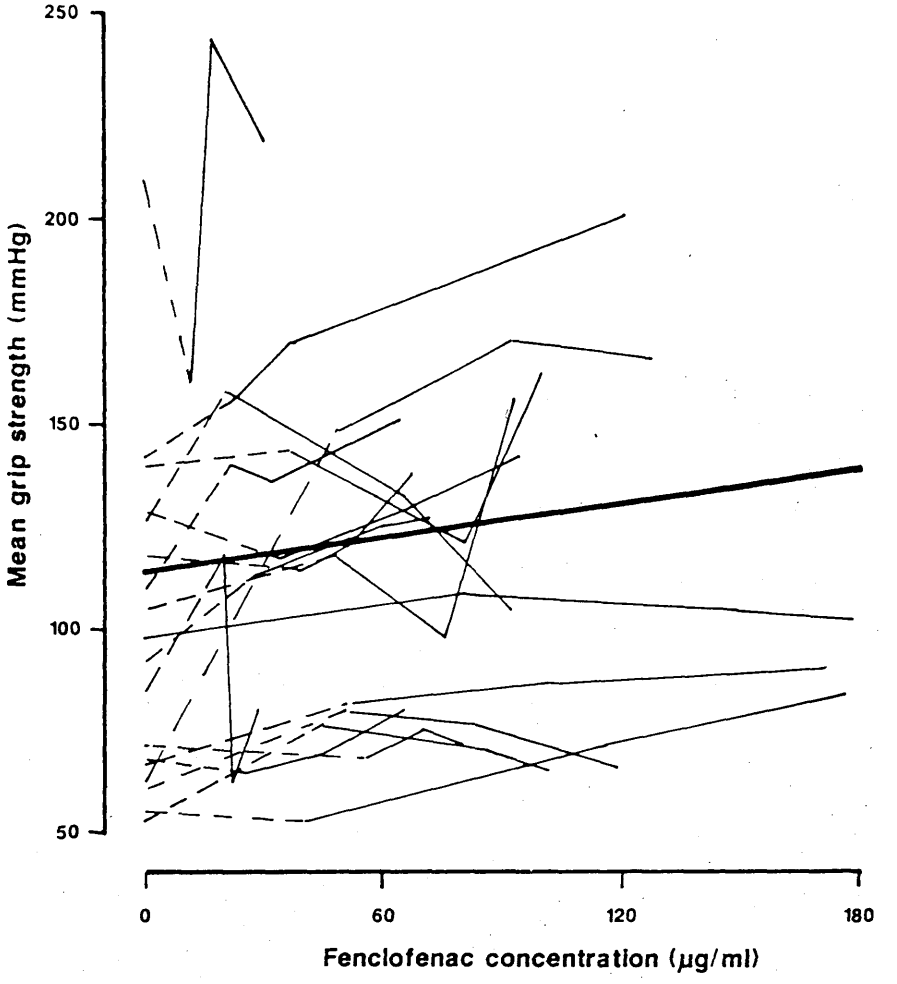
* slopes significantly different from zero ($p<0.05$).

TABLE 5.12 Summary of the results for fenclofenac dose and concentration-response data fitted to Model 2 using GLIM (Baseline data omitted)

Clinical effect measurement	DOSE			TOTAL CONCENTRATION			FREE CONCENTRATION		
	Slope (SE) (units/mg/day x10^{-3})	Median intercept (range)	C_{det}	Slope (SE) (units/µg/ml x10^{-2})	Median intercept (range)	C_{det}	Slope (SE) (units/µg/ml)	Median intercept (range)	C_{det}
Ritchie Articular Index	-2.6 (1.5)	16 (4-34)	Ø.765	-5.1 (2.2)	17 (6-34)	Ø.772	-3.4 (2.4)	15 (3-32)	Ø.761
Duration of morning stiffness (min)	-42* (18)	146 (64-47Ø)	Ø.813	-67* (26)	146 (39-466)	Ø.8Ø9	-42 (28)	122 (26-434)	Ø.795
Mean grip strength (mmHg)	9.Ø (5.Ø)	112 (58-197)	Ø.881	13.8 (8.6)	114 (54-2Ø5)	Ø.879	8.5 (7.8)	122 (62-2Ø8)	Ø.875
Pain score (cm)	-1.Ø* (Ø.4)	5.4 (3.1-9.3)	Ø.714	-1.7* (Ø.5)	5.5 (2.7-9.4)	Ø.713	-1.4* (Ø.5)	4.8 (2.6-8.8)	Ø.713

SE is the standard error in the estimate of the slope

* slopes significantly different from zero ($p<Ø.Ø5$)



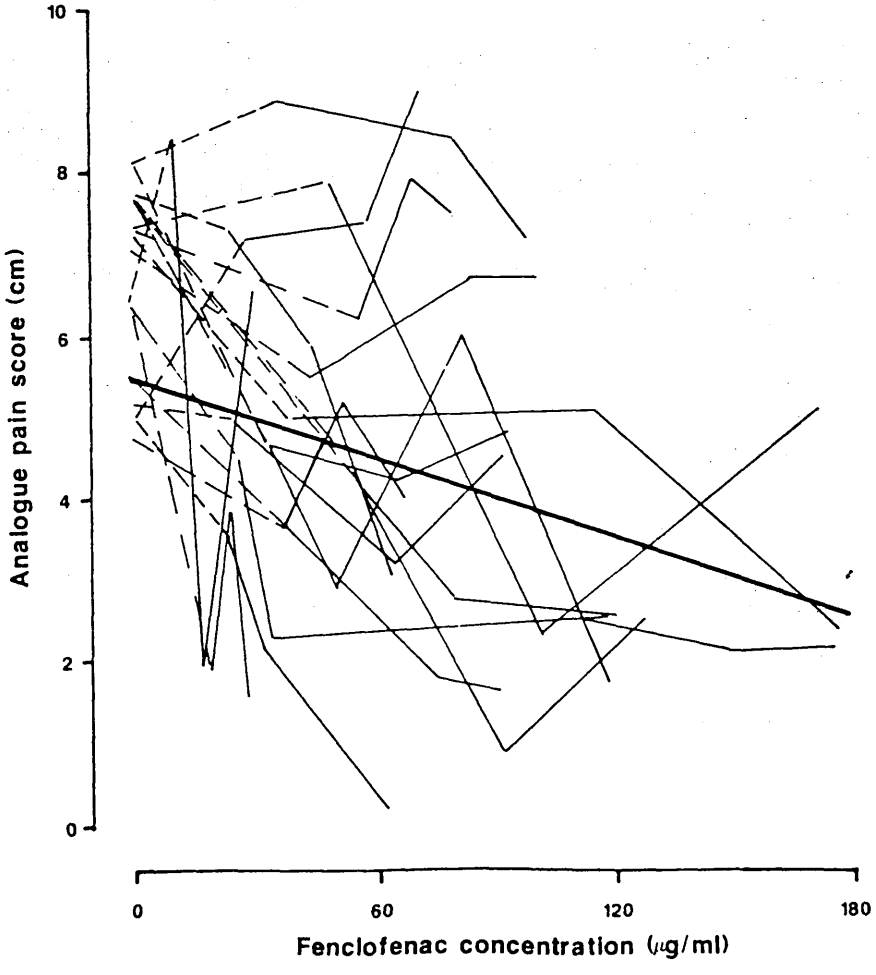


FIGURE 5.11 Individual total fenclofenac concentration-response data. The bold continuous line gives the average slope of improvement

TABLE 5.13 Comparison of the residual sum of squares (SSQres) for clinical response data fitted to the full and reduced models in terms of dose, total or free concentration

		DOSE	TOTAL	FREE
ARTICULAR INDEX				
Model:	1	580*	647	661
	2	1112	1083*	1134
MORNING STIFFNESS				
Model:	1	84060*	104800	105400
	2	140800*	144100	154300
GRIP STRENGTH				
Model:	1	4940*	6079	5990
	2	11490*	11690	12120
PAIN SCORE				
Model:	1	57*	61	60
	2	82	80*	82

* the lowest SSQres for the comparison between dose, total and free concentration

gave the lowest SSQres when data were fitted to the full model. In general, the SSQres was lowest for concentration when data were fitted to Model 2. In addition, the difference in SSQres between Model 1 and Model 2 was always less for concentration than for dose, indicating that concentration does explain some of the inter-individual variability in the response.

Plots of the residuals $(y_i-\hat{y}_i)$ against the predicted effect or concentration indicated that with grip strength and the duration of morning stiffness, baseline values were not fitted well assuming a linear model. With all data included, baseline measurements were overpredicted for grip strength and underpredicted for the duration of morning stiffness. There did not appear to be any trends in the residuals with the other rheumatological measures. These discrepancies indicated that either it was inappropriate to include the baseline values (carried out under non-blinded conditions) or that the data should more appropriately be fitted to an E_{max} model (Holford & Sheiner, 1982).

These data (baseline measurements were included) were fitted to a linear and a nonlinear (E_{max}) model using the program NONMEM (Equations 4.16 & 4.17). A comparison of the E_{max} and linear model was made on the basis of the difference in the objective values (Table 5.14). The best improvement in the objective value using the E_{max} model was that associated with the analogue pain score analysed in terms of total and free concentration.

TABLE 5.14 Comparison of the objective value for fenclofenac dose, free and total concentration - response data fitted to E_{max} and linear models using NONMEM

	ARTICULAR INDEX			MORNING STIFFNESS			GRIP STRENGTH			PAIN SCORE		
	Linear	E_{max}	D	Linear	E_{max}	D	Linear	E_{max}	D	Linear	E_{max}	D
DOSE	377	374	3	753	745	8	542	541	1	157	150	7
TOTAL	375	371	4	748	746	2	543	541	2	163	151	12
FREE	383	374	9	752	748	4	550	542	8	169	152	17

D is the difference in the objective values for the fit to the linear and E_{max} models

The final NONMEM parameter estimates for the E_{max} model are summarised in Table 5.15. Intersubject variability (ie the variance parameters) and the residual intra-subject error were very large. The standard errors of most parameter estimates were also relatively large. $C_{50\%}$ was most poorly estimated. The structural model parameters (SE in the estimate) were best defined for analogue pain score eg. for total concentration E_{max} was 5.0(0.9)cm , $C_{50\%}$ was 69(27)µg/ml and C_{0} was 6.8(0.3)cm. For all effects in terms of dose, total or free concentration, the residual unexplained variability was large and a reflection of the the known variability in some of these response measures (Chapter 3). The residual error, which is also is also due to model mispecification and true intra-subject variability, was slightly larger when data were fitted to the linear model.

5.3.3 Side-effects, biochemistry and haematology

Side-effects reported are given in Table 5.16. These were minor in nature and consisted of gastrointestinal, central nervous system and dermatological complaints. None were so serious as to require discontinuation of treatment or withdrawal from the study. There did not appear to be any

TABLE 5.15 NONMEM parameter estimates (SE) for fenclofenac dose, total and free concentration-response data fitted to the E_{max} model

RITCHIE ARTICULAR INDEX

Parameter	Dose	Total	Free
E_{max}	-12(5)	-14(7)	-11(8)
var	Ø.33(Ø.91)	Ø.33(Ø.34)	Ø.34(Ø.39)
$EC_{50\%}$	5Ø7(829) [a]	42(59) [b]	113(146) [c]
var	ID	ID	ID
$C_Ø$	23(3)	24(3)	23(3)
var	Ø.Ø95(Ø.Ø58)	Ø.1ØØ(Ø.Ø47)	Ø.1ØØ(Ø.Ø56)
ϵ	36(14)	33(12)	35(12)

DURATION OF MORNING STIFFNESS (minutes)

Parameter	Dose	Total	Free
E_{max}	-163(325)	-241(64)	-112(49)
var	ID	ID	Ø.57(Ø.92)
$EC_{50\%}$	534(185Ø) [a]	79(67) [b]	1Ø(8) [c]
var	1Ø(24)	Ø.18(Ø.13)	Ø.21(Ø.21)
$C_Ø$	218(45)	218(32)	217(4Ø)
var	Ø.28(Ø.31)	Ø.18(Ø.13)	Ø.21(Ø.21)
ϵ	433Ø(417Ø)	631Ø(348Ø)	564Ø(468Ø)

Key: var = variance parameter for the preceding structural parameter
ϵ = the residual error
ID = parameter was indeterminate
a = units are mg/day
b = units are μg/ml
c = units are ng/ml

$\sqrt{\text{var}}$ gives as estimate of the inter-individual coefficient of variation in the structural model parameter
$\sqrt{\epsilon}$ gives the estimate of the random additive error

TABLE 5.15 NONMEM parameter estimates (SE) for fenclofenac dose, total and free concentration-response data fitted to the E_{max} model

MEAN GRIP STRENGTH (mmHg)

Parameter	Dose	Total	Free
E_{max}	54(61)	39(14)	26(8)
var	ID	Ø.79(Ø.69)	Ø.7Ø(Ø.65)
$EC_{5Ø\%}$	273Ø(51ØØØ)[a]	76(67)[b]	144(1Ø8)[c]
var	ID	ID	ID
$C_Ø$	1ØØ(2)	1ØØ(1Ø)	99(1Ø)
var	Ø.12(Ø.Ø6)	Ø.14(Ø.Ø5)	Ø.14(Ø.Ø6)
ϵ	288(1Ø9)	269(87)	281(9Ø)

ANALOGUE PAIN SCORE (cm)

Parameter	Dose	Total	Free
E_{max}	-4.1(2.4)	-5.Ø(Ø.9)	-3.3(1.Ø)
var	Ø.52(1.14)	ID	Ø.56(Ø.47)
$EC_{5Ø\%}$	795(136Ø)[a]	69(27)[b]	1Ø5(113)[c]
var	ID	2.47(1.51)	ID
$C_Ø$	6.7(Ø.3)	6.8(Ø.3)	6.8(Ø.3)
var	ID	ID	ID
ϵ	1.9(Ø.5)	1.9(Ø.5)	1.9(Ø.5)

$\sqrt{var}$ gives as estimate of the inter-individual coefficient of variation in the structural model parameter

$\sqrt{\epsilon}$ gives the estimate of the random additive error in the response

TABLE 5.16 Side effects reported on each dose and associated total fenclofenac trough concentrations. Patient number is given in parenthesis.

Side effect	Dose (mg/day)		
	600	1200	1800
Indigestion	13(5)	20(5)	93(3)
Vomiting		81(16)	101(12)
Drowsiness			52(5),108(6)
Headache	51(15)		
Dizzy spells	51(6)		
Haematuria			78(7)
Hot flushes	41(14)		
Slight rash		29(13)	
Mild skin irritation			93(1)
Blotches on skin		71(9)	140(2)

total concentration. In addition, patients with very high free concentrations (due to non-linear binding above 100µg/ml) reported no adverse effects.

Biochemical and haematological indices which showed a change from baseline are given in Table 5.17. There appeared to be a dose related increase in creatinine, although values remained within the normal range. This effect may be of some clinical significance as creatinine concentrations tend on the whole to be lower in patients with rheumatoid arthritis due to a reduction in its production (Nived et al, 1983). There was also some evidence of a dose related reduction in the white blood cell count, but again values remained within the normal range. There was a reduction in bilirubin, red blood cell count and platelet count, but these changes did not reach significance. There was a significant reduction in alkaline phosphatase on the highest dose. The reduction was most dramatic in patients with high initial values. These patients also attained relatively high trough fenclofenac concentrations.

5.4 DISCUSSION

Despite the observation that the binding of fenclofenac to plasma proteins is concentration dependent over the range of total concentrations encountered in this study, 12 hour total trough concentrations were consistent with linear kinetics. Even below 100µg/ml there was a slight increase in

TABLE 5.17 Summary of biochemical and haematological indices

Indice	Baseline	Dose (mg/day) 600	1200	1800	Friedman two-way analysis of variance	
Creatinine (μmol/l)	78 (55-154)	80 (54-179)	82 (56-185)	84 (62-165)	1200>600 1800>1200	p<0.05 p<0.05
Bilirubin (μmol/l)	5 (3-12)	4 (2-11)	4 (2-8)	4 (1-7)		
Alkaline phosphatase (IU/l)	102 (60-182)	103 (62-163)	100 (67-159)	96 (58-141)	1800<baseline	p<0.05
Red blood cells ($x10^{12}$/l)	4.38 (3.44-5.50)	4.35 (3.28-5.43)	4.21 (3.66-5.24)	4.25 (3.61-5.16)		
White blood cells ($x10^{9}$/l)	10.2 (5.9-13.1)	7.9 (5.0-12.3)	8.3 (5.4-12.3)	7.3 (5.0-12.7)	1200<baseline 1800<baseline	p<0.05 p<0.05
Platelets ($x10^{9}$/l)	416 (242-985)	357 (244-694)	393 (202-887)	355 (215-765)		

the free fraction with increasing total concentration, in contrast to previous observations (Brewster & Muir 1978). Three patients showed a dramatic non-linear increase in the free drug concentration over the three doses (Patients 14, 15 and 18). Assuming that the clearance of free drug remains constant, there should be a linear increase in free concentrations and a non-linear increase in total concentrations. These results suggest that in some patients there might be saturation of hepatic metabolism. In terms of total concentration this effect may be masked in part due to saturation of binding sites on plasma protein.

The elimination half-life determined from the single dose study ranged from 11 to 33 hours (median 20 hours). This average value is slightly shorter than the elimination half-life determined in healthy volunteers (mean 27 hours, range 20-38 hours, Henson et al; 1980). The median clearance (range) of total drug in patients was 0.62(0.33-1.49)l/h, higher than that found in healthy volunteers with a mean (SD) of 0.38 (0.12)l/h. It is possible, however, that 48 hours was too short a sampling time to get an accurate estimate of the elimination half-life or clearance and this may explain the underprediction of trough concentrations at steady state.

The variability in fenclofenac clearance (coefficient of variation was approximately 50%) determined from the initial single dose studies (Table 5.6) is reflected in the range of trough concentrations at steady

state (Figure 5.4). For a drug such as fenclofenac with a low extraction ratio, the elimination is dependent on the free fraction of drug in the blood and the intrinsic clearance of free drug (Wilkinson & Shand; 1975). In general, the free fraction of fenclofenac was fairly consistent between patients for a given total concentration, but the free fraction was much higher in one patient with a very low albumin concentration (30g/l) and non-linear binding was evident at much lower total concentrations. Rheumatoid arthritis is a disease not only of the joints but is also associated with dramatic systemic effects. Alterations in the production and catabolism of plasma proteins occur, and it is possible that there are changes in the configuration of these protein molecules. The higher value of clearance determined in this study may be a result of lower albumin concentrations in patients with rheumatoid arthritis compared to healthy individuals, but there was no correlation between total fenclofenac clearance and albumin concentration. It would be interesting to compare the relationship across a wider range of albumin concentrations.

These results suggest that factors affecting the intrinsic clearance of fenclofenac may be important determinants of the total clearance. There was a trend towards a decrease in the apparent clearance of total fenclofenac with increasing age and alkaline phosphatase. Alkaline phosphatase is often raised in patients with rheumatoid arthritis. Indeed, at the beginning of this

study, alkaline phosphatase was above the normal range in 7 of the 18 patients.

Serum alkaline phosphatase is composed of isoenzymes derived from the liver, bone and the intestine. In normal adults 50% is synthesised in the liver and 50% is derived from bone, reticuloendothelial and vascular sources. A correlation between alkaline phosphatase and the number of osteoblastic cells in bone has been reported (Teaford & White; 1964). The raised levels in patients with rheumatoid arthritis may be due to effects of the disease on bone or on the liver. It may be assumed that the decrease in fenclofenac clearance is related to a diffuse effect of the disease on the liver, also associated with an increased production of alkaline phosphatase. Together with a significant reduction in white cell count and the previous observations that during long term treatment there was a reduction in the ESR (Akyol, Anderson & Thompson, 1977) these observations lend substance to the proposal that fenclofenac possesses some disease modifying activity. Indeed, in animal studies fenclofenac was more effective against chronic immunologically-mediated inflammation than against acute inflammation (Phillips, 1980).

There was a decrease in alkaline phosphatase with dose which was most dramatic in patients with high initial levels. This effect has been noted in studies with benoxaprofen (Jones, 1982). It was proposed that this was due to an effect on the production of alkaline phosphatase

by osteoclasts, directly or indirectly. Raised alkaline phosphatase in rheumatoid arthritis, however, has been shown to be of hepatic origin (Mills & Sturrock, 1982). There was, however, no evidence that the clearance of fenclofenac increased over the treatment period in the patients who showed the most dramatic reduction in alkaline phosphatase.

Despite the observed inter-subject variability in the pharmacokinetics of fenclofenac, clinical response was explained equally well by dose as by total concentration irrespective of the model used (linear or non-linear). This is probably due to the marked 'noise' or intra-subject variability in clinical response. Although some patients showed little or no improvement, others showed a dramatic response to fenclofenac. The more complex linear model (Model 3) which describes the data in terms of an individual intercept and slope had to be rejected in favour of the simpler model (Model 2). If there was a relationship between total concentration or free concentration and clinical response across the patient group, the full model should have been more appropriate for explaining the response in terms of dose but not for response in terms of total or free concentration. The full model was not significantly better for dose, but in general, the increase in the SSQres was greatest for dose as a result of removing the individual slope parameter. Although comparison of the results of the GLIM analysis with and without baseline measurements indicated that the data would be more appropriately fitted

to an E_{max} model, the variability in the data often resulted in the rejection of the more complex non-linear model in favour of the simple linear model. In addition, the parameters were always poorly defined and therefore not very meaningful.

Across the concentration or dose range encountered clinically, the simplest linear model predicts an improvement in clinical response with increments in dose or concentration within an individual patient for at least two clinical effect parameters. Due to the lack of response in some patients, the slope of improvement (which is an average value for all patients) is not very dramatic (Table 6.10): reduction in morning stiffness of 25 minutes; reduction in the analogue pain score of 0.6cm, both as a result of increasing the dose from 1200 to 1800mg.

If one considers the fit to the E_{max} model, which is perhaps more realistic, the concentration necessary to achieve 50% of the maximum reduction in the analogue pain score, $C_{50\%}$ (SE) was 795(1360)mg/day, 68.9(26.7)µg/ml and 105(113)ng/ml for dose, total and free concentration respectively; ie, somewhere between the 600 and 1200mg doses. And the maximum reduction in the pain score was 4, 5 and 3cm for dose total and free concentration respectively.

In conclusion, these results suggest that fenclofenac could have been given in doses above 1200mg/day with the expectation that on average there would be an improvement in symptoms. Despite the considerable inter-subject

variability in the kinetics of fenclofenac, these results indicate that knowledge of plasma concentrations (total or free) adds little to the explanation of clinical response. Although subjectively most patients with trough total concentrations above 100µg/ml showed an improvement in symptoms, the analysis did not indicate minimum effective or toxic concentrations.

CHAPTER 6

NAPROXEN, PHARMACOKINETICS AND CLINICAL RESPONSE

6.1 INTRODUCTION

In the last chapter, knowledge of concentration was found to offer little advantage over dose in the description of the clinical response to fenclofenac in patients with rheumatoid arthritis. Increments in dose or concentration, however, were associated with reductions in the duration of morning stiffness and the analogue pain score. This was investigated further with another NSAID, naproxen and this chapter presents the results of a dose ranging controlled study of naproxen in patients with rheumatoid arthritis. The general approach was the same as that described in the previous chapter for fenclofenac. Attention was directed towards the determination of the variability in the pharmacokinetics of naproxen and the general aim was to evaluate whether knowledge of total or free drug concentrations could contribute to the explanation of clinical response or toxicity.

Two previous controlled studies have investigated the relationship between dose and clinical response (Luftschein et al, 1979; Day et al, 1982). Luftschein and colleagues found a significant linear improvement with dose in only 2 of 12 outcome measures (joint swelling and a joint pain and tenderness score). A concentration response relationship was not investigated. Day et al (1982) gave three doses of naproxen (250, 750 and 1500mg/day) to 24 patients (some were stable on gold or penicillamine). They were able to

demonstrate a linear dose response relationship in 5 of 9 clinical response measures (joint count, patients' pain assessment, activities of daily living, grip strength and patients' and doctors' global assessments). Using a parametric ranking technique, there appeared to be a linear relationship between the percentage of responders and trough total concentration. However the advantage of knowledge of concentration over dose could not be tested.

The specific aims of this study were to investigate the following:

a) The relationship between the dose of naproxen and plasma concentration (total and free).

b) The relationship between naproxen clearance and any specific patient factor.

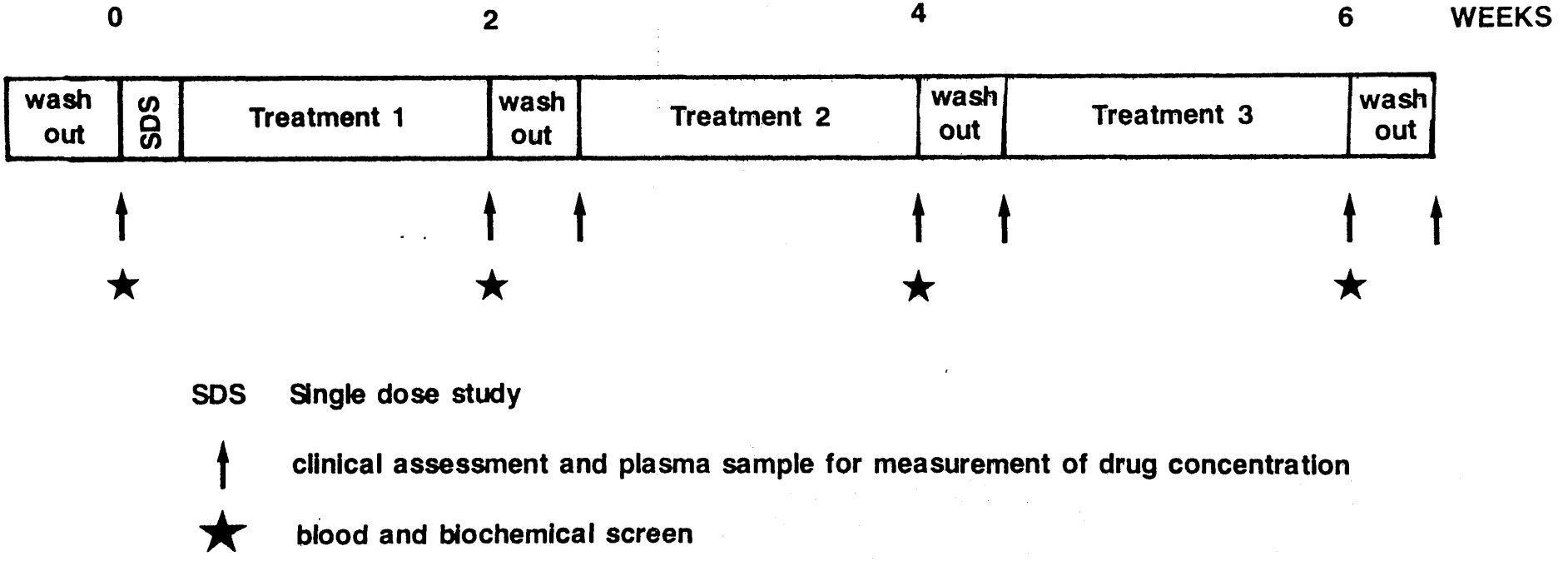
c) The relationship between the dose of naproxen and/or the plasma concentration (total and free) and clinical response and/or toxicity.

6.2 PATIENTS AND METHODS

6.2.1 Study design

The basic study design was identical to that described for fenclofenac (Chapter 5) and is outlined in Figure 6.1. The doses of naproxen were: 500, 1000 and 1500mg/day. Rheumatological assessments were identical to those used in the study of fenclofenac (Chapter 3, Appendix I).

FIGURE 6.1 Study outline



6.2.2 Patients

Eighteen outpatients with 'definite' or 'classical' rheumatoid arthritis (Ropes et al, 1959) complied with the protocol and completed the study. This completed 3 randomised blocks of 6 for the order of the three doses. Thirteen patients were female and five were male. Their ages ranged from 43 to 74 years and the disease duration ranged from 6 months to 23 years. Individual patient characteristics are given in Table 6.1 together with previous NSAID therapy. None of the patients was receiving corticosteroids or any other second line drug. Patients were included in the study only if there was a 'flare' after the withdrawal of their previous NSAID for at least 3 days. Patient 18, however stopped taking piroxicam one week before the start of the study. Table 6.2 presents the rheumatological measures at the end of this initial wash-out period and gives an indication of the severity of the disease.

6.2.3 Single dose study

The initial wash-out period was followed by a single 1000mg dose study (4x250mg tablets). The conditions being indentical to those of the fenclofenac study. A control 40ml blood sample was taken from all patients before the dose to determine naproxen binding parameters (Chapter 3). Subsequently, 10ml samples were taken at the times given for fenclofenac up to 48 hours. All blood samples were handled and stored as described for fenclofenac (Chapter 5). Total

TABLE 6.1 Patient characteristics (naproxen study)

Patient number	Sex	Age (yr)	Weight (kg)	Height (cm)	Disease duration	ESR (mm/hr)	Albumin (g/l)	Creatinine (μmol/l)	Alk phos (IU/l)	Smoking	Previous NSAID
1	M	69	59.0	155	0.5	-	35	110	204	Y	none
2	F	63	71.2	158	0.5	103	38	89	162	N	ibuprofen+mefenamic acid
3*	F	67	58.0	163	8.0	30	41	68	99	N	naproxen
4*	F	68	53.0	150	3.0	40	43	111	108	N	ibuprofen
5	F	48	60.0	162	4.0	48	43	59	132	Y	fenoprofen
6	M	43	61.5	177	1.5	62	38	78	107	Y	diclofenac+ketoprofen
7*	F	63	74.8	152	2.5	86	42	138	107	N	naproxen
8	F	62	73.0	163	23.0	43	42	87	136	N	diclofenac
9	F	60	49.0	158	2.0	64	42	76	111	N	naproxen
10	F	54	48.0	152	12.0	50	43	53	114	Y	fenoprofen+indomethacin
11	F	72	77.0	160	4.0	50	41	110	77	N	diclofenac
12	M	54	52.0	165	6.0	31	37	86	130	Y	diclofenac
13	M	63	76.0	168	3.0	74	36	120	114	N	naproxen+ketoprofen
14	M	69	69.0	188	2.5	54	34	67	142	Y	naproxen+indomethacin
15	F	74	58.0	178	10.0	23	41	102	120	N	fenoprofen
16	F	65	69.0	163	4.0	40	34	77	135	N	
17	F	43	65.0	158	3.0	10	38	71	101	Y	ibuprofen
18	F	47	47.0	152	5.0	22	42	53	103	N	piroxicam

Key: Alk phos = alkaline phosphatase

* patients receiving cimetidine

TABLE 6.2 Disease severity assessed by rheumatological measures at the end of the initial wash-out period (naproxen study)

Patient	Ritchie Articular index (Ø-78)	Duration of morning stiffness (min)	Grip strength (mmHg) LH	RH	Analogue pain score (Ø-1Øcm)
1	15	24Ø	78	1Ø7	5.5
2	11	12Ø	126	148	4.9
3	1Ø	12Ø	89	7Ø	8.5
4	44	18Ø	65	82	6.8
5	17	9Ø	82	42	6.5
6	38	9Ø	57	6Ø	9.8
7	55	3Ø	64	53	9.6
8	32	36Ø	93	115	7.9
9	2Ø	1Ø	6Ø	55	4.9
1Ø	52	9Ø	51	47	4.2
11	16	18Ø	8Ø	12Ø	7.6
12	25	18Ø	1Ø8	122	6.5
13	1Ø	3Ø	135	128	2.6
14	12	4Ø	146	112	2.6
15	26	12Ø	83	87	5.6
16	21	45	72	59	5.7
17	12	18Ø	86	89	2.Ø
18	31	2Ø	67	83	8.4

and free naproxen were determined as outlined in Chapter 2.

6.2.4 Randomised treatment period

During the randomised treatment period each dose of naproxen was given for 12 days at a time. Naproxen (250mg) and placebo were identical in appearance. On each dose, patients took three tablets two times a day; at 10.00 and 22.00 hours.

Rheumatological assessments were carried out and blood samples were taken for the measurement of naproxen concentrations (total and free) and for standard biochemical and haematological screens throughout the study as detailed for fenclofenac (Figure 6.1). Blood samples for drug analysis were handled as above for the single dose study.

6.2.5 Data analysis

(i) Single dose study

Total or free concentration-time profiles were fitted to one and two compartment models with first order or zero order absorption (Models 1-4, Appendix II) using non-linear least squares regression analysis (Chapter 4). The most appropriate model was chosen on the basis of the criteria given in Chapter 4. For total concentration the error was assumed to be constant, while the free concentration was weighted proportional to the reciprocal of the fitted concentration ($1/\hat{c}_i$).

(ii) Binding studies

Binding data were fitted to the Langmuir isotherm with

two independent binding sites using non-linear least squares regression analysis (Chapter 4). Free concentration as the dependent variable was weighted as above.

(iii) Dose and concentration-response analysis

The rheumatological assessments used in these analyses were the same as those used in Chapter 5. Data analysis techniques were identical to those for fenclofenac.

6.3 RESULTS

6.3.1 Pharmacokinetics and protein binding

(i) Total naproxen pharmacokinetics

Total concentration-time profiles after a single dose of 1000mg naproxen were in general fitted well to a two compartment model with a zero order input (Table 6.3). Parameter estimates are presented in Table 6.4. Apparent clearance ranged from 0.22 to 1.22 l/h (median 0.58 l/h). The parameter values, however, could not be used to predict total trough concentrations at steady state on each dose.

There was a non-linear increase in total naproxen trough concentrations. Mean concentrations (±SD) were 36.5(±7.1), 49.2(±8.0) and 56.4(±9.5) µg/ml on 500, 1000 and 1500 mg/day respectively. Individual trough concentrations are presented in Figure 6.2a. The non-linear increase was consistent for all patients. The variability in total concentrations was small compared to the range of clearance determined from the single dose study.

TABLE 6.3 Comparison of AIC values for total naproxen concentration-time profiles fitted to Models 1-4

Patient	One compartment model first order	One compartment model zero order	Two compartment model first order	Two compartment model zero order
1	101	100*	110	103
2	135	123	131	116*
3	118	101	99*	106
4	132	120	132	119*
5	122	104	122	99*
6	102	101	136	78*
7	114	114	96*	102
8	127	134	121*	136
9	152	174	174	123*
10	115	120	119	104*
11	122	106	117	89*
12	111	93	128	78*
13	106	96*	109	100
14	111*	116	131	130
15	131	119*	132	124
16	135	100	117	88*
17	103	103	86	83*
18	135	122	139	119*

* indicates the lowest AIC value for comparison of the four models

TABLE 6.4 Individual parameter estimates (SE) for total naproxen concentration-time profiles fitted to a two compartment model with zero order input

Patient	V_1 (l)	α (h^{-1})	β (h^{-1})	k_{21} (h^{-1})	Cl (l/h)	C_{det}	df
1	10.2 (0.6)	0.09 (0.03)	0.001 (0.103)	0.005 (0.119)	0.254	0.947	8
2	7.7 (0.5)	0.43 (0.05)	0.033 (0.008)	0.218 (0.033)	0.499	0.958	10
3	8.0 (0.2)	0.15 (0.02)	0.035 (0.012)	0.070 (0.018)	0.616	0.964	9
4	7.4 (1.2)	0.19 (0.28)	0.026 (0.029)	0.111 (0.234)	0.322*	0.943	9
5	6.7 (0.2)	0.29 (0.01)	0.042 (0.004)	0.142 (0.007)	0.583	0.982	10
6	9.5 (0.4)	0.36 (0.08)	0.047 (0.009)	0.189 (0.058)	0.855	0.996	10
7	9.5 (0.5)	0.22 (0.02	0.035 (0.007)	0.140 (0.016)	0.522*	0.989	10
8	9.4 (0.4)	0.11 (0.01)	0.031 (0.005)	0.059 (0.007)	0.558	0.934	10
9	7.5 (0.4)	0.21 (0.02)	0.039 (0.008)	0.118 (0.015)	0.521	0.988	10
10	7.1 (0.2)	0.76 (0.02)	0.092 (0.005)	0.569 (0.020)	0.878	0.978	10
11	8.8 (0.4)	0.16 (0.01)	0.026 (0.006)	0.076 (0.009)	1.225	0.993	10
12	8.8 (0.1)	0.28 (0.07)	0.048 (0.028)	0.155 (0.050)	0.766	0.995	10
13	11.2 (1.2)	0.82 (0.12)	0.065 (0.021)	0.712 (0.062)	0.837	0.974	10
14	14.3 (2.6)	0.10 (0.05)	0.035 (0.017)	0.089 (0.056)	0.574	0.809	10
15	7.9 (1.0)	0.17 (0.11)	0.004 (0.061)	0.024 (0.112)	0.224	0.928	10
16	9.1 (0.5)	0.30 (0.13)	0.042 (0.009)	0.152 (0.017)	0.754	0.988	10
17	6.2 (0.6)	0.51 (0.13)	0.057 (0.061)	0.193 (0.061)	0.934	0.994	9
18	7.7 (0.3)	0.23 (0.02)	0.026 (0.004)	0.135 (0.009)	0.341	0.949	10
median	8.4	0.22	0.037	0.141	0.578		
range	(6.2-14.3)	(0.09-0.82)	(0.001-0.092)	(0.005-0.712)	(0.224-1.22)		

* patients receiving cimetidine

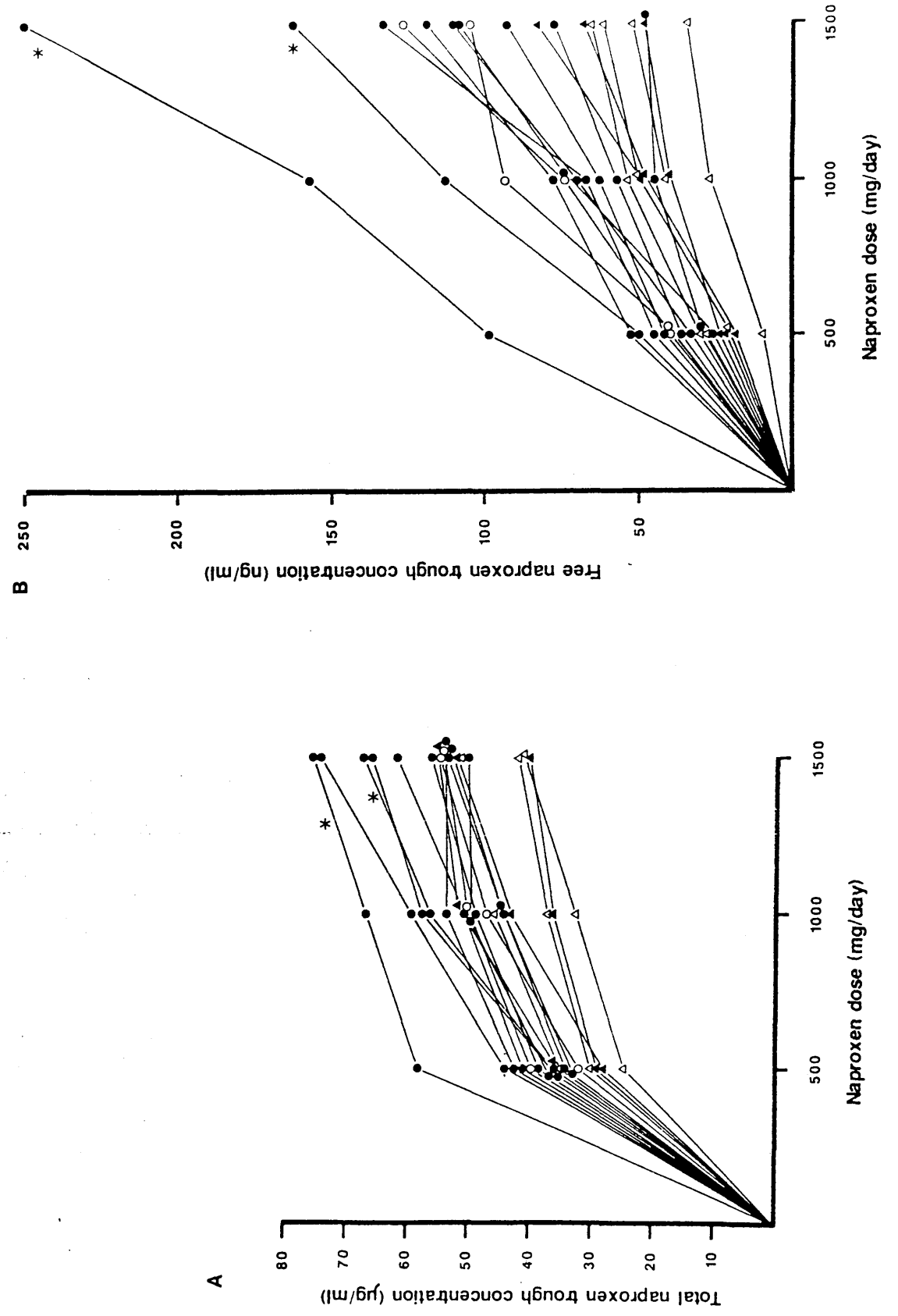


FIGURE 6.2 Individual total and free naproxen trough concentrations at steady state on each dose. Female nonsmoker (●) and smoker (○), male nonsmoker (▲) and smoker (△).
* patients receiving cimetidine

(ii) Free trough concentrations at steady state

The relationship between the dose of naproxen and the free drug concentration was linear: mean trough concentrations (±SD) were 34.2(±15.2), 63.9(±25.9) and 95.1(±40.6) ng/ml on 500, 1000 and 1500 mg/day respectively. Individual free trough concentrations are presented in Figure 6.2b. The variability in total concentrations was small in comparison to the variability in free drug concentrations. The free concentration of naproxen tended to be higher in females and lower in smokers. In addition, the free concentration was considerably higher in two patients who were receiving cimetidine throughout the study (Figure 6.2b). The differences were not so dramatic for total concentration (Figure 6.2a).

The free fraction in trough samples ranged from 0.032% at a total concentrations of 25µg/ml to 0.4422% at a total concentration of 75µg/ml (Figure 6.3): the percentage of naproxen bound to plasma albumin over this concentration range exceeded 99.5%.

(iii) Protein binding studies

To explain the kinetics of naproxen it was necessary to determine the free concentration-time profile. Instead of measuring the free naproxen concentration in each of the single dose study plasma samples, the binding of naproxen was investigated over a much wider concentration range by spiking the control plasma taken after the initial wash-out period with naproxen to give concentrations over the range 25 to 500µg/ml using equilibrium dialysis (Chapter 2). This

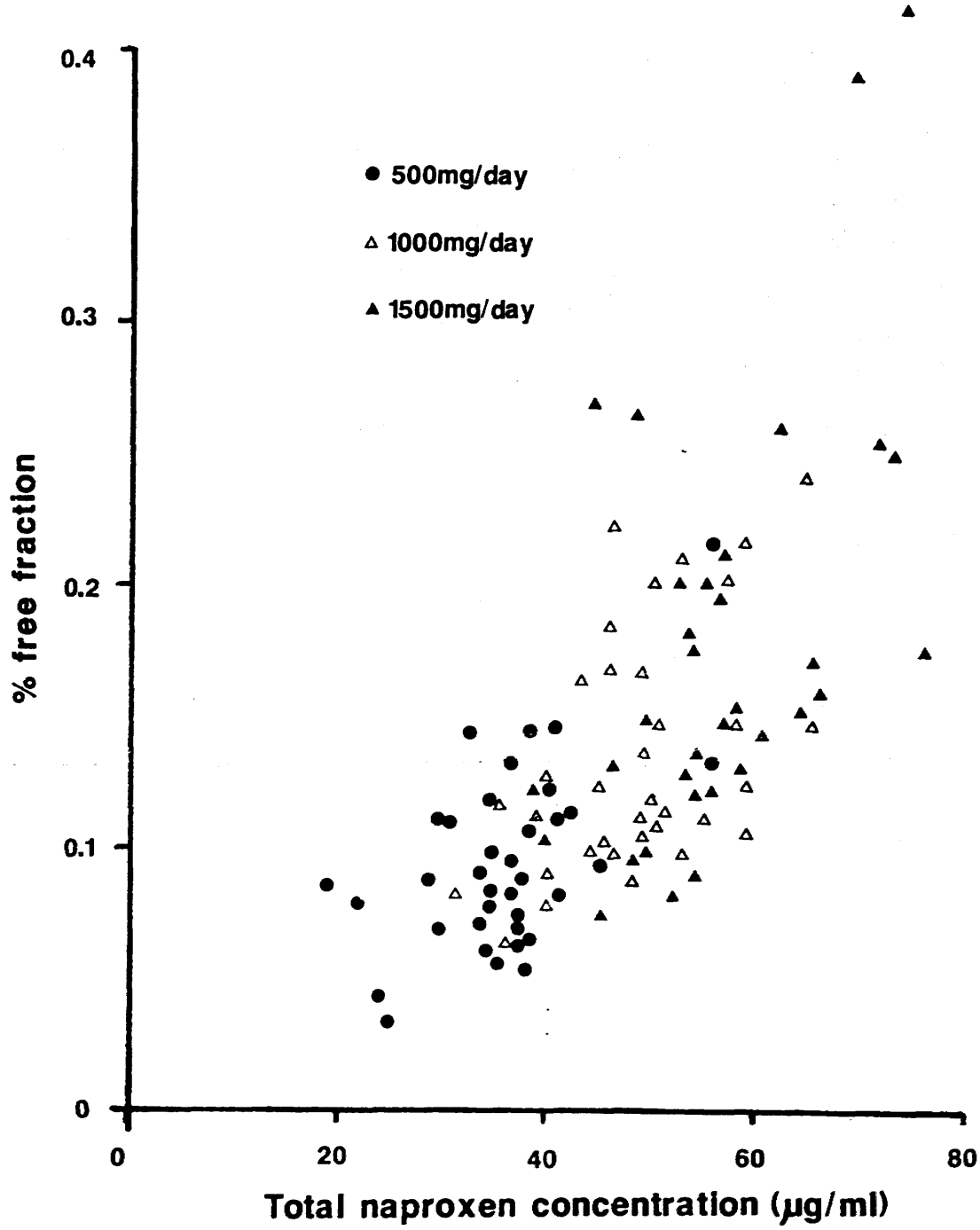


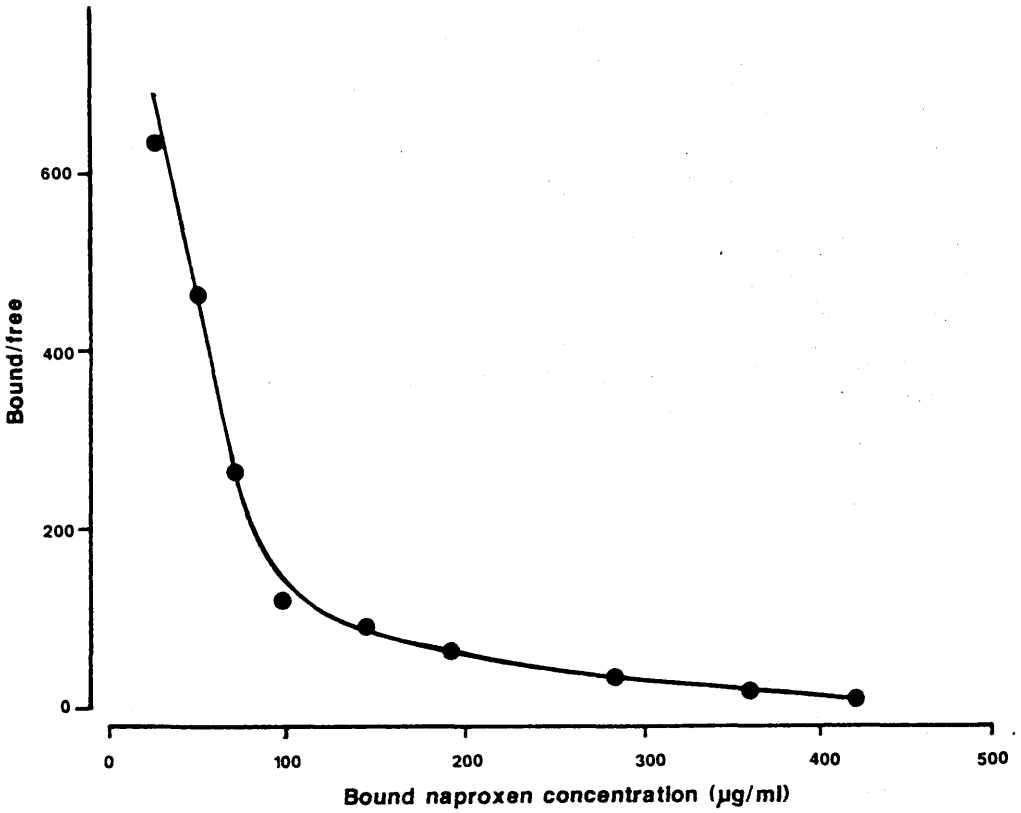
FIGURE 6.3 The free fraction of naproxen plotted against the total concentration in trough samples at steady state on each dose.

allowed the estimation of the parameters of an appropriate binding model assuming that the interaction obeyed the law of mass action. The parameters could then be used to determine the free concentration corresponding to a particular total concentration.

The individual binding data plotted in the form of a modified Scatchard plot (bound/free against bound) indicated that naproxen was bound to at least two distinct binding sites. The binding parameters, determined graphically were used as initial estimates for the non-linear least squares regression analysis.

The binding data for a representative patient, plotted in the form of Scatchard and fitted to the Langmuir equation rearranged in terms of free concentration, are given in Figure 6.4. A summary of the individual binding parameters (expressed in µg/ml naproxen) are presented in Table 6.5. The mean binding capacities for the high and low affinity sites were 73(+15) and 473(+53)µg/ml respectively. The dissociation constants were 0.060(+0.025) and 6.2(+1.5)µg/ml for the high and low affinity sites respectively. The standard error in the estimate of some of the individual parameters (especially the dissociation constant for the high affinity site) was often large. The variability in the mean parameter values may in part, therefore be a result of poorly defined individual parameter estimates.

By fitting the data from all patients simultaneously using NONMEM (Chapter 4), mean binding parameters and their variances within the patient population could be determined.



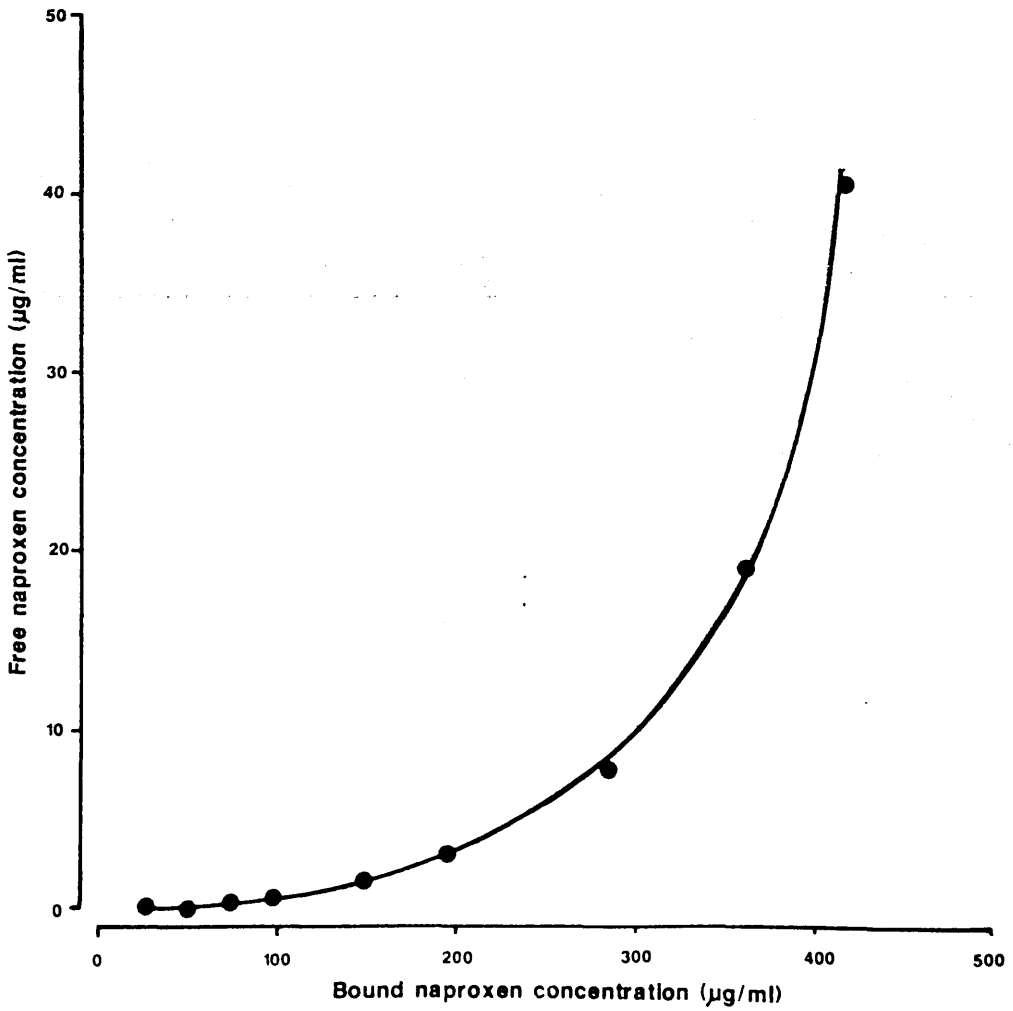


FIGURE 6.4 Binding data for a representative patient (14)

A plotted in the form of a modified Scatchard plot

B Fitted to the Langmuir isotherm rearranged with free concentration as the dependent variable

TABLE 6.5 Individual naproxen plasma protein binding parameter estimates (SE)

Patient	nP_1 (μg/ml)	K_{d1} (μg/ml)	nP_2 (μg/ml)	K_{d2} (μg/ml)	C_{det}	df
2	66 (4)	0.071 (0.009)	448 (36)	7.0 (1.0)	0.823	4
3	71 (6)	0.051 (0.008)	500 (15)	6.1 (0.7)	0.999	4
4	73 (4)	0.051 (0.006)	550 (40)	6.1 (0.8)	0.996	3
5	84 (11)	0.050 (0.006)	500 (9)	5.5 (0.6)	0.999	3
6	79 (2)	0.048 (0.002)	443 (25)	5.5 (0.6)	0.961	4
7	80 (5)	0.086 (0.009)	457 (12)	5.8 (0.6)	0.987	4
8	67 (1)	0.092 (0.426)	470 (8)	4.5 (9.0)	0.999	4
9	75 (8)	0.118 (0.026)	546 (26)	8.9 (1.3)	0.997	4
10	87 (5)	0.039 (0.008)	514 (7)	5.9 (0.4)	0.999	4
11	87 (3)	0.048 (0.003)	407 (42)	5.4 (1.0)	0.963	3
12	47 (7)	0.030 (0.015)	438 (7)	4.5 (0.5)	0.995	4
13	83 (6)	0.087 (0.010)	392 (7)	7.8 (1.1)	0.973	4
14	68 (6)	0.045 (0.017)	414 (6)	6.8 (0.5)	0.998	4
15	40 (5)	0.021 (0.015)	442 (20)	3.3 (0.4)	0.993	4
16	56 (4)	0.088 (0.013)	426 (23)	6.4 (0.7)	0.800	4
17	88 (3)	0.068 (0.006)	488 (16)	9.2 (0.8)	0.999	4
18	90 (12)	0.044 (0.020)	574 (28)	7.2 (1.3)	0.998	4
mean	73	0.060	473	6.2		
SD	15	0.025	53	1.5		

The results of the NONMEM analysis are shown in Table 6.6. The population average binding parameters were similar to those obtained from the mean of the individual parameter values; the inter-subject variability in the dissociation constant for the high affinity site was still very large.

If it is assumed that albumin is the major binding protein, nP_1 and nP_2 should theoretically be related to the concentration of albumin. Using a simple model:

$$nP_1 = \Theta_1.alb \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6.1)$$

$$nP_2 = \Theta_2.alb \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6.2)$$

where Θ_1 and Θ_2 are constants which relate the binding capacity for the high and low affinity sites to the individual albumin concentration (alb), the objective value was reduced, indicating an improvement in the 'goodness of fit' for the same number of parameters. The results of this analysis are also shown in Table 6.6. The estimate of the inter-subject variability in K_{d1} was still relatively large, but smaller compared to the previous model. The program was unable to determine the inter-subject variability in K_{d2}. A plot of free against total concentration using the NONMEM binding parameters with a range of albumin concentrations is given in Figure 6.5.

(iv) Free naproxen pharmacokinetics

Free drug concentration-time profiles were generated using NONMEM binding parameters (adjusted for individual albumin concentration) from the total concentrations after the single 1000mg dose of naproxen. The program used is given in Appendix III.

TABLE 6.6 Naproxen plasma protein binding parameters estimates (SE) using NONMEM

	nP_1 (µg/ml)	Θ_1 (µg/ml/g/l)	K_{d1} (µg/ml)	nP_2 (µg/ml)	Θ_2 (µg/ml/g/l)	K_{d2} (µg/ml)	CV	Obj
Model 1	74.6 (6.8)	-	0.0648 (0.0110)	484 (1.4)	-	6.34 (0.81)	21	-181.0
var	3.9 (2.3)		0.0024 (0.0007)	653 (3930)		ID		
Model 2	-	1.97 (0.12)	0.0699 (0.0078)	-	12.1 (0.4)	6.85 (0.64)	19	-226.7
var	3.1 (1.8)		0.0013 (0.0003)	498 (237)		ID		

$nP_1 = \Theta_1.alb$

$nP_2 = \Theta_2.alb$

CV is the estimate of the coefficient of variation in the residual error
Obj is the objective value

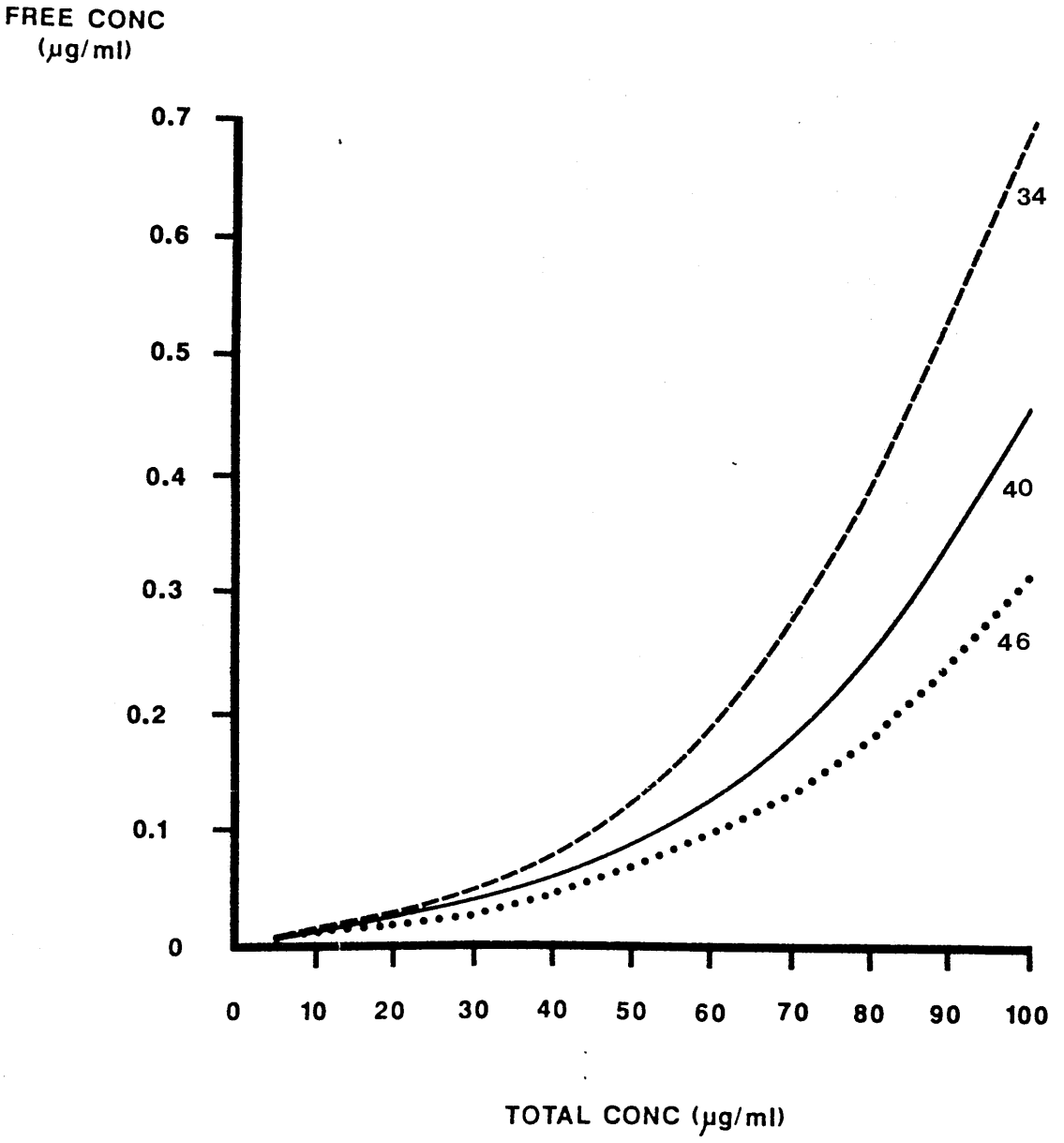


FIGURE 6.5 Free naproxen concentration plotted against total naproxen concentration determined using the NONMEM binding parameters over the albumin concentration range 34-46g/l

Free concentration-time profiles were best fitted by a two compartment model. The fit was improved when a zero order input was used instead of the usual first order input (Table 6.7). The pharmacokinetic parameters for the data fitted to this model (Model 4, Appendix II) are given in Table 6.8. Representative profiles of naproxen (total and free) and desmethylnaproxen (DMN) after a single 1000mg dose are given in Figure 6.6. Although DMN was detected in the plasma after the single dose of naproxen, the levels were close to the limit of detection and in most cases, could not be detected after 12 hours. The kinetics of the metabolite could not be determined. The volume of distribution of the central compartment for free naproxen was obviously very large as a result of the very low free concentrations. The clearance of free drug was considerably higher than the clearance of total drug, again due to the fact that a large fraction of the total drug is bound to plasma albumin.

(v) Correlation between patient factors and naproxen clearance

The relationship between the clearance of free naproxen and various patient factors such as sex, age, creatinine, alkaline phosphatase and smoking were investigated using general linear regression and correlation techniques. There was a weak but significant reduction in clearance with increasing age (Figure 6.7a). The clearance also tended to be lower in females and in patients on cimetidine. It tended to be higher in smokers. There was no correlation between the clearance of naproxen and weight in the group as a whole (Figure 6.7b).

TABLE 6.7 Comparison of first order and zero order input on the AIC values for free naproxen concentration - time profiles fitted to a two compartment model

Patient	first order	zero order
1	-39.9	-46.3*
2	-47.9	-64.3*
3	-34.1	-64.4*
4	-62.Ø	-78.8*
5	-24.Ø	-32.8*
6	-97.2*	-96.1
7	-41.1	-5Ø.7*
8	-53.6	-66.6*
9	-47.8*	-47.2
1Ø	-45.8	-54.6*
11	-42.4	-73.8*
12	-62.Ø*	-59.Ø
13	-54.3	-72.Ø*
14	-44.9	-55.3*
15	-24.7	-54.2*
16	-97.2*	-72.6
17	-6Ø.4	-117.1*
18	-34.8	-51.9*

* indicates the lowest AIC value

TABLE 6.8 Parameter estimates (SE) for naproxen free concentration - time profiles fitted to a two compartment model with a zero order input

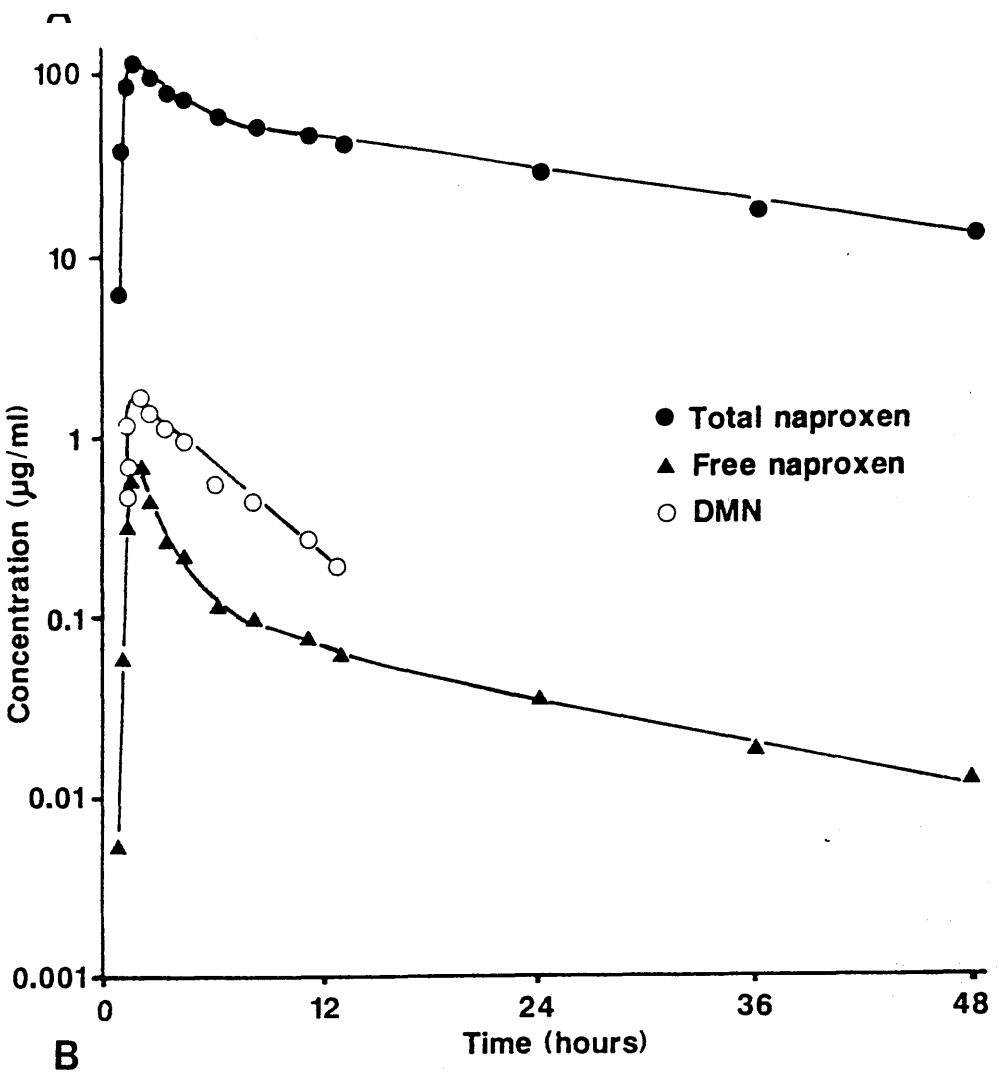
Patient	T_{lag} (h)	T (h)	V_1 (l)	α (h^{-1})	β (h^{-1})	k_{21} (h^{-1})	V_{ss} (l)	Cl (l/h)	C_{det}	df
1	0.23 (0.01)	2.52 (0.18)	387 (114)	2.73 (2.73)	0.098 (0.017)	0.308 (0.071)	2462	339	0.958	8
2	0.45 (0.01)	0.80 (0.07)	1062 (88)	0.75 (0.11)	0.053 (0.006)	0.150 (0.024)	3809	283	0.986	10
3	1.01 (0.04)	3.06 (0.23)	1052 (80)	0.58 (0.06)	0.075 (0.008)	0.150 (0.015)	2560	308	0.984	9
4	1.03 (0.01)	0.62 (0.03)	1366 (46)	0.37 (0.03)	0.046 (0.005)	0.111 (0.016)	3232	206 *	0.996	9
5	0.52 (0.14)	0.96 (0.38)	1443 (273)	0.41 (0.09)	0.056 (0.016)	0.099 (0.024)	3412	334	0.906	9
6	0.70 (0.01)	0.38 (0.02)	1929 (91)	0.58 (0.05)	0.061 (0.004)	0.134 (0.014)	5427	513	0.996	10
7	0.02 (0.67)	0.55 (0.52)	1620 (134)	0.32 (0.07)	0.046 (0.014)	0.090 (0.039)	2024	268 *	0.971	10
8	0.25 (0.04)	2.00 (0.11)	2104 (84)	0.30 (0.01)	0.048 (0.002)	0.080 (0.004)	4418	377	0.935	10
9	0.22 (0.02)	0.54 (0.15)	1086 (121)	0.35 (0.05)	0.052 (0.007)	0.085 (0.011)	2400	231	0.971	10
10	0.76 (0.04)	3.04 (0.82)	2958 (785)	0.28 (0.14)	0.064 (0.028)	0.093 (0.067)	4813	572	0.848	10
11	0.42 (0.03)	0.23 (0.07)	1860 (159)	0.31 (0.03)	0.038 (0.006)	0.074 (0.009)	4746	303	0.990	10
12	0.38 (0.04)	0.47 (0.11)	1464 (152)	0.56 (0.13)	0.074 (0.013)	0.170 (0.049)	3361	358	0.980	10
13	1.34 (0.06)	1.76 (0.27)	3975 (278)	0.90 (0.07)	0.079 (0.007)	0.682 (0.058)	5098	416	0.934	10
14	0.06 (0.04)	1.02 (0.10)	2504 (140)	0.39 (0.03)	0.046 (0.005)	0.126 (0.011)	5835	351	0.928	10
15	1.26 (0.06)	1.12 (0.15)	1018 (88)	0.43 (0.05)	0.030 (0.013)	0.046 (0.021)	3974	283	0.975	10
16	0.86 (0.01)	1.39 (0.08)	1227 (90)	0.59 (0.06)	0.061 (0.004)	0.129 (0.014)	3538	342	0.986	10
17	1.78 (0.02)	2.21 (0.07)	1060 (48)	0.76 (0.05)	0.071 (0.006)	0.120 (0.012)	3368	474	0.999	9
18	1.12 (0.05)	1.78 (0.15)	1546 (64)	0.27 (0.01)	0.040 (0.002)	0.073 (0.004)	3432	228	0.957	10
Median	0.61	1.05	1454	0.42	0.055	0.116	3485	336		
range	(0.02-1.78)	(0.23-3.06)	(389-3975)	(0.23-2.7)	(0.030-0.98)	(0.046-0.680)	(2024-5835)	(206-572)		

* patients receiving cimetidine

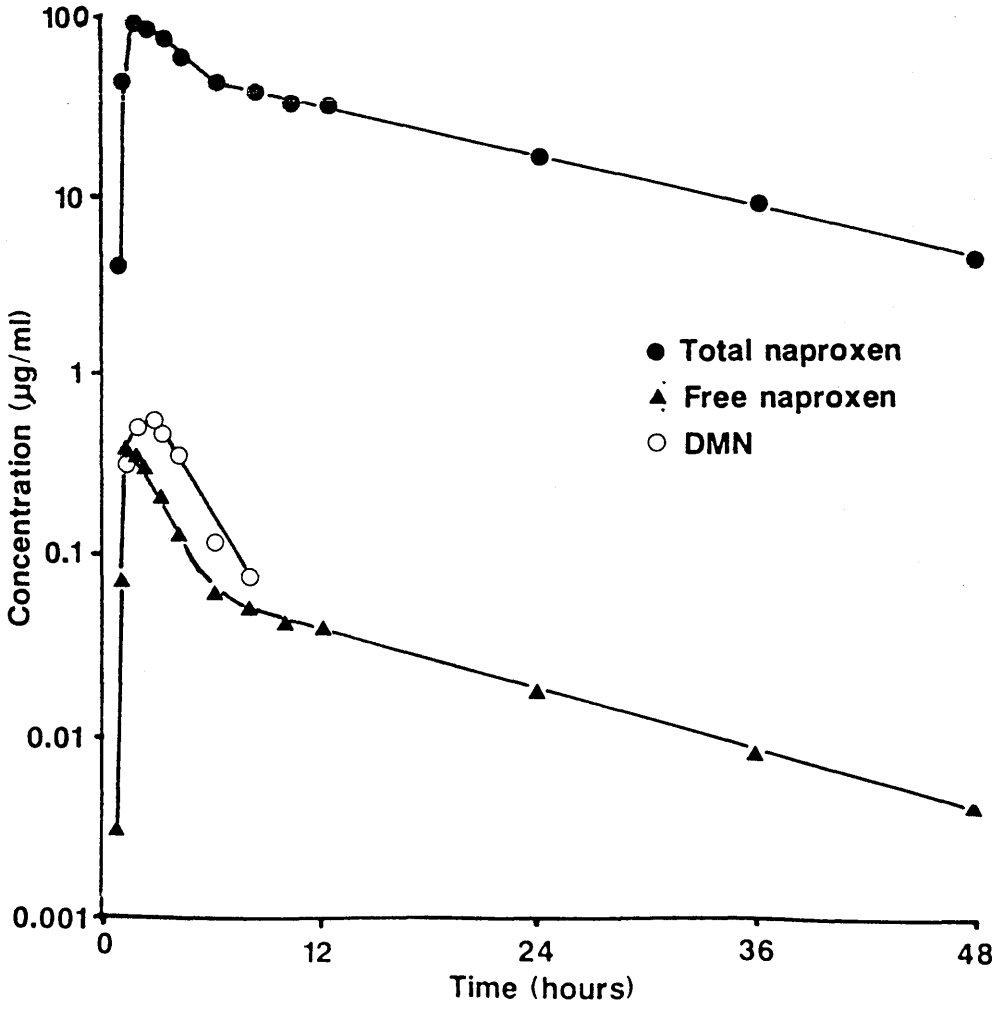
FIGURE 6.6 Representative profiles of total naproxen, free naproxen and DMN in plasma after a single 1000mg dose

A Patient 2

B Patient 6


100
10
1
0.1
0.01
0.001
Concentration (µg/ml)
Total naproxen
Free naproxen
DMN
0
12
24
36
48
Time (hours)

B


100
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Concentration (µg/ml)
Total naproxen
Free naproxen
DMN
0
12
24
36
48
Time (hours)

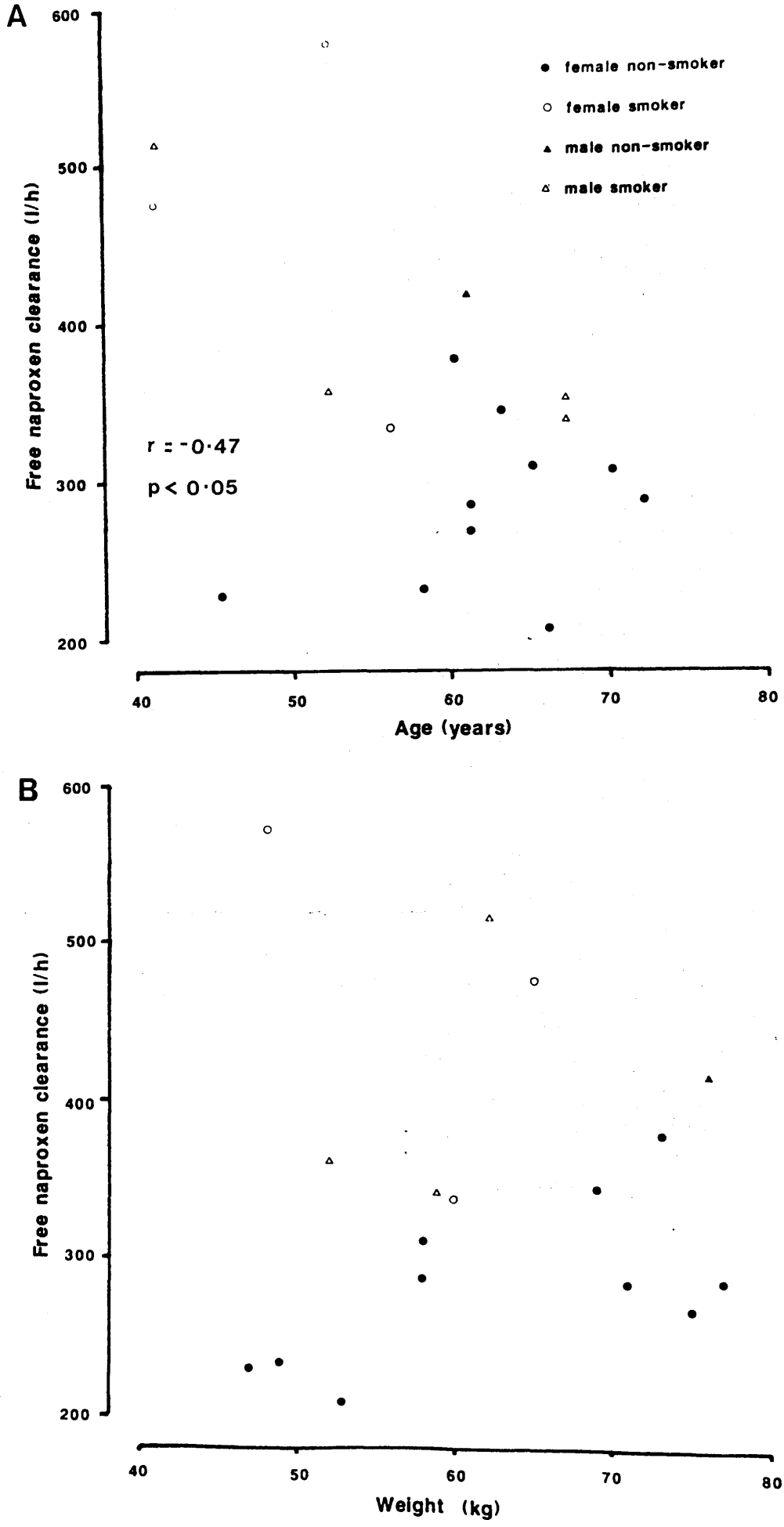

FIGURE 6.7 The apparent clearance of free naproxen plotted against age and weight

(vi) Prediction of free concentrations at steady state

The pharmacokinetic parameters determined for free drug were used to predict free trough concentrations at steady state (the steady state equation is given in Appendix II for the two compartment model) and the error in the prediction at each dose was tested using a paired t-test. The individual pharmacokinetic parameters gave unbiased predictions although the predictions were not very precise (Figure 6.8).

(vii) Prediction of total concentration at steady state

The total concentration at steady state corresponding to the predicted free concentration was determined from the Langmuir isotherm (Equation 4.14) with the NONMEM binding parameters individualised for albumin concentration. These predicted total concentrations were compared to the observed total concentrations using a paired t-test. The prediction errors for total concentration are given in Figure 6.9. The underprediction of the total concentration was most dramatic at the lowest dose, suggesting that there is a quantitative alteration in the binding of naproxen to the high affinity site on albumin. There was no difference in the albumin concentration at the end of the treatment periods compared to the initial wash-out period. Prediction of total drug concentrations at steady state indicated that the binding parameters determined with plasma obtained at the start of the study were inconsistent with the actual binding during repeated dosing with naproxen.

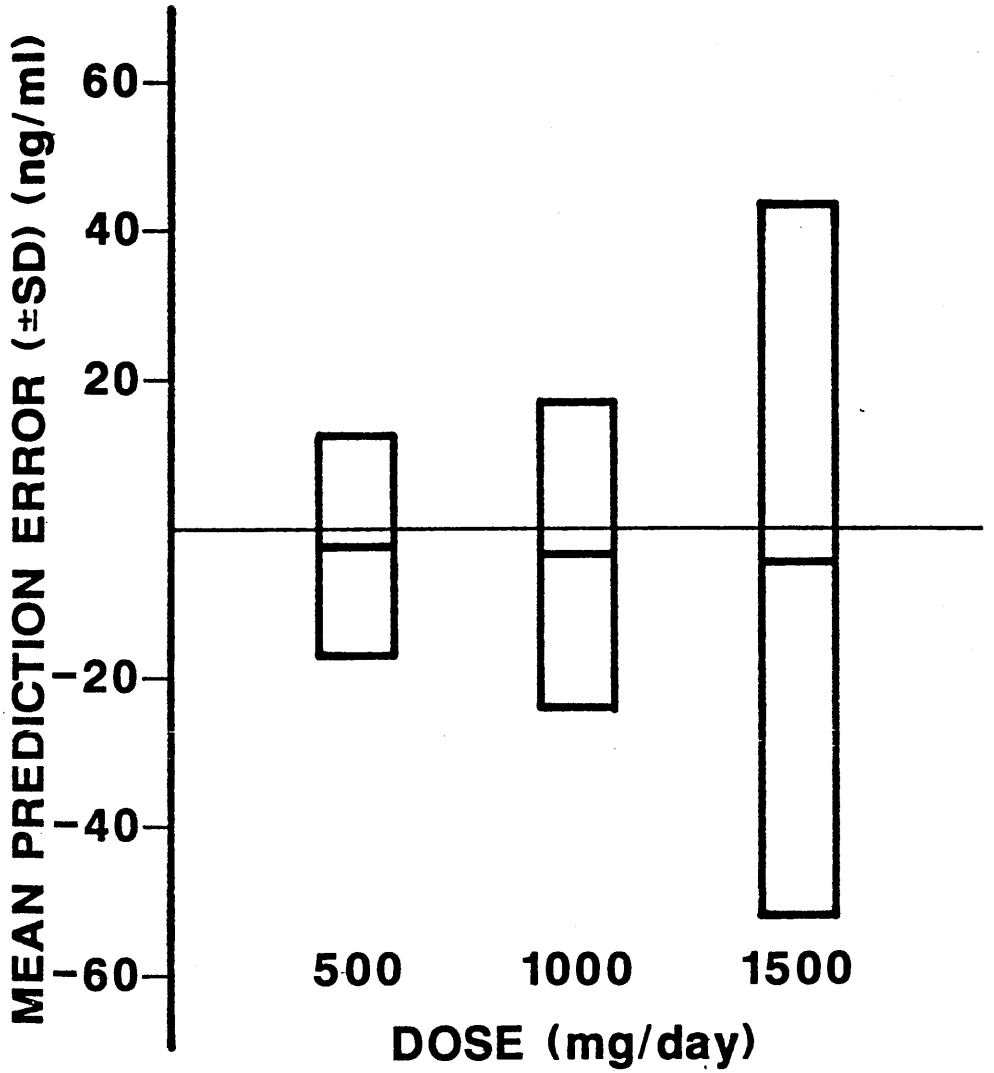

FIGURE 6.8 Mean error (±SD) in the prediction of free naproxen trough concentration at steady state (error=predicted-observed)

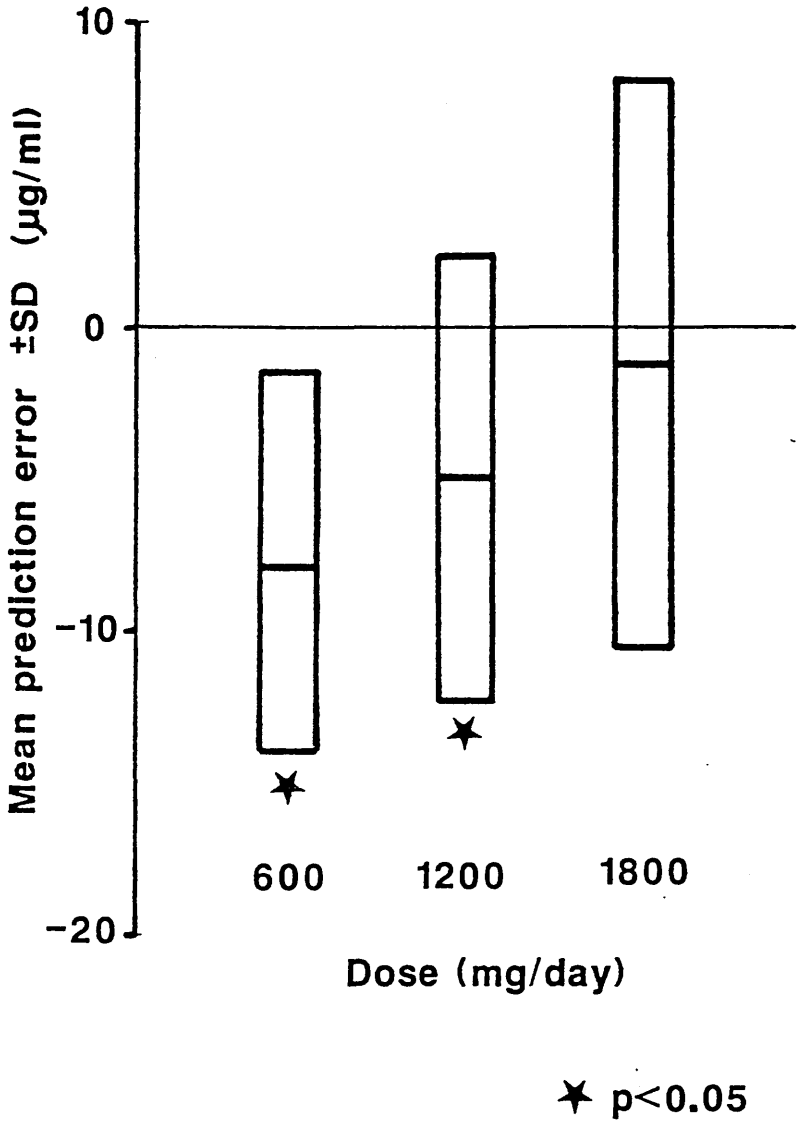

FIGURE 6.9 Mean error (±SD) in the prediction of total naproxen trough concentration at steady state

(viii) DMN concentrations in plasma and binding to plasma proteins

There was indirect evidence that DMN does not bind significantly to albumin in the presence of the parent drug. DMN could be measured easily in buffer after dialysis of steady state trough samples. The concentration of DMN in buffer was of the same order as the concentration of naproxen. DMN was rarely detected in trough plasma samples at steady state, even on the 1500mg dose. In one patient, however, DMN was detected at concentrations of about 0.15µg/ml (limit of detection 0.08µg/ml). The concentration of DMN in buffer after dialysis was 0.02-0.08µg/ml. Thus if DMN is not bound to plasma protein the original concentration in plasma was 0.04-0.16µg/ml. However if this metabolite did compete with naproxen for binding to albumin, the free fraction would have been higher in steady state plasma samples than in the initial spiked plasma samples.

6.3.2 Dose and concentration-response relationships

There were no significant time or treatment order effects. However, one patient appeared to improve dramatically throughout the 6 weeks of the study (Patient 4). The mean (SD) total concentration of naproxen in samples taken at the end of each washout period was 6.1(2.5), 7.4(2.8) and 7.3(3.4) µg/ml after 500, 1000 and 1500mg respectively. As with the fenclofenac study the assessments carried out at this time could not be included in the analysis to account for any week to week variability in

individual disease activity.

The response data, plotted in terms of dose for each response measurement are given in Figure 6.10. Friedman two way analysis of variance indicated a significant improvement in all clinical effects on 1000 & 1500mg/day when compared to the initial washout period (Table 6.9). On average there was an improvement in symptoms from 500 to 1000mg/day, but there was virtually no further improvement on increasing the dose to 1500mg/day. Grip strength showed significant improvement on increasing the dose from 500 to 1500mg/day. In addition, there was no difference between the summed efficacy score on any of the three doses. Only 1000mg and 1500mg produced an effect which was significantly different from the baseline.

(i) GLIM analysis

The dose, total and free concentration-response data were fitted to the three possible linear models using GLIM, with and without baseline measurements included. The most appropriate model was chosen on the basis of the F value (Chapter 4). Table 6.10 gives the results for the total concentration-response data (without baseline measurements) fitted to the linear models. As with the fenclofenac data the subject effect accounted for a large percentage of the total sum of squares, in particular for grip strength and the Ritchie Articular Index. The final parameter values for data fitted to the reduced model (Model 2, Equation 4.22) are given in Table 6.11 (with baseline measurements) and 6.12 (without baseline measurements).

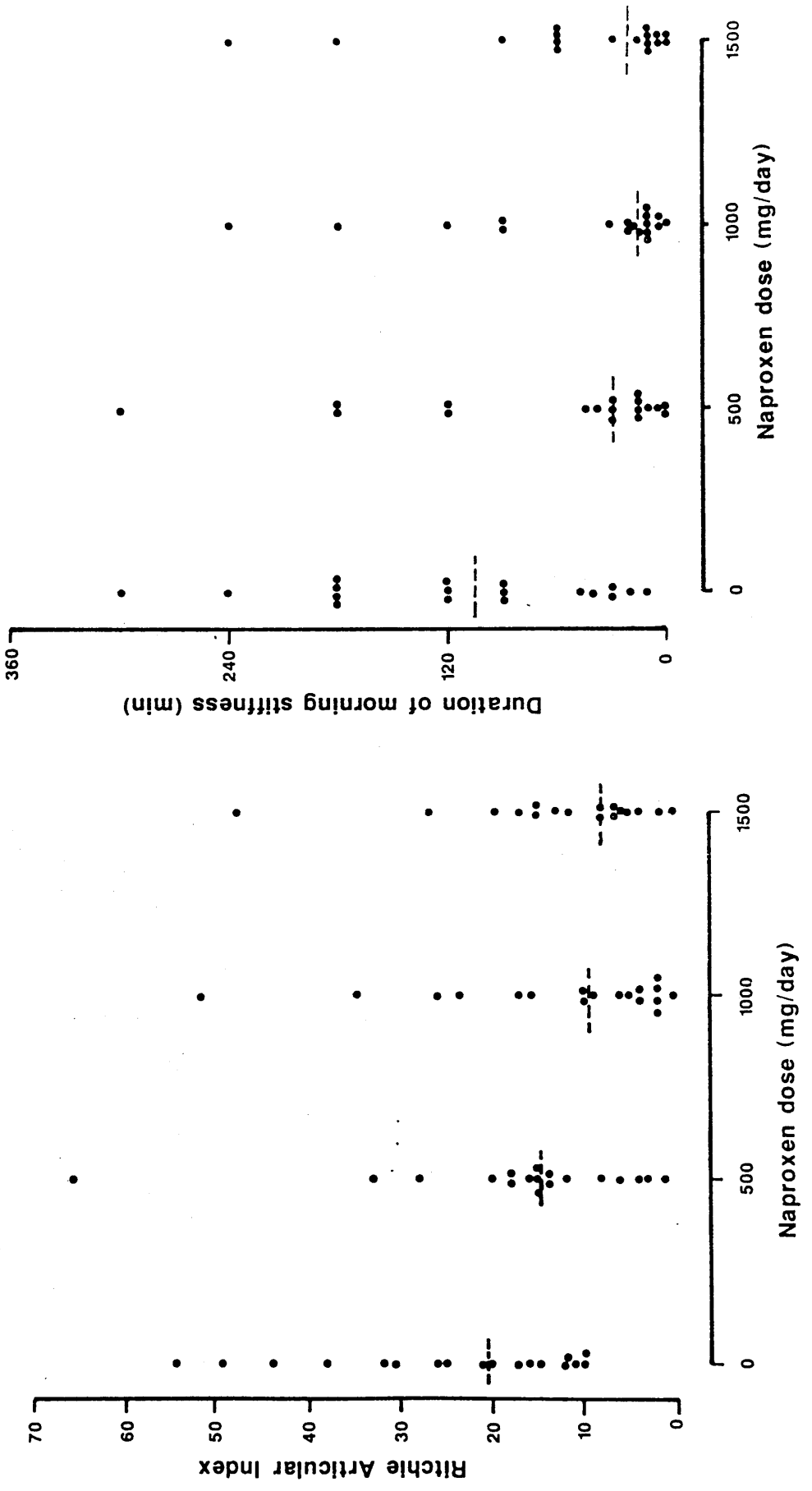

FIGURE 6.10 Summary of clinical measures after the initial wash-out period and after 12 days treatment with each dose of naproxen

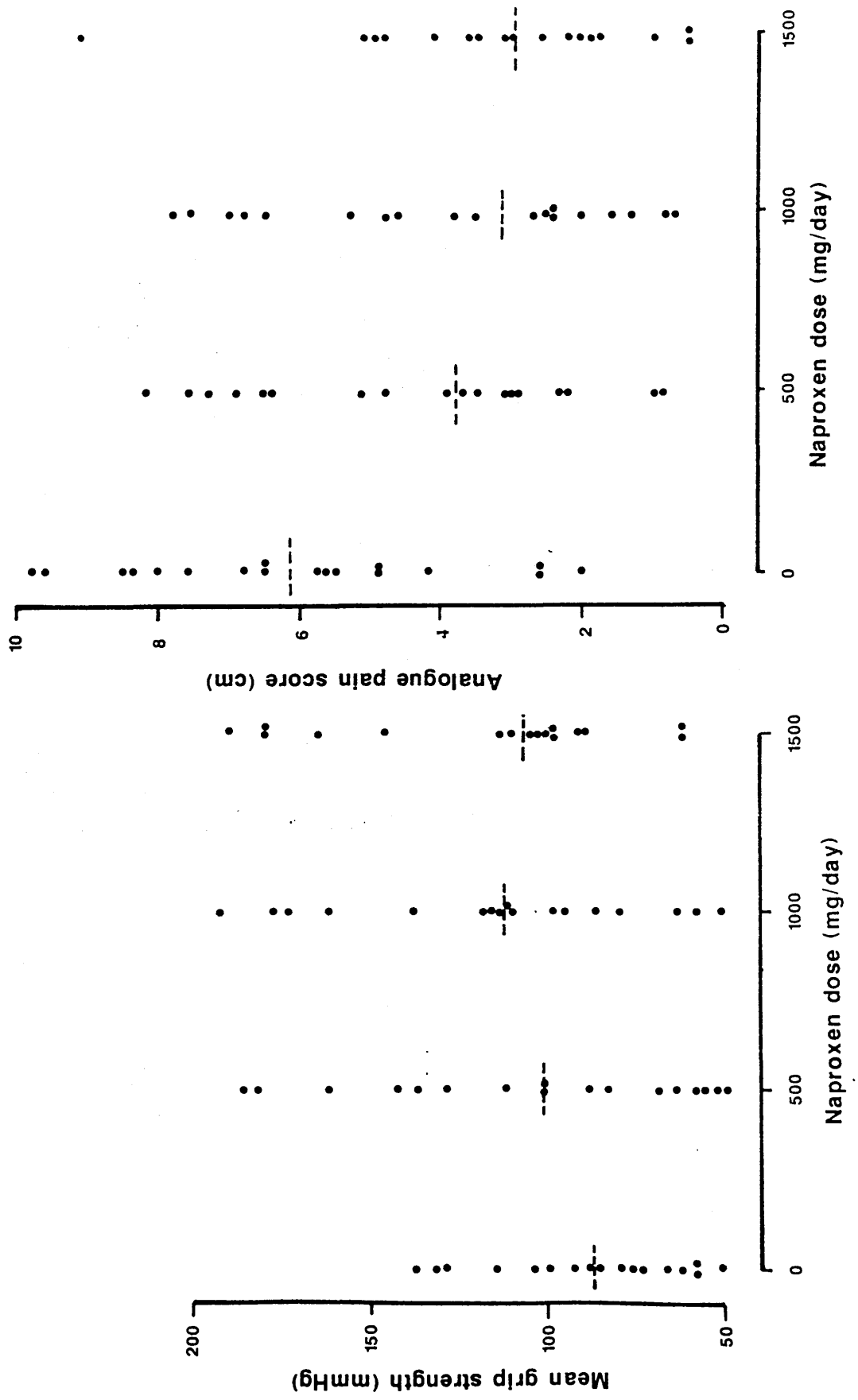

FIGURE 6.10 Summary of clinical measures after the initial wash-out period and after 12 days treatment with each dose of naproxen

TABLE 6.9 Summary of clinical response with increments in naproxen dose. The data are expressed as medians and the range is given in parenthesis.

Clinical effect	Dose (mg/day) baseline	500	1000	1500	Friedman two way analysis of variance	
Ritchie Articular Index	20.5 (10-55)	15.0 (1-66)	7.5 (0-52)	10.0 (0-48)	500<baseline 1000,1500<baseline	p<0.05 p<0.01
Duration of morning stiffness (hr)	105 (10-300)	30 (0-300)	15 (0-240)	15 (0-240)	1000<baseline 1500<baseline	p<0.05 p<0.01
Mean grip strength (mmHg)	85 (58-137)	101.5 (52-186)	112 (48-193)	104 (60-190)	1500>500 1000,1500>baseline	p<0.05 p<0.01
Analogue pain score (cm)	6.1 (2.0-9.8)	3.8 (0.9-7.7)	3.1 (0.7-7.8)	2.9 (0.5-9.1)	1000<baseline 1500<baseline	p<0.05 p<0.01

TABLE 6.10 Comparison of different linear models to describe naproxen total concentration-response data (baseline data omitted)

RITCHIE ARTICULAR INDEX

Linear model	SSQres	df_1, df_2	F value	p value	C_{det}
Total SS	9278				
Model 1	533				Ø.943
Model 2	1451	17,17 (1)	1.72	NS	Ø.844*
Model 3	1653	1,34 (2)	4.73	<Ø.Ø5	Ø.822

DURATION OF MORNING STIFFNESS

Linear model	SSQres	df_1, df_2	F value	p value	C_{det}
Total SS	272400				
Model 1	18280				Ø.933
Model 2	67950	17,17 (1)	2.72	NS	Ø.750
Model 3	70030	1,34 (2)	1.Ø4	NS	Ø.743*

Linear models:

1. $Effect_i = a_i + b_i.C$
2. $Effect_i = a_i + B.C$
3. $Effect_i = a_i$

Model for comparison is given in parenthesis

* denotes the most appropriate model

TABLE 6.10 Comparison of different linear models to describe naproxen total concentration-response data (baseline data omitted)

MEAN GRIP STRENGTH

Linear model	SSQres	df_1, df_2	F value	p value	C_{det}
Total SS	9159Ø				
Model 1	144Ø				Ø.984
Model 2	463Ø	17,17 (1)	2.22	NS	Ø.949*
Model 3	5923	1,34 (2)	9.5Ø	p<Ø.Ø1	Ø.935

ANALOGUE PAIN SCORE

Linear model	SSQres	df_1, df_2	F value	p value	C_{det}
Total SS	26Ø				
Model 1	52				Ø.8ØØ
Model 2	1Ø7	17,17 (1)	1.Ø2	NS	Ø.588*
Model 3	128	1,34 (2)	6.95	<Ø.Ø1	Ø.5Ø8

Linear models:

1. $Effect_i = a_i + b_i.C$

2. $Effect_i = a_i + B.C$

3. $Effect_i = a_i$

Model for comparison is given in parenthesis

* denotes the most appropriate model

When baseline measurements were included (Table 6.11), the slope of improvement was significant for all response measurements when compared to the simplest model (Model 3). In general, the reduced model described the clinical effect measurements in terms of dose or free concentration best, but for analogue pain score, the full model (Model 1) was more appropriate. In terms of total concentration, the full model was significantly better for analogue pain score and grip strength. The coefficient of determination was always higher for total concentration than for dose or free concentration.

Without the baseline measurements (Table 6.12) for all response measures, Model 1 had to be rejected in favour of the reduced (constant slope) model (Model 2). As with the fenclofenac data, this analysis resulted in a flattening of the slope of improvement for response measurements in terms of dose or free concentration, in some cases the slope was no longer significantly different from zero. The slope was significant only for the improvement in grip strength and pain score in terms of dose. In terms of free concentration only the reduction in pain score was significant. The results of this analysis for total concentration were, however, very similar to those obtained with baseline data included. The slope was significant for all responses except the duration of morning stiffness. The analysis of clinical effect in terms of total naproxen concentration always gave a higher value of the coefficient of determination than dose or free concentration.

Articular index, grip strength and analogue pain score

TABLE 6.11 Summary of the results for naproxen dose and concentration - response data fitted to Model 2 using GLIM (baseline measurements included)

Clinical response measurement	DOSE Slope(SE) $x10^{-3}$ (units/mg/day)	DOSE Median intercept (range)	DOSE C_{det}	TOTAL CONCENTRATION Slope(SE) $x10^{-1}$ (units/µg/ml)	TOTAL CONCENTRATION Median intercept (range)	TOTAL CONCENTRATION C_{det}	FREE CONCENTRATION Slope(SE) $x10^{-2}$ (units/ng/ml)	FREE CONCENTRATION Median intercept (range)	FREE CONCENTRATION C_{det}
Ritchie Articular Index	-7.74 (1.61)	20 (10-61)	0.796	-2.25 (0.39)	21 (12-64)	0.821	-8.60 (2.15)	18 (9-64)	0.775
Duration of morning stiffness (min)	-42.8 (11.5)	82 (45-272)	0.657	-13.2 (2.79)	90 (58-248)	0.696	-50.4 (14.9)	72 (29-270)	0.644
Mean grip strength (mmHg)	19.8 (3.35)	90 (43-160)	0.888	5.67 (0.80)	84 (37-156)	0.906	22.4 (4.6)	92 (44-162)	0.873
Analogue pain score (cm)	-1.96 (0.34)	6.0 (4.3-9.1)	0.738	-0.54 (0.11)	6.2 (4.6-9.4)	0.768	-2.24 (0.58)	5.6 (3.5-9.2)	0.751

SE is the standard error in the estimate of the slope

All slopes are significant

TABLE 6.12 Summary of the results for naproxen dose and concentration - response data fitted to Model 2 using GLIM (baseline data excluded)

Clinical response measurement	DOSE Slope (SE) $x10^{-3}$ (units/mg/day)	DOSE Median intercept (range)	DOSE C_{det}	TOTAL CONCENTRATION Slope (SE) $x10^{-1}$ (units/µg/ml)	TOTAL CONCENTRATION Median intercept (range)	TOTAL CONCENTRATION C_{det}	FREE CONCENTRATION Slope (SE) $x10^{-2}$ (units/ng/ml)	FREE CONCENTRATION Median intercept (range)	FREE CONCENTRATION C_{det}
Ritchie Articular Index	-3.77 (2.28)	15 (6-59)	0.831	-2.25* (1.04)	20 (12-67)	0.844	-3.88 (2.82)	13 (5-60)	0.835
Duration of morning stiffness (min)	-14.2 (15.3)	45 (18-274)	0.749	-7.23 (7.08)	70 (35-292)	0.751	-16.2 (18.1)	50 (10-273)	0.748
Mean grip strength (mmHg)	10.0* (4.15)	95 (40-197)	0.945	5.70* (1.85)	82 (30-164)	0.949	8.56 (5.89)	104 (46-183)	0.940
Analogue pain score (cm)	-1.32* (0.62)	4.8 (2.9-8.9)	0.565	-0.74* (0.28)	7.5 (4.1-10)	0.591	-1.70* (0.75)	4.8 (2.0-9.0)	0.571

SE is the standard error in the estimate of the slope

* the slope is significantly different from zero ($p<0.05$)

are plotted against total concentration with the regression line indicated in Figure 6.11. These graphs illustrate the considerable inter and intra-subject variability in the data. On average the GLIM analysis indicated small reductions in the analogue pain score and the Ritchie Articular Index of 0.94cm and 3 respectively on increasing the dose from 500 to 1000mg/day assuming the average total trough concentration. The reduction is even smaller on increasing the dose from 1000 to 1500mg/day.

A higher coefficient of determination was obtained by fitting the response data in terms of log dose or log free concentration. Comparison of the residual sum of squares for each response index fitted to Models 1 and 2 in terms of dose, log dose, total and free concentration and log free concentration are shown in Table 6.13. With data fitted to Model 2, there is little difference between the SSQres for total concentration and log free concentration. The increase in the SSQres by removing the individual intercept parameter, was in general greater for log dose than for total concentration or log free concentration.

(ii) NONMEM analysis

As the response data appeared to plateau with increasing dose, a hyperbolic or E_{max} model was investigated using NONMEM (baseline data were included). For comparison the data were also fitted to a linear model. The objective value for the response data fitted in terms of dose, total or free concentration are given in Table 6.14. It is clear that E_{max} model did not offer any improvement over the

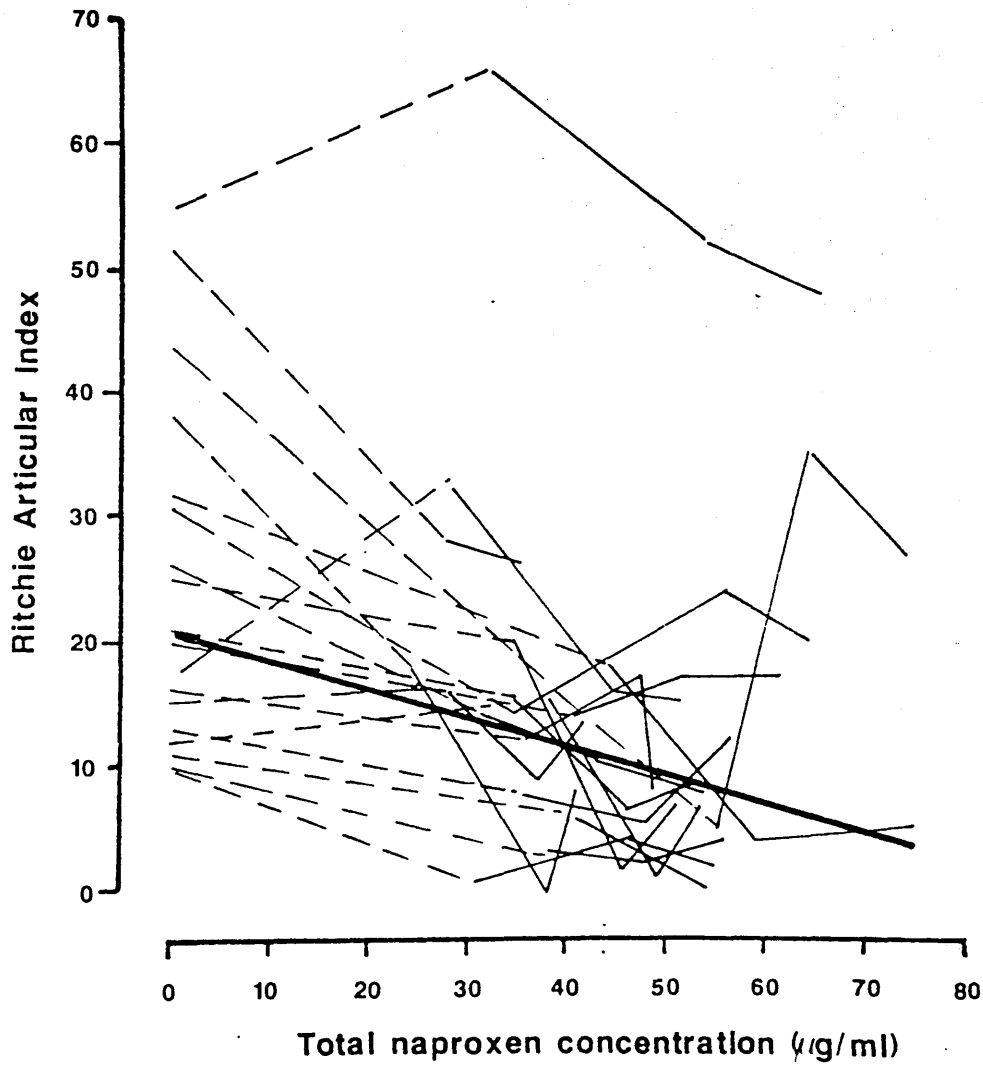

FIGURE 6.11 Individual response plotted against total naproxen concentration. The bold continuous line indicates the average slope of improvement

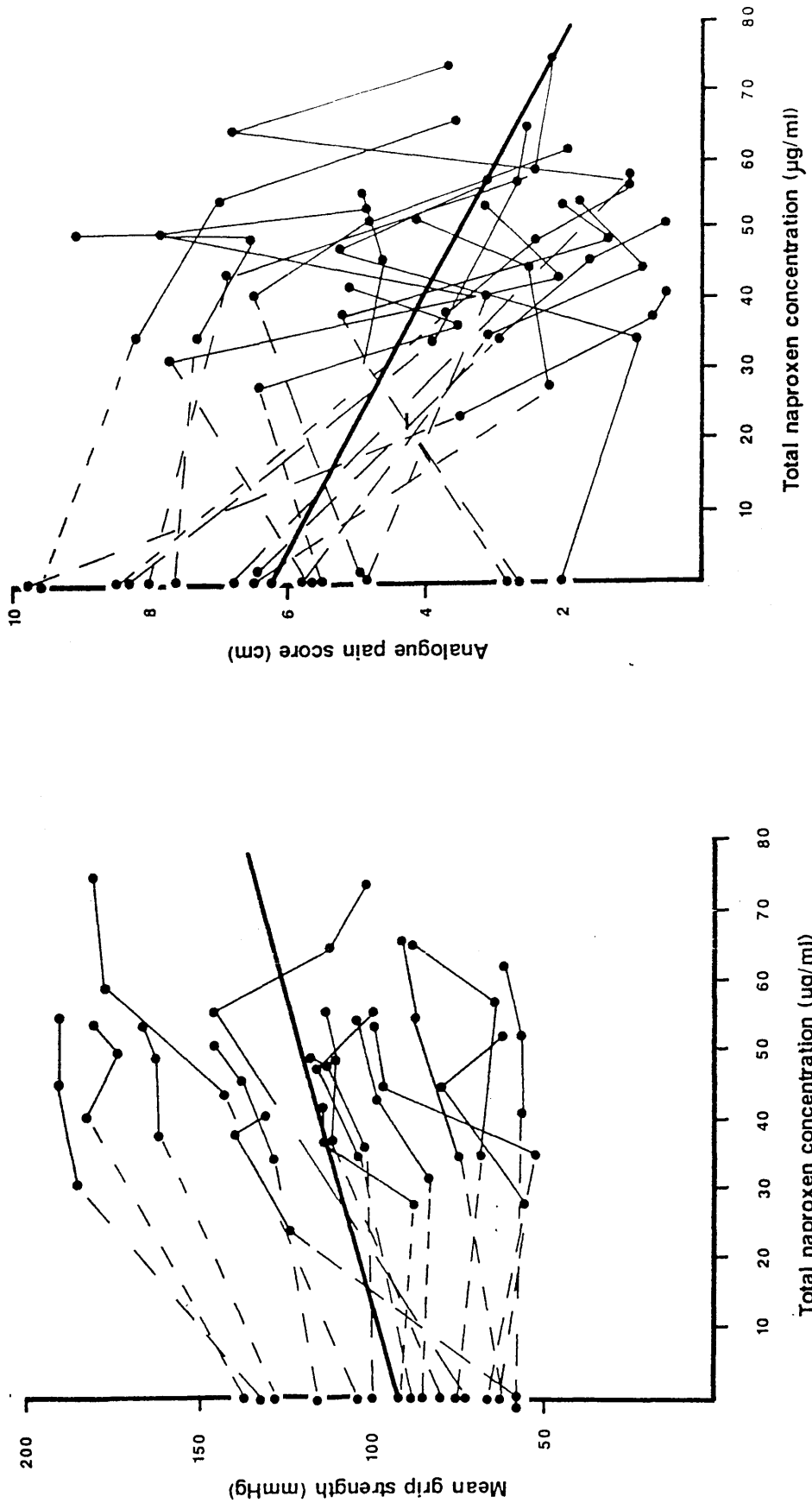

FIGURE 6.11 Individual response plotted against total naproxen concentration. The bold continuous line indicates the average slope of improvement

TABLE 6.13 Comparison of the residual sum of squares for dose and concentration - response data fitted linear models 1 and 2 using GLIM

Model	DOSE	LOG DOSE	TOTAL	FREE	LOG FREE
Ritchie Articular Index:					
Model 1	701	500	533	768	650
Model 2	1530	1502	1451	1565	1409
Morning stiffness:					
Model 1	19750	19060	18280	23010	23700
Model 2	68290	67970	67950	68510	66390
Mean grip strength:					
Model 1	1631	1217	1440	1908	1637
Model 2	5055	4911	4630	5499	4822
Analogue pain score:					
Model 1	55	46	53	52	48
Model 2	113	113	107	112	109

Linear models:

1. $Effect_i = a_i + b_i.C$

2. $Effect_i = a_i + B.C$

TABLE 6.14 Comparison of naproxen dose and concentration - response data fitted to a linear and E_{max} model using NONMEM

	Objective value		
	Linear	E_{max}	D
a) ARTICULAR INDEX			
dose	397	391	6
total	392	390	2
free	406	390	16
b) MORNING STIFFNESS			
dose	663	657	6
total	656	654	2
free	666	657	9
c) GRIP STRENGTH			
dose	514	492	22
total	499	515	-16
free	521	495	26
d) PAIN SCORE			
dose	180	178	2
total	180	177	3
free	191	182	9

D is the difference in the objective value for data fitted to the linear and E_{max} models

linear model for total concentration.

The E_{max} model appeared to be more appropriate for the articular index and grip strength in terms of free concentration and for grip strength in terms of dose. However, although the objective value and examination of residual plots indicated an improvement in the fit of the data in these cases with the E_{max} model, the parameters were in general more poorly defined than the parameters of the linear model, especially the estimate of $EC_{50\%}$. However, the residual error (ϵ) was smaller for all responses (except the pain score) in terms of dose or free concentration with the E_{max} model.

The parameter values for the responses analysed in terms of dose are given in Table 6.15. The value of $EC_{50\%}$ was approximately 200-300mg/day. E_{max} was -14 (6), -78 (31) minutes, 37 (9)mmHg and -4.9 (2.7)cm for articular index, morning stiffness, grip strength and pain score respectively.

The results for the analysis of the response data in terms of free concentration are given in Table 6.16. The estimate of $EC_{50\%}$ was in general around 20ng/ml (the average free concentration of 500mg/day was 34ng/ml). E_{max} was similar to that for dose.

6.3.3 Side-effects, biochemistry and haematology

Few side effects were reported during the study. All were minor and did not require any change in treatment. One patient complained of constipation on all three doses and

TABLE 6.15 NONMEM parameter estimates (SE) for naproxen dose - response data fitted to the E_{max} model

Parameter	Articular index	Morning stiffness (min)	Grip strength (mmHg)	Analogue pain score (cm)
E_{max} (units)	-14 (6)	-78 (31)	37 (9)	-4.9 (2.7)
var	ID	0.85(0.38)	0.35(0.19)	0.18(0.51)
$EC_{50\%}$ (mg)	273 (478)	244 (324)	207 (284)	1020 (1180)
var	3.3(11.3)	4.6(12)	10(20)	ID
E_{0} (units)	25 (3)	115 (19)	86 (6)	6.1 (0.5)
var	0.20(0.09)	0.19(0.11)	0.09(0.02)	0.012(0.020)
ϵ	42(15)	2200(951)	78(29)	3.6(0.7)

Key: var = the variance parameter
ϵ = the residual unexplained error

$\sqrt{\text{var}}$ x100 gives the coefficient of variation of the structural model parameter

$\sqrt{\epsilon}$ is an additive error

TABLE 6.16 NONMEM parameter estimates (SE) for naproxen free concentration - response data fitted to the E_{max} model

Parameter	Articular index	Morning stiffness (min)	Grip strength (mmHg)	Analogue pain score (cm)
E_{max} (units)	-15 (3)	-83 (42)	40 (7)	-2.5 (1.0)
var	ID	0.30(0.46)	0.10(0.20)	0.20(0.22)
$EC_{50\%}$ (ng/ml)	22 (8)	20 (29)	24 (21)	3 (10)
var	4.8(5.5)	ID	5.3 (4.4)	ID
E_{0} (units)	22 (3)	114 (18)	87 (6)	6.1 (0.5)
var	0.23(0.10)	0.22(0.12)	0.10(0.02)	0.007(0.020)
ϵ	37(16)	2170(943)	118(39)	3.9(0.8)

Key: var = the variance parameter
ϵ = the residual unexplained error

$\sqrt{\text{var}}$ x100 gives the coefficient of variation of the structural model parameter

$\sqrt{\epsilon}$ is an additive error

also complained of insomnia on the 1000mg and 1500mg doses (patient 2). The other side effects reported were nausea on 500mg (patient 18) and 1000mg (patient 4 and 18) and lightheadedness on 1500mg (patient 11). There did not appear to be any relationship between side effects and dose, total or free naproxen concentration. There were no changes in any biochemical or haematological measurements on any dose.

6.4 DISCUSSION

The non-linear relationship between the dose and plasma total naproxen concentration agrees with the results of other studies of the kinetics of naproxen (Runkel et al, 1974 & 1976). The associated linear increase in the free concentration indicates that the intrinsic clearance of naproxen remained constant over the dose range. According to the physiological model proposed by Wilkinson & Shand (1975), the nonlinearity can be attributed to the concentration dependent plasma protein binding. Saturation of the high affinity binding site occured with total concentrations of above approximately 70µg/ml. The clearance of total naproxen is not constant but increases with increasing total concentration. This phenomenon occurs if the elimination of a drug is restricted to the free fraction. The clearance of total naproxen was considerably less then liver blood flow (0.58 l/h) at the free fractions encountered after therapeutic doses.

The NONMEM protein binding parameter estimates can be

compared with those previously quoted after converting to molar concentrations and taking the reciprocal of the dissociation constant. The values for n, nP and K (the association constant) from published data and from the NONMEM analysis for patients in this study are given in Table 6.17, together with the binding parameters determined in a single healthy individual. It is obvious that the mean NONMEM binding parameters are not entirely consistent with the results of others for the binding of naproxen in plasma. This is not entirely unexpected for the reasons discussed in Chapter 4. The binding affinity for isolated HSA (40g/l) was considerably higher than the binding to human plasma. Although in this study naproxen binding in patients with rheumatoid arthritis was similar to that in one healthy volunteer, the affinity for the primary binding site was higher in the healthy individual. Naproxen was bound with much higher affinity than fenclofenac (3.29 compared with $0.54\mu M^{-1}$) to the primary binding site but the affinity for the secondary binding sites was lower than for fenclofenac (0.034 compared to $0.135\mu M^{-1}$).

The variability in total naproxen trough concentrations was quite small compared to free drug concentration. Total concentration is dependent on the individual clearance of free naproxen and plasma protein binding. The much smaller variability in total concentrations suggests that plasma protein binding masks some of the variability in the clearance of naproxen. The variability in free drug concentrations reflects the inter-individual differences in

TABLE 6.17 Naproxen protein binding constants

	n_1	nP_1 (μM)	K_1 (μM^{-1})	n_2	nP_2 (μM)	K_2 (μM^{-1})	Reference
Plasma	-	419	Ø.9Ø	-	3322	Ø.Ø16	Runkel et al, 1976
BSA	1.2	-	Ø.78	6.7	-	Ø.ØØ2	Kaneo et al, 1981
HSA	1.2-1.7	-	48-81	4.4-6.Ø	-	6.7-1Ø	Calvo & Dominguez-Gill, 1983
Plasma	Ø.55	324	3.29	3.5	21Ø4	Ø.Ø34	patients with rheumatoid arthritis (NONMEM analysis)
Plasma	Ø.64	388	8.49	4.4	27Ø3	Ø.Ø37	healthy individual

Key: K is the equilibrium association constant
BSA is bovine serum albumin
HSA is human serum albumin

the intrinsic clearance of naproxen. Free drug clearance was slightly less than that for a group of male healthy volunteers (Upton et al, 1984).

A number of factors appear to affect the elimination of naproxen. In this study, the significance of each factor could not be assessed due to the small number of patients. However the observed reduced clearance of free naproxen in older patients agrees with two previous studies (Upton et al, 1984 & McVerry et al, 1986). In healthy volunteers, a negligible fraction of naproxen is excreted unchanged in the urine (Upton et al, 1980b). In the elderly, impaired renal function may lead to the accumulation of naproxen glucuronide. This metabolite is readily hydrolysed in vivo, liberating the parent drug. This phenomen may in part explain the reduced clearance of free naproxen observed in elderly patients (Upton et al, 1980b) and may also be important in patients with renal failure. However, the following observations may indicate that other factors may be important:

1. The clearance of free naproxen tended to be lower in female patients. This could not be explained in terms of weight because there was no difference in weight between males and females.

2. Cimetidine, which is an hepatic enzyme inhibitor, may also influence the elimination of naproxen; two patients receiving this drug achieved the highest free concentrations and clearance of free drug was low. Both patients, however, were female. This may be worth further investigation as patients receiving NSAIDs are often prescribed H_2-antagonists

for prophylaxis or treatment of dyspepsia and peptic ulceration. Previously, cimetidine was reported to have no effect on the kinetics of naproxen, but only total drug concentrations were considered (Holford et al, 1981). Plasma concentrations of indomethacin, on the other hand, were lower when given in combination with cimetidine (Howes et al, 1983). In this case, the absorption of indomethacin appeared to have been affected.

3. Free concentrations in smokers tended to be lower and the clearance of free drug tended to be high. Most of the smokers, however, were male. It is not possible to distinguish the effect of smoking from sex related differences or the effect of age.

Free naproxen concentrations were generated over the range of total concentrations encountered in this study using the NONMEM binding parameters assuming an average albumin concentration of 40g/l. The generated free fractions are shown in Figure 6.12 together with the actual free fractions measured at steady state. It is clear that the binding in control plasma spiked with naproxen is not consistent with the plasma protein binding *in vivo*. The reasons for this are unclear but it is possible that it is due to differences in the patients' clinical state: during a 'flare' (without treatment) and during active treatment. There are a number endogenous and exogenous factors which might be responsible including total protein or albumin concentration, free fatty acids, bilirubin and other drugs, especially NSAIDs.

1. If the **albumin** concentration was lower after

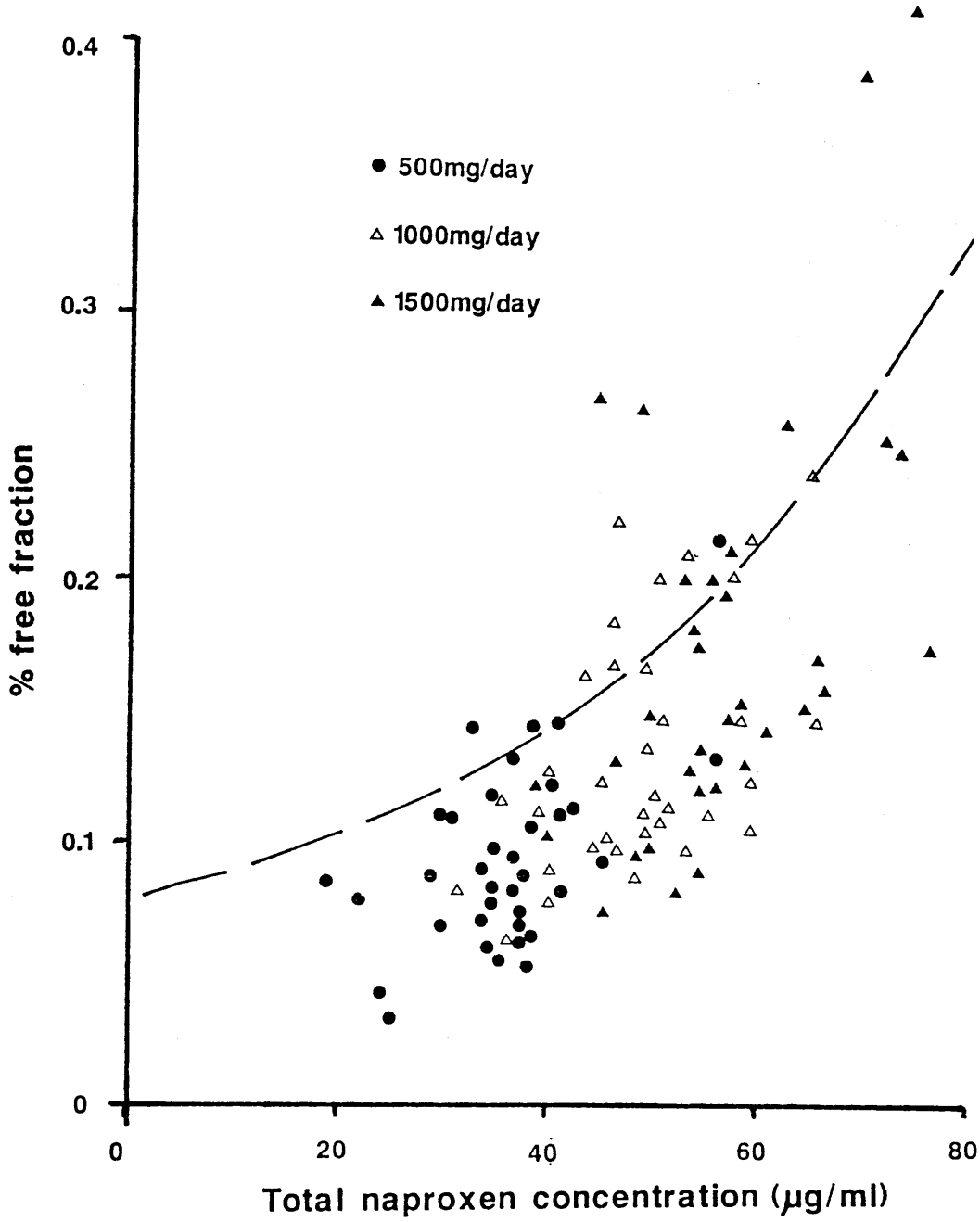

FIGURE 6.12 The free fraction of naproxen over the concentration range encountered using the NONMEM binding parameters (assuming an average albumin concentration of 40g/l) together with the actual free fractions in samples taken at steady state

withdrawal of therapy, the free fraction would be higher. However, there was no change in the albumin concentration between the end of the initial wash-out period and on any of the three doses.

2. If **free fatty acids** were raised due to the withdrawal of anti-inflammatory therapy, this might result in higher free fractions. Free fatty acids in plasma are in general bound to albumin in large amounts and with higher affinity than most drugs (Ashbrook et al, 1975). At a molar ratio of 4 (palmitic acid to albumin) there was a 50% reduction in the affinity constant and number of binding sites for the high affinity site resulting in higher free fractions of naproxen (Calvo & Dominguez-Gil, 1983). Under normal conditions free fatty acid concentrations have been found to fluctuate, the fatty acid to albumin ratio ranging from 0.5 to 1.5 (Court, Dunlop & Leonard, 1971). A study of the binding of valproic acid indicated that palmitic acid reduced the affinity constant but not the number of binding sites (Monks & Richens, 1979). Free fatty acid concentrations, however, could not be measured.

3. **Prostaglandins** (PGH_2 and TXA_2) have been found to bind covalently to human plasma albumin (Maclouf et al, 1980). Prostaglandin synthesis was inhibited during treatment periods but not during the wash-out period.

4. **Bilirubin** has been implicated as another factor which will compete with naproxen for binding to plasma albumin (Held, 1980). However, in this study there was no difference in bilirubin concentrations at the end of the

initial wash-out period compared to the treatment periods.

5. Other **NSAIDs** can interfere with the binding of naproxen (Kaneo et al, 1981). At a molar concentration of $3x10^{-6}M$, flufenamic acid produced the largest increase in the free fraction of naproxen in a solution of bovine serum albumin, followed by flurbiprofen, indomethacin and phenylbutazone. Aspirin at this concentration had no effect (Kaneo et al, 1981). However, when aspirin and naproxen were given together at therapeutic doses, there was an increase in the clearance of naproxen which was related to displacement of naproxen from binding sites (Segre et al, 1974). Since patients were withdrawn from previous therapy for at least 3 days it unlikely that sufficient concentrations of the previous NSAID would have been present to cause any significant alteration in the binding of naproxen. Patients were allowed to take paracetamol during wash-out periods, however, paracetamol is not bound to any significant extent in plasma (Gazzard et al, 1973).

It is interesting to note that the binding determined in one healthy individual gave better predictions of free concentrations in patients at steady state. The binding parameters were similar to the mean binding parameters determined from the NONMEM analysis of the 18 patients, except for the affinity constant for the high affinity site (Table 6.17). This requires further investigation: it would have been helpful, to have determined the binding of naproxen in age matched controls. This result taken alone, however, suggest thats an endogenous substance which

interferes specifically with the binding of naproxen to the high affinity site was present in higher concentrations when patients were withdrawn from therapy.

It was not clear whether the binding of fenclofenac to plasma proteins was different in plasma after withdrawal of therapy or during treatment. From Figure 5.9 there is a suggestion the free fraction was higher in control plasma than in trough samples at steady state. However, the mean binding parameters were only determined in control plasma from 5 out of the 18 patients.

Despite the quantitative difference in the binding of naproxen, free concentration-time profiles gave unbiased predictions of free concentrations at steady state. Free concentration, however, was not predicted with any degree of precision. The reasons for this are unclear: it is possible again that the binding parameters did not give a good description of the actual free concentrations during the single dose study.

On average the difference in response on the three doses of naproxen was small and comparable with a previous study of naproxen where 250, 750 and 1500mg/day were given to patients with rheumatoid arthritis (Day et al, 1982). Their results indicated that the average difference between the pre-study flare and 250mg/day was much greater than the difference between 250 and 1500mg/day. Similarily in this study the greatest difference in response was between no treatment and 500mg/day.

Since there was considerable inter-subject variability

in the data, a linear modelling approach was used which allowed for individual disease severity. Analysis with baseline data included indicated significant improvements with increments in dose, total or free concentration. However since the largest difference in the clinical response measurements occured between 'no treatment' and the lowest dose, it is certain that the linear relationship between dose or concentration and response is weighted by this baseline observation. Analysis of response data without baseline data indicated that this was indeed the case for dose and free concentration (for all responses except the analogue pain score) but there was still a significant linear improvement in 3 out of 4 response measurements with increments in concentration. However, with only three data points per individual and considerable intra-subject variability, the full model (which includes an individual slope for each patient) which was probably more realistic had to be rejected.

The significance of the linear relationship between clinical response and total concentration should be viewed in the light of the non-linear relationship between naproxen dose and total concentration. A non-linear relationship between dose and concentration appears to parallel a non-linear relationship between dose (or free concentration) and clinical response. As an example, a patient with a grip strength of 90mmHg and an analogue pain score of 7.2cm before treatment can expect on average to achieve an improved grip of 118mmHg and a reduction in the pain score

to 3.6cm with a trough total naproxen concentration of 49µg/ml (mean trough on 1000mg/day). Succesive increments in dose from 500 to 1000mg/day and 1000 to 1500mg/day produced on average 34.5% and 14.6% increases in the mean trough concentration respectively. With a linear relationship between the total naproxen concentration and clinical effect in this patient, it is obvious that successive increments in dose will lead to less than proportional improvements in response. For example if the patient above was given 1500mg/day, assuming that the trough concentration achieved was 56µg/ml, a grip of 122mmHg and analogue pain score of 3.0cm could be expected, a small improvement over the 1000mg/day dose.

In order to compare the analysis for total concentration with dose and free concentration, the data were also analysed using GLIM in terms of log dose and log free concentration. This analysis indicated that the log of the free concentration gave a slightly better description of the response data than log dose or total concentration.

It is normally assumed that the free concentration in plasma reflects the free concentration at the receptor site. In this situation it may be assumed that the free concentration in plasma at the end of a dosing interval at steady state will be in equilibrium with the free concentration in synovial fluid. In vitro the concentration of naproxen required to give 50% inhibition of prostaglandin E_2 synthesis was 0.25µg/ml in human synovial microsomes

cultures (Robinson et al, 1980). In this study the free concentration of naproxen in in trough plasma samples ranged from 0.009 to 0.25µg/ml. Thus the free concentrations achieved clinically in plasma are close to those necessary to produce significant inhibition of prostaglandin synthesis. However these $EC_{50\%}$'s for the inhibition of prostaglandin synthesis *in vitro* do not compare with the estimate determined in this study (20ng/ml). This is not surprising due to the variability in the response data.

The oxidative metabolite, DMN, although present in plasma in very low concentrations in comparison to total naproxen achieves similar concentrations to free naproxen. Some metabolites are biologically active and contribute sigificantly to the clinical response (Atkinson & Strong, 1977). The evidence in animal models of inflammation, however, suggests that DMN has little pharmacological activity (Syntex, personal communication).

There was a linear increase in free concentration as the dose was increased up to 1500mg/day. It it is possible, however, that as the dose is increased the metabolic capacity of the liver may become saturated and there will be a nonlinear increase in the free concentration. Although this is not evident from the study of large doses of naproxen (Runkel et al, 1976), these investigators only measured total naproxen concentrations. Although there was no significant increases in toxicity with higher free concentrations in the present study, caution should be exercised especially in the elderly with further increments

in dose. All side-effects were reported by female patients which may be a result of the fact that in general, the clearance of free naproxen was lower and free concentrations were higher in the female patients.

Day and colleagues (1982) took a different approach in order to determine whether there was a naproxen concentration-effect relationship. To reduce some of the inter-subject variability in the response measurements, they used a non-parametric ranking method to obtain a 'summed efficacy score', and by arbitrarily defining patients as responders or non-responders for each dose, the authors showed that the proportion of responders increased at higher total naproxen concentrations. However, since there was a dose response relationship, their analysis did not really distinguish a concentration-response relationship from a dose-response relationship. The significance of the improvement with increments in concentration was uncertain. It was interesting to note, however, that while there appeared to be a linear relationship between total naproxen concentration and the percentage responders, the relationship with free concentration appeared to be non-linear, reaching a maximum effect with free concentrations of 0.36µg/ml. They found that 76% of patients with trough concentrations above 50µg/ml had been classed as responders.

In conclusion, the pharmacokinetics of total naproxen are non-linear due to saturation of plasma protein binding: the kinetics of free naproxen are linear. There was considerable variability in the pharmacokinetics of free

naproxen and increasing age was associated with a decrease in the clearance of free naproxen.

There was a linear relationship between clinical response and total concentration as both reach a plateau as the dose increases. The response in terms of dose or free concentration (except the analogue pain score) was described better by an E_{max} model than by a linear model. The parameters, however, were poorly estimated as there were only four data points per individual and the difference in effects between doses or concentrations was very small. Assuming that the free concentration in plasma is in equilibrium with the free concentration at the receptor site, these results suggest that increments in the dose of naproxen over 1000mg/day will lead to only a slight improvement in the clinical response in the majority of patients. Finally, there appears to be little advantage in taking account of inter-individual differences in the pharmacokinetics of naproxen to explain clinical response.

7.1 INTRODUCTION

In the previous two chapters, the variability in the pharmacokinetics of two NSAIDs, fenclofenac and naproxen, were investigated to determine whether the response to NSAIDs is more closely related to plasma concentration than to dose. More information, however, may be obtained by determining drug concentrations closer to their site of action.

In inflammatory joint disease, the NSAID site of action is in the synovium. Although it is not often possible to measure drug 'levels' in the synovium, the synovial fluid does provide an accessible sampling site which may represent the 'levels' in synovial tissue more closely than plasma. Variable patient response to NSAIDs may reflect differences in the levels of free or bound drug achieved in this fluid.

The most effective treatment of 'infective arthritis' may be based on the determination of antibiotic concentrations in synovial fluid. Less attention has been directed at the measurement of synovial fluid concentrations of NSAIDs, and no studies have attempted to correlate clinical response to drug concentrations achieved in this fluid. No relationship was found between indomethacin plasma concentration and clinical response (Ekstrand et al, 1980) and there was no difference in the pharmacokinetics of indomethacin in responders and non-responders (Baber et al, 1979).

This chapter presents a single dose study of a slow

release preparation of indomethacin (Indocid-R) which aimed to:

a) quantitate the pharmacokinetics of indomethacin in plasma and synovial fluid after a single dose of Indocid-R.

b) assess whether concentrations of indomethacin in synovial fluid can be determined from plasma concentrations.

7.2 PATIENTS AND METHODS

7.2.1 Patients

Seven patients with 'definite' or 'classical' rheumatoid arthritis (Ropes et al, 1959) and one patient with osteoarthritis took part in the study. All had knee effusions requiring aspiration. Five were female and three were male. Their ages ranged from 40 to 85 years (median 58). All other patient details are given in Table 7.1. Patients were withdrawn from any previous NSAID therapy for three days prior to the study. Paracetamol was supplied to relieve any pain experienced during this wash-out period.

7.2.2 Indocid-R

This was given as a single Indocid-R capsule, containing 75mg of indomethacin in pellet form. 50mg is formulated in enteric coated pellets designed for gradual release in an alkaline environment and 25mg is contained in uncoated pellets available for immediate release in the acid

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TABLE 7.1. Indocid-R plasma and synovial fluid kinetic study: patient characteristics

Patient	Disease	Sex	Age (yr)	Weight (kg)	Height (cm)	Albumin (g/l) P	Albumin (g/l) SF	ESR (mm/h)
1	RA	F	47	66	158	40	19	10
2	RA	M	78	74	158	33	23	67
3	RA	F	40	51	145	39	24	9
4	RA	F	54	71	163	41	33	33
5	OA	M	61	114	182	41	20	6
6	RA	M	85	60	170	41	23	57
7	RA	F	54	81	158	40	24	-
8	RA	F	62	69	158	40	7	28

Key: P = plasma
SF = synovial fluid

RA = rheumatoid arthritis
OA = osteoarthritis

environment of the stomach.

7.2.3 Study design

At the end of the three day wash-out period patients took a single tablet of Indocid-R with 100ml of water at least 2 hours after a light breakfast. Blood (10ml) was taken from an indwelling intravenous cannula at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 9, 12 and 24 hours and collected into heparinised containers. Samples of synovial fluid (10ml) were obtained by separate joint aspirations carried out under aseptic conditions at 0, 3, 6, 12 and 24 hours after the dose.

Blood and synovial fluid samples were centrifuged at 2000rpm and plasma and cell free synovial fluid were stored at -20°C. Indomethacin concentrations were determined in plasma and synovial fluid by HPLC as outlined in Chapter 2. In addition, a standard biochemical screen was carried out at the start of the study and albumin and total protein concentrations were determined in synovial fluid.

7.2.4 Data analysis

The individual patient plasma and synovial fluid concentration-time data were fitted using weighted non-linear least squares regression analysis (Chapter 4). Concentration was weighted proportional to the reciprocal of the fitted concentration ($1/\hat{c}_i$).

7.3 RESULTS

7.3.1 Plasma pharmacokinetics

The absorption of indomethacin from the Indocid-R preparation was in general rapid after a variable lag time. The mean peak concentration was 3.12μg/ml. There was a rapid distribution phase, followed by a slower elimination phase and the individual data were fitted to a two compartment model with zero order absorption and a time lag (Model 4, Appendix II). Representative plasma profiles are shown in Figure 7.1.

Parameter estimates for individual data fitted to Model 4 are given in Table 7.2. In some cases the parameters were not well defined. If there was a substantial lag time, there were fewer data points to provide information on the distribution and elimination of the drug. The T_{lag} ranged from Ø.24 to 1.45 hours and T ranged from Ø.6 hours to 2.5 hours. The terminal elimination half-life ranged from 3.8 to 9.8 hours and the average estimate of CL/F derived from the parameters was 8.4 l/h and ranged from 3.9 to 9.6 l/h. There was little evidence that there was a sustained release of indomethacin.

There was no correlation between the clearance of indomethacin and age or albumin concentration. There was a possible relationship between clearance and weight, but this was not significant (Ø.592, $p<Ø.122$).

FIGURE 7.1 Representative indomethacin plasma concentration-time profiles after a single dose of Indocid-R

A Patient 2
B Patient 3
C Patient 4

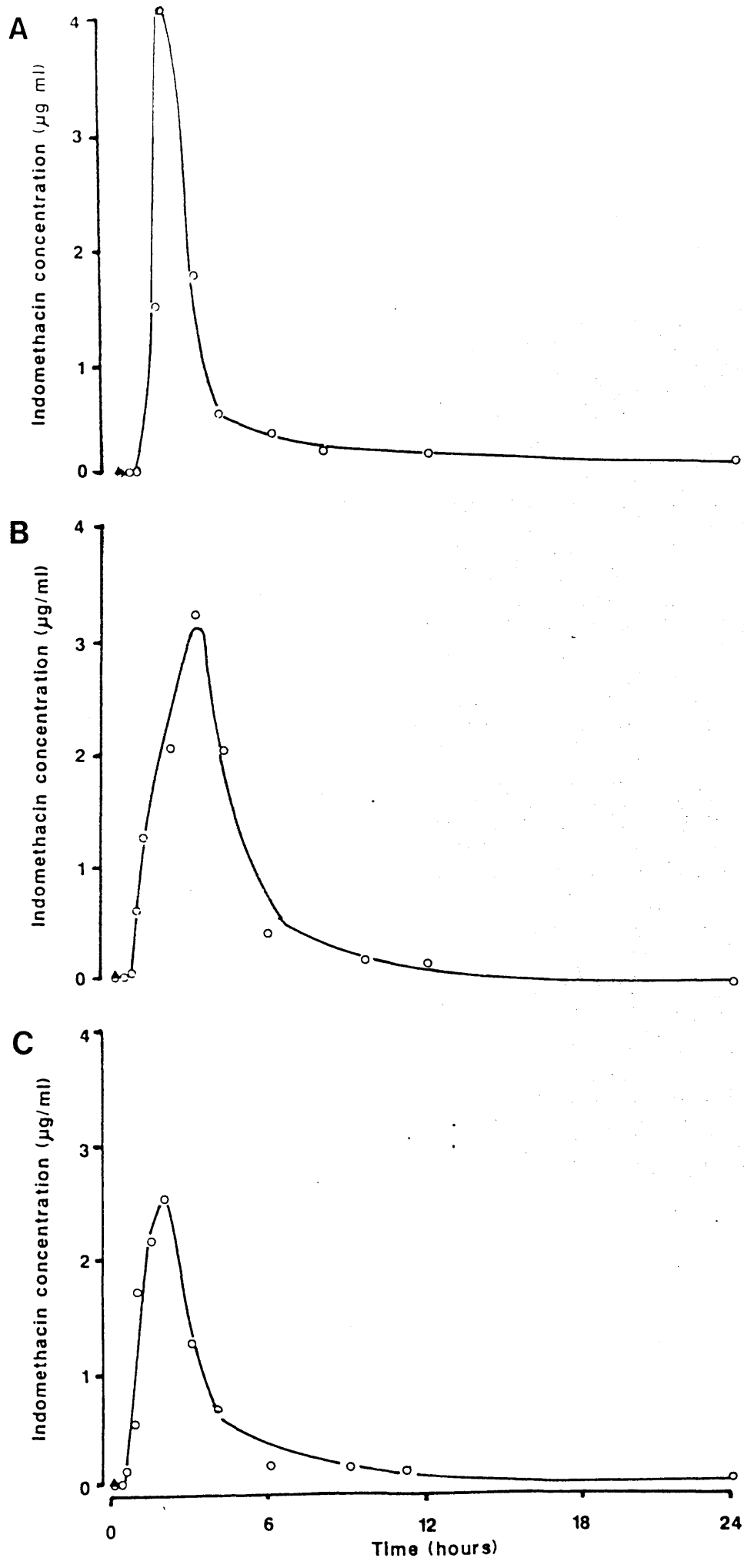

A
Indomethacin concentration (µg ml)
4
3
2
1
0
B
Indomethacin concentration (µg/ml)
4
3
2
1
0
C
Indomethacin concentration (µg/ml)
4
3
2
1
0
0
6
12
18
24
Time (hours)

TABLE 7.2 Individual parameter estimates (SE) for indomethacin plasma concentration-time data after a single dose of Indocid-R fitted to a two compartment model with zero order input

Patient	T_{lag} (h)	T (h)	V_1 (l)	α (h^{-1})	β (h^{-1})	k_{21} (h^{-1})	V_2 (l)	V_{ss} (l)	Cl (l/h)	C_{det}	df
1	1.42(0.02)	1.24(0.09)	12.0(0.5)	0.59(0.03)	0.104(0.005)	0.148(0.007)	10.8	22.8	5.01	0.985	7
2	1.34(0.02)	1.04(0.11)	6.6(1.2)	1.94(0.32)	0.120(0.019)	0.178(0.028)	21.3	27.9	8.63	0.999	7
3	0.49(0.01)	3.06(0.22)	7.7(1.3)	1.12(0.24)	0.114(0.028)	0.167(0.046)	13.9	21.6	5.89	0.973	7
4	0.60(0.05)	1.22(0.18)	15.8(2.1)	0.86(0.15)	0.083(0.026)	0.117(0.047)	29.2	45.0	9.65	0.967	7
5	0.37(0.01)	0.90(0.01)	16.8(0.2)	0.91(0.09)	0.122(0.014)	0.206(0.022)	23.3	40.1	9.03	0.998	7
6	0.28(0.07)	2.18(0.23)	10.9(1.0)	0.70(0.07)	0.184(0.022)	0.356(0.041)	5.1	16.0	3.94	0.936	6
7	0.24(0.01)	0.57(0.04)	13.6(0.9)	0.94(0.08)	0.079(0.023)	0.116(0.033)	30.7	44.3	8.69	0.997	7
8	1.44(0.11)	1.22(0.09)	12.0(1.5)	1.44(0.26)	0.070(0.013)	0.146(0.026)	55.2	67.2	8.26	0.998	7
median	0.54	1.22	12.0	0.92	0.109	0.158	22.3	34.0	8.44		
range	0.24-1.44	0.57-3.06	6.6-16.8	0.59-1.94	0.070-0.184	0.116-0.356	5.1-55.2	16.0-67.2	3.94-9.65		

7.3.2 Synovial fluid pharmacokinetics

The synovial fluid concentration-time profiles could be approximated to a simple input and output function. The indomethacin concentration-time profiles were considerably flatter in synovial fluid. The peak concentration in synovial fluid occured later and was on average 21% of that in plasma. By six hours the concentration in synovial fluid exceeded that in plasma. The ratio of indomethacin concentration in synovial fluid to that in plasma over the 24 hours is given in Table 7.3.

There was no correlation between concentrations in synovial fluid and synovial fluid albumin concentration at any time point. There was a positive correlation between the ratio of albumin in synovial fluid to that in plasma and the ratio of indomethacin in synovial fluid to that in plasma only at 6 hours (patient 8 not included as the albumin concentration was suspect; a synovial fluid sample was not taken from patient 4 at 6 hours). Representative plasma and synovial fluid profiles are shown in Figure 7.2.

Two pharmacokinetic models were proposed to describe the kinetics in plasma and synovial fluid and are presented in (Figure 7.3). In both cases the plasma kinetics are described by a two compartment model with zero order absorption: Model 5 assumes that concentrations of indomethacin in synovial fluid are representative of the profile of drug in the kinetically defined peripheral compartment whereas Model 6 assumes that the synovial fluid represents a distinct compartment which does not affect the

TABLE 7.3 Ratio of indomethacin concentration in synovial fluid to the concentration in plasma

	Time (h)			
Patient	3	6	12	24
1	0.09	0.79	1.63	1.63
2	0.39	2.74	3.61	4.34
3	0.10	1.40	1.17	1.81
4	0.66	-	1.38	1.36
5	0.60	1.11	2.24	1.51
6	-	1.01	-	1.35
7	0.65	1.36	1.85	0.70
8	0.30	1.46	-	0.69
median	0.40	1.41	1.98	1.68
range	0.09-0.65	0.79-2.74	1.17-3.61	0.70-4.39

kinetics of indomethacin in plasma. The equations describing the concentration in plasma and synovial fluid are given in Appendix II (Models 5 and 6).

(i) Model 5

Plasma pharmacokinetic parameters determined for the fit of individual data (Table 7.2) were used to calculate the concentration of indomethacin in the kinetically defined peripheral compartment. These calculated or predicted concentrations (pred) and synovial fluid concentrations (obs) are given in Table 7.4. The correlation between observed and predicted concentrations with time was tested using GLIM (Chapter 4). The most appropriate linear model was:

$$obs = b_i.pred \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 7.1$$

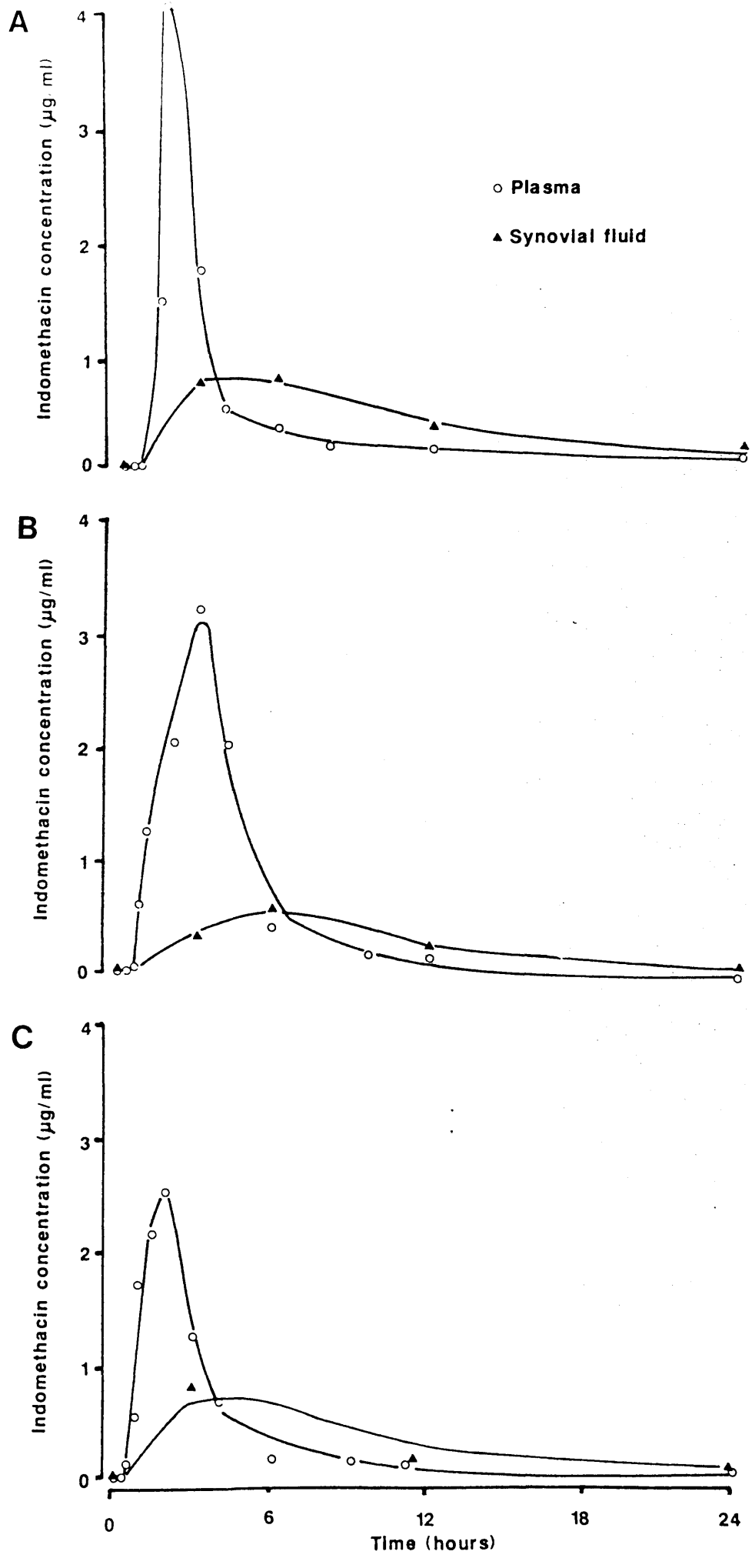
where b_i is an individual slope. The individual slopes ranged from Ø.33 to 1.98. If there was no error in the prediction of the synovial fluid concentrations, the slope should be unity. However, this does indicate that in general, concentrations in synovial fluid change in parallel with those predicted in the peripheral compartment.

The error in the prediction was also tested using the Wilcoxon sign rank test. Although Figure 7.4 indicates that there was a trend towards a greater overprediction of the concentration in synovial fluid at the later time points, there was no significant difference between predicted and observed concentrations. This was possibly due to a number of factors:

a) the number of individuals was too small and the magnitude

FIGURE 7.2 Representative indomethacin plasma and synovial fluid concentration-time profiles after a single dose of Indocid-R

A Patient 2
B Patient 3
C Patient 4


A
B
C
Indomethacin concentration (µg/ml)
Plasma
Synovial fluid
0
1
2
3
4
0
6
12
18
24
Time (hours)

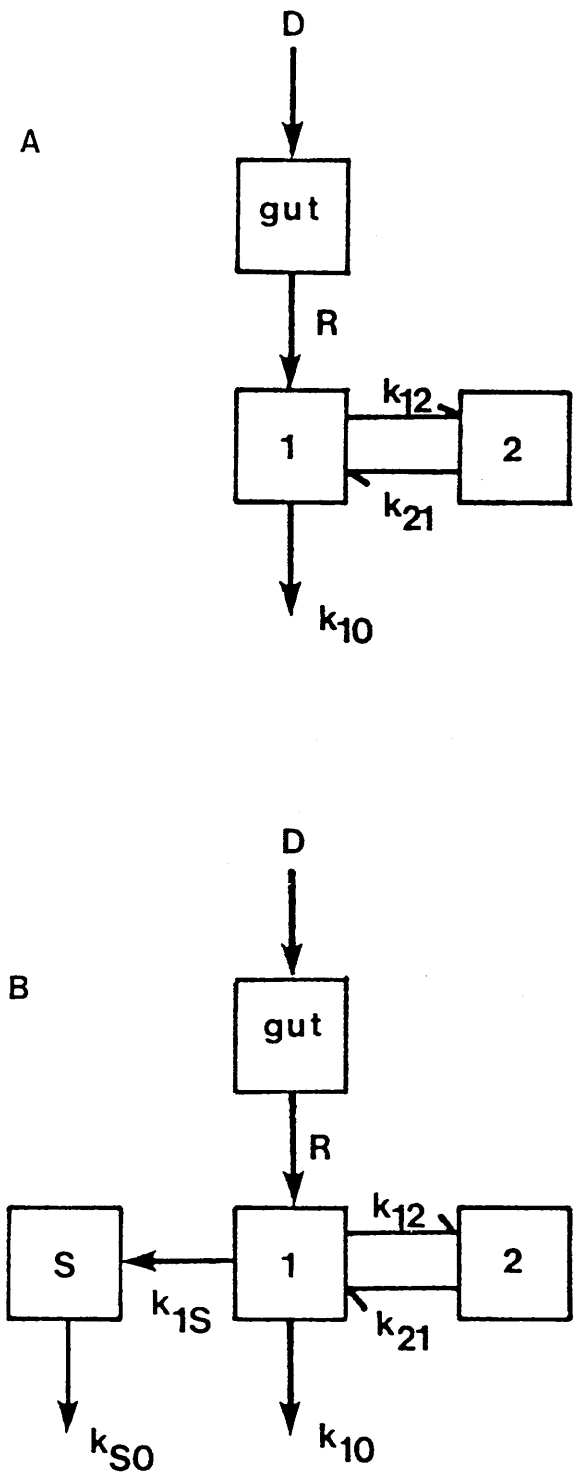


FIGURE 7.3 Pharmacokinetic models proposed to describe the concentration of indomethacin in plasma and synovial fluid

A Model 5

B Model 6

TABLE 7.4 Observed and predicted indomethacin concentrations in synovial fluid

Patient		Time (h) 3	6	12	24
1	O	Ø.339	Ø.663	Ø.36Ø	Ø.Ø88
	P	Ø.663	1.Ø96	Ø.68Ø	Ø.2ØØ
2	O	Ø.694	Ø.784	Ø.343	Ø.136
	P	Ø.471	Ø.431	Ø.21Ø	Ø.Ø5Ø
3	O	Ø.33Ø	Ø.567	Ø.272	Ø.Ø56
	P	Ø.712	Ø.712	Ø.375	Ø.Ø93
4	O	Ø.82Ø	-	Ø.126	Ø.Ø38
	P	Ø.36Ø	-	Ø.257	Ø.Ø91
5	O	Ø.463	Ø.345	Ø.19Ø	Ø.Ø53
	P	Ø.678	Ø.563	Ø.311	Ø.Ø67
6	O	-	Ø.831	-	Ø.Ø31
	P	-	2.48Ø	-	Ø.Ø96
7	O	Ø.477	Ø.3Ø8	Ø.1Ø2	Ø.Ø33
	P	Ø.471	Ø.426	Ø.268	Ø.1Ø4
8	O	Ø.6Ø9	Ø.576	-	Ø.Ø5Ø
	P	Ø.188	Ø.328	-	Ø.Ø96

Key: O = observed synovial fluid concentration

P = concentration predicted in the peripheral compartment (Model 4)

Concentrations are in µg/ml

of the errors was large.

b) poorly defined pharmacokinetic parameters.

Since this analysis was inconclusive, the individual plasma and synovial fluid concentration - time data were fitted simultaneously to Model 5. The results of this analysis are given in Table 7.5. In this situation, the synovial fluid data are fitted together with the plasma data, and therefore influence the estimates of the plasma pharmacokinetic parameters. Comparison with the results obtained by fitting the plasma data to Model 4 indicates that there are some differences in the parameters. On average, Cl/F is slightly higher when the data were fitted to model 6. V_2 estimated for Model 5 is similar to that derived from the parameters for plasma data fitted to Model 4. In Table 7.5, k_{12} and k_{21} are the input and output rate constants for synovial fluid. The elimination from synovial fluid was in general slower (median half-life 3.7 hours) than the input (median half-life 2.5 hours).

(ii) Model 6

The results for the plasma and synovial fluid data fitted simultaneously to Model 6 are given in Table 7.6. Comparison of this analysis with that of plasma alone indicates that some of the parameters describing the concentration in plasma are slightly different due to the addition of the synovial fluid data. The calculated apparent clearance, however, was virtually identical. The parameters describing the profile in synovial fluid are $k_{S\emptyset}$ and k_{1S}/V_S. As the volume of synovial fluid was not known, the input

TABLE 7.5 Indocid-R: individual parameter estimates (SE) for plasma and synovial fluid concentration-time data fitted to Model 5

Patient	V_1 (l)	α (h^{-1})	β (h^{-1})	k_{21} (h^{-1})	V_2 (l)	k_{12} (h^{-1})	CL (l/h)	C_{det}	df
1	11.7 (1.2)	0.625 (0.102)	0.109 (0.013)	0.159 (0.027)	20.2 (3.4)	0.146	5.01	0.985	11
2	7.2 (1.2)	1.767 (0.249)	0.101 (0.008)	0.151 (0.013)	14.0 (1.9)	0.535	8.51	0.999	11
3	8.6 (1.1)	0.964 (0.144)	0.120 (0.018)	0.169 (0.026)	20.7 (3.6)	0.230	5.89	0.977	11
4	14.8 (3.2)	1.021 (0.232)	0.161 (0.026)	0.238 (0.045)	18.0 (10.2)	0.254	10.22	0.967	10
5	16.8 (1.2)	0.908 (0.090)	0.119 (0.010)	0.201 (0.021)	36.5 (3.7)	0.288	9.03	0.998	11
6	9.8 (2.2)	0.918 (0.252)	0.190 (0.051)	0.435 (0.129)	16.8 (14.8)	0.272	3.93	0.945	8
7	13.1 (1.1)	1.015 (0.096)	0.112 (0.014)	0.165 (0.022)	34.0 (5.4)	0.274	9.02	0.997	11
8	9.7 (2.9)	2.025 (0.713)	0.120 (0.014)	0.255 (0.040)	23.2 (5.1)	0.973	9.24	0.990	10
median	10.8	0.990	0.120	0.185	20.4	0.273	8.76		
range	7.2-16.8	0.625-2.02	0.101-0.190	0.151-0.435	14.0-36.5	0.146-0.937	3.93-10.22		

TABLE 7.6. Indocid-R: individual parameter estimates (SE) for plasma and synovial fluid concentration-time data fitted to Model 6

Patient	V_1 (l)	α (h^{-1})	β (h^{-1})	k_{21} (h^{-1})	$k_{S\emptyset}$ (h^{-1})	k_{1S}/V_S ($h^{-1}l^{-1}$)	CL (l/h)	C_{det}	df
1	12.1 (Ø.9)	Ø.59 (Ø.Ø5)	Ø.1Ø4 (Ø.Ø1Ø)	Ø.147 (Ø.Ø15)	Ø.166 (Ø.Ø26)	Ø.ØØ7 (Ø.ØØ1)	5.Ø5	Ø.986	1Ø
2	6.8 (1.3)	1.86 (Ø.11)	Ø.117 (Ø.Ø19)	Ø.173 (Ø.Ø29)	Ø.133 (Ø.Ø14)	Ø.Ø23 (Ø.ØØ4)	8.55	Ø.999	1Ø
3	7.7 (Ø.6)	1.12 (Ø.11)	Ø.116 (Ø.Ø11)	Ø.171 (Ø.Ø17)	Ø.18Ø (Ø.Ø17)	Ø.Ø12 (Ø.ØØ2)	5.85	Ø.976	1Ø
4	11.9 (3.5)	1.14 (Ø.34)	Ø.Ø85 (Ø.Ø32)	Ø.122 (Ø.Ø48)	Ø.362 (Ø.Ø86)	Ø.Ø21 (Ø.Ø68)	9.45	Ø.97Ø	9
5	16.8 (1.3)	Ø.9Ø (Ø.11)	Ø.117 (Ø.Ø18)	Ø.198 (Ø.Ø38)	Ø.198 (Ø.Ø24)	Ø.ØØ7 (Ø.ØØ1)	8.93	Ø.998	1Ø
6	1Ø.4 (2.7)	Ø.83 (Ø.24)	Ø.193 (Ø.Ø61)	Ø.4Ø9 (Ø.129)	Ø.346 (Ø.33Ø)	Ø.Ø15 (Ø.Ø15)	4.11	Ø.944	7
7	13.6 (Ø.8)	Ø.94 (Ø.Ø8)	Ø.Ø74 (Ø.Ø2Ø)	Ø.11Ø (Ø.Ø29)	Ø.228 (Ø.Ø24)	Ø.ØØ8 (Ø.ØØ1)	8.56	Ø.997	1Ø
8	12.1 (1.4)	1.41 (Ø.23)	Ø.Ø74 (Ø.Ø12)	Ø.151 (Ø.Ø26)	Ø.398 (Ø.Ø71)	Ø.Ø2Ø (Ø.ØØ3)	8.36	Ø.998	9
median	12.1	1.Ø3	Ø.11Ø	Ø.161	Ø.213	Ø.Ø14	8.56		
range	6.8-16.8	Ø.59-1.86	Ø.Ø74-Ø.193	Ø.11Ø-Ø.4Ø9	Ø.133-Ø.398	Ø.ØØ7-Ø.Ø23	4.11-9.45		

rate constant K_{1S} could not be estimated. The half-life for elimination from from synovial fluid ($\ln 2/k_{S\emptyset}$) ranged from 2.3 to 5.8 hours (median 3.7 hours). If the kinetics of indomethacin in synovial fluid are equivalent to those in the peripheral compartment, a correlation between K_{21} and $k_{S\emptyset}$ might be expected. There was, however, no correlation between these parameters. However, this is perhaps not surprising as there was some error in the determination of both of these parameters.

A comparison of the 'goodness of fit' for the simultaneous analysis of plasma and synovial fluid data fitted to models 5 and 6 is given in Table 7.7. There was very little difference in the residuals for the two models, although Model 5, which has one parameter less, often gave a lower AIC value.

7.3.3 Plasma and synovial fluid concentrations at steady state

The average parameters determined from the individual fits were used to predict the indomethacin concentration profile in plasma and synovial fluid at steady state on once daily dosing. The concentrations predicted at steady state were almost superimposable on the the profile after a single dose. If the model is appropriate and the kinetics are linear, there will be no accumulation of indomethacin in plasma or synovial fluid with the envisaged dosing regimen.

TABLE 7.7 Comparison of the 'goodness of fit' for indomethacin concentration-time data fitted to models 5 and 6

	Model 5			Model 6		
Patient	df	$WSSQ_{res}$	AIC	df	$WSSQ_{res}$	AIC
1	11	Ø.2Ø8	-7.4*	1Ø	Ø.2Ø1	-7.2
2	11	Ø.Ø88	-57.1*	1Ø	Ø.Ø89	-49.7
3	11	Ø.275	-3.8*	1Ø	Ø.229	-1.Ø
4	1Ø	Ø.437	-3.6*	9	Ø.262	-3.3
5	11	Ø.Ø72	-52.Ø*	1Ø	Ø.Ø71	-5Ø.2
6	8	Ø.662	19.5*	7	Ø.663	21.6
7	11	Ø.2ØØ	-33.5*	1Ø	Ø.Ø91	-28.3
8	1Ø	Ø.19Ø	-31.8	9	Ø.Ø38	-55.Ø*

Key: df = degrees of freedom

$WSSQ_{res}$ = weighted residual sum of squares

* indicates the lowest AIC value

7.4 DISCUSSION

It appears that under the conditions of this study Indocid-R is not a very efficient slow release preparation. While absorption was often delayed, subsequent profiles resembled those obtained after a single 75mg dose of standard indomethacin (Schoog, Laufen & Dessain, 1981; Yeh et al, 1982). The mean peak concentration was slightly lower than has been observed in healthy male volunteers after a single 75mg dose of standard indomethacin. However, peak concentrations after Indocid-R were only 1.47 and 2.14µg/ml respectively in these studies (Schoog et al, 1981; Yeh et al, 1982). The similarity between Indocid-R and standard preparations of indomethacin has been noted previously (Adams et al, 1982). Peak concentrations for Indocid-R and 50mg standard indomethacin were not significantly different when corrected for dose. The only noticeable difference was a slightly delayed peak concentration.

The apparent clearance or indomethacin determined from the fit to the two compartment model was in general slightly higher than that determined in healthy volunteers after doses of up to 100mg of standard indomethacin (Alvan et al, 1975). This may be due to a reduction in bioavailability of the Indocid-R preparation or the lower albumin concentration in patients. There was no correlation between the clearance of indomethacin and albumin concentration.

The analysis of plasma concentration-time data did not take account of possible enterohepatic recirculation which

has been reported to range from 24-115% of an intravenous dose of indomethacin (Kwan et al, 1976). Although terminal concentrations were sometimes erratic, the data were fitted relatively well to a two compartment model. In fact, there were probably too few data points to identify enterohepatic recirculation.

The mean plasma kinetic parameters predict that on average, there will be virtually no accumulation of indomethacin on multiple dosing with Indocid-R once daily, in agreement with the results of others (Schoog et al, 1982; Verbesselt et al, 1983).

The profile of indomethacin in synovial fluid in this study was very similar to that observed for standard indomethacin (Emori et al, 1973). There was a similar delay: peak concentrations in synovial fluid were 25% of that in plasma, compared to 21% in this study and concentrations exceeded those in plasma after 4 hours.

In all studies investigating the distribution of NSAIDs in synovial fluid and plasma, the levels in synovial fluid initially were lower than those in plasma and peak concentrations were delayed. Free drug in synovial fluid is less available for elimination compared to free drug in plasma so a gradient develops across the synovial membrane during the elimination phase and the concentration in synovial fluid remains higher than that in plasma. The comparative profiles of indomethacin in plasma and synovial fluid are consistent with this general description.

There has been no previous report of the simultaneous

fitting of plasma and synovial fluid data to an integrated pharmacokinetic model. Most investigators have assumed that the concentration profile in synovial fluid is consistent with the profile of the drug in the peripheral compartment of a two compartment model. Ray et al (1979), however, in a study of carprofen (a propionic acid derivative), suggested that the peripheral compartment concentrations (predicted from the parameters determined by fitting the plasma concentration-time data to a two compartment model) did not give a good description of concentrations in synovial fluid. However, no statistical test of the difference between the predicted and observed concentrations was carried out. In addition, it was unlikely that adequate parameter estimates determining concentrations in the peripheral compartment could have been obtained from the plasma concentration-time data available.

Aarons et al (1986) proposed an alternative pharmacokinetic model to describe the concentration of flurbiprofen in synovial fluid. The total concentration at any time could be simulated fairly well by assuming that the levels were determined by the free concentration in plasma, a diffusion constant (R) for the movement of free drug across the synovial membrane, the total concentration of binding protein in synovial fluid and the volume of synovial fluid. However, the actual value of R was not be determined in this study as the volume of synovial fluid was not known and the model could not be tested by fitting plasma and synovial fluid data as only one synovial fluid concentration

was available per patient.

The rate of input tended to be faster than rate of elimination of indomethacin from the synovial fluid (data fitted to Model 5). The range of values was quite large. The kinetics of NSAIDs in synovial fluid may be related to clinical factors such as synovial blood flow, endothelial vascular permeability to albumin, diffusion of free drug across the synovium, synovial fluid and synovial pH. Inflammatory disease may influence both synovial blood flow and vascular permeability and it may influence the structure of the synovial tissue.

The clearance of ^{133}Xenon from the joint is an indirect measure of synovial blood flow (Dick, 1972) and it would have been useful to compare this with the rate of input of the drug into synovial fluid. Alternatively it might have been interesting to have determined the disease activity in the knee joint since this will affect blood flow. Aarons et al (1986) found, however, no correlation between the concentration of flurbiprofen in synovial fluid and Xenon clearance but there was a weak positive correlation between the synovial fluid concentration and a thermographic measure of disease activity. The converse was found for phenylbutazone: concentrations were lower in patients with more actively inflamed joints (Farr and Willis, 1977). These relationships, however, were based on single paired observations often taken at different times during the dosing interval. Aarons et al (1986) concluded that the diffusion of free drug across the synovium was an important

determinant of synovial fluid drug concentration. Acidic NSAIDs accumulate in inflamed tissues in animals (Graf et al, 1975), and in patients, oxyphenbutazone 'levels' were higher in synovial tissue from patients with severe inflammation than in those with little or no inflammation (Gaucher et al, 1983). Thus greater relief may be attained in patients with more severe inflammation.

There have been few studies of the binding of NSAIDs in synovial fluid, but the binding of piroxicam was equivalent in plasma and synovial fluid for the same total albumin concentration (Trnavska, Trnavsky & Zlnay; 1984). However, the binding of salicylate was reduced in synovial fluid compared to plasma due to a alteration in the binding to the high affinity site (Trnavska & Trnavsky; 1980). The free indomethacin concentration was not determined in this study. If one assumes that the binding constants for indomethacin are equivalent in plasma and synovial fluid, the free concentrations must be considerably higher than those in plasma by six hours after the dose. The measurement of free concentrations in plasma and synovial fluid or a comparison of the binding profiles of indomethacin in the two fluids would have provided useful additional information on the distribution of indomethacin. The free drug concentration in corresponding plasma and synovial fluid samples has been reported to be equivalent for a number of NSAIDs (Rosenthal, Bayles & Fermont-Smith, 1964; Whitlam et al, 1981). Aarons et al (1986), however, found that the free fraction of flurbiprofen in plasma and synovial fluid was

the same.

At a total concentration of 2.5µg/ml, the free fraction of indomethacin was 5% in synovial fluid and 4% in plasma (Wanwimolruk, Brooks & Birkett, 1983). Thus the free concentration or indomethacin on average will range from 2ng/ml at 24 hours to 31ng/ml at around 4 hours. Sturge et al (1978) found that concentrations of 14.3ng/ml were required to give 50% inhibition of PGE_2 production by rheumatoid synovial fragments in vitro. Robinson (1980) quotes an $IC_{50\%}$ of 1.8ng/ml for PGE_2 production in rheumatoid synovial cultures. Thus residual concentrations in synovial fluid at 24 hours are possibly still sufficient to inhibit PGE_2. Other prostaglandins may contribute to the inflammatory response eg PGI_2 and TXA_2 and the production of these may be inhibited to a greater or lesser extent.

The rate of elimination of indomethacin from synovial fluid is faster than that from plasma. In most previous studies, especially with the short half-life NSAIDs, the elimination from synovial fluid has been reported to be slower than that from plasma (Sholkoff et al, 1967; Emori et al, 1973; Chalmers, Glass and Marchant, 1980; Glass & Swannell, 1980; Caruso et al 1980) and it has been suggested that the drug will therefore accumulate in synovial fluid. In many studies, however, a comparison was made between the distribution phase in plasma and the elimination phase in synovial fluid. Others have found that the elimination from plasma and synovial fluid was similar (Makela, Lempianen & Ylijoki, 1981; Dromgoole et al, 1982).

The mean kinetic parameters predict that there will be no accumulation of indomethacin in synovial fluid with once daily dosing of Indocid-R.

In conclusion, this study indicates that the kinetics of indomethacin in plasma can in some cases give a good description of the profile in synovial fluid. With a larger number of plasma samples, a more accurate estimate of the plasma pharmacokinetics might have improved the prediction of concentrations in synovial fluid. The extended clinical response to these short half-life NSAIDs may well be explained in terms of the equilibrium delay which will exist for free drug between synovial fluid and plasma during the elimination phase. Indocid-R, however appears to offer little advantage over standard preparations of indomethacin in terms of giving sustained plasma concentrations. The range of input and output rate constants indicates that there is some considerable inter-individual variability in the kinetics of indomethacin between plasma and synovial fluid and this might explain some of the variability in clinical response. The relationship between the free concentration in synovial fluid and clinical response should be investigated further.

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

The number of NSAIDs available to the rheumatologist has increased dramatically over the last decade or so. The choice and dose of drug remains, however, largely empirical. Newer NSAIDs are generally accepted for clinical use if they show similar efficacy and reduced toxicity in comparative studies with older established NSAIDs. The new drugs are seldom investigated with a view to establish a dose or concentration-response relationship.

Comparative studies of NSAIDs in rheumatoid arthritis suggest that patient response is highly variable and unpredictable (Huskisson et al, 1976; Scott et al, 1982). For example Scott et al (1982) found significant differences between patients but no significant difference between drugs. In addition, they identified a significant drug-patient interaction and they suggested that this indicated that some patients are particularly suited to one drug but not to another. Two studies were unable to explain this variability in pharmacokinetic terms (Capell et al, 1977; Baber et al, 1979) but inter-individual differences in disease severity were not taken into account. In other studies the response to an increase in dose or concentration has been difficult to detect (Orme et al, 1976; Ekstrand et al, 1980; Grennan et al, 1983).

This thesis reinforces the view that pharmacokinetic information contributes little to the understanding of variability in clinical response even when individual differences in disease severity are taken into account and

that there is little to choose between dose, total or free concentration in the description of clinical response.

Conventional analysis of the relationship between dose and clinical response (using analysis of variance), indicated that in general, there was no significant difference between the three doses for either naproxen or fenclofenac. This reflected the considerable intra- and inter-subject variability in patient response and its measurement. A much larger number of patients would have been necessary to demonstrate a dose response relationship with this type of analysis. However, when a linear modelling approach was used (GLIM, Baker & Nelder, 1978) the analysis indicated that for both drugs there was an improvement in response with increments in dose. In the case of fenclofenac significant improvement was seen in the duration of morning stiffness and the analogue pain score. With increments in naproxen dose there was a significant improvement in the mean grip strength and the analogue pain score.

Since naproxen and fenclofenac demonstrated considerable pharmacokinetic variability it might have been expected that concentration would have explained some of the response variability. This however was not the case. Using the same linear modelling approach, the most appropriate linear model for both dose and concentration allowed for individual variability in terms of an individual intercept (disease severity before treatment). The improvement in symptoms with increments in dose or concentration (if any)

was described by a common slope. In general, the 'goodness of fit' to this model was similar for dose and concentration but it was possible to show some added benefit from concentration.

An improved fit for response when analysed in terms of naproxen total concentration was due to the non-linear increase in trough concentrations, a consequence of saturation of binding sites on plasma proteins. Therefore a direct comparison to test for the effect of inter-subject variability in the kinetics of naproxen could not be made using this analysis. There was, however, a linear increase in the free drug concentration and the analysis in terms of free drug indicated that there was only slight improvement over dose in the explanation of some of the rheumatological measures.

The largest difference in clinical response was often observed between 'no treatment' and the lowest dose. This was more apparent for naproxen than for fenclofenac. It was not clear as to whether this indicated that the concentrations were close to those necessary to produce a maximum response or whether these measures, carried out under non-blinded conditions, were exaggerated by their subjective nature.

If baseline data were included in the analysis, a hyperbolic or E_{max} model was more appropriate in some cases. The concentration required to give 50% of the maximum response was approximately 70µg/ml for fenclofenac (close to

the average concentration achieved on the 1200mg/day dose). For naproxen, however, the free concentration required to give 50% of the maximum response was estimated as 20ng/ml (the average free concentration on 500mg/day was 34ng/ml). The errors in the estimate of the parameters of the E_{max} model were large.

The linear model was more appropriate for total naproxen concentration. This appeared to be due to the fact that total concentration and clinical response moved in parallel towards a plateau. Although, it is generally assumed that the free concentration of drug in the blood is pharmacologically active, the results of the naproxen study suggest that clinical response is more closely related to total concentration. Support for this notion has been provided by Grennan et al (1983) who found that the maximum response to ibuprofen occured at a dose of 1600mg/day, a dose of 2400mg producing no further improvement. The kinetics of ibuprofen were non-linear and these workers suggested that binding to plasma proteins might mimic binding to the enzyme at their site of action in inflamed tissues. Similarly Day et al (1982) found that while there was a linear relationship between total naproxen concentration and response, the relationship between free concentration and response appeared to reach a plateau.

There was some difference in the response measures which were able to detect significant differences between doses or concentrations for the two drugs. These differences may have

occured, however, by chance or may have been related to differences in the patient groups. While the duration of morning stiffness (a relatively insensitive measure of anti-inflammatory activity) showed no difference over the three doses of naproxen, there was a linear relationship between the dose (or concentration) of fenclofenac and the reduction in morning stiffness. Grip strength was also a useful measure. The intra-subject variability was in general smaller for grip strength, but this was offset by the fact that changes in grip tended to be small and dependent on the degree of underlying damage to joints. Patients with severe deformity showed little response. Despite its subjective nature, the analogue pain score in general appeared to be the most sensitive measure to detect changes in symptoms with increments in dose or concentration.

Some patients in these studies showed wide swings in their disease severity throughout the study period making it almost impossible to distinguish a dose or concentration response relationship. This type of variability in the disease may explain why comparative studies of NSAIDs have suggested that patient response to different drugs is variable and unpredictable (Huskisson et al, 1976; Scott et al, 1982). If it is assumed that all NSAIDs share a common mode of action, equipotent doses should produce equivalent responses in the same patients on a given day.

A number of factors may influence the pharmacokinetics of NSAIDs. Differences in protein binding or hepatic

metabolic activity will affect the total concentration achieved in plasma. The clearance of fenclofenac appeared to be reduced in older patients and those with raised alkaline phosphatase. The clearance of naproxen, on the other hand, was reduced in the elderly and appeared to be lower in female patients. There were no apparent dose or concentration related side-effects, however the small number of patients precluded any formal analysis.

In general, pharmacokinetic variability appears to contribute very little to the total variability in clinical response. This conclusion has been reached by others despite different analytical approaches. Grennan et al (1983) concluded that there was no advantage in knowing plasma concentrations, but their analysis took no account of inter-individual disease severity. Brooks et al (1975) came to the same conclusion; in this case a parallel design was used.

In the future some of the problems encountered in this thesis could be overcome by:

1. Normalising the response in different patients with a range of disease severity eg expressing response as a percentage change from a baseline flare.

2. Using more stringent inclusion criteria to give a more homogeneous group of patients in terms of disease severity.

3. The inclusion of a placebo period, or an additional dose, especially towards the lower end of the therapeutic range. A larger number of observations within the same

individual would allow a better description of the data.

If clinical response to NSAIDs in rheumatoid arthritis is due solely to the inhibition of prostaglandin synthesis and if the factors which affect the pharmacokinetics of the drugs are known, it should theoretically be possible to determine the optimum dose of a particular NSAIDs for an individual patient. In this thesis only eighteen patients were studied with each drug and it was not possible to determine accurately the contribution of any particular patient factor such as age, sex, smoking etc. However, if a larger population of patients was studied a clearer picture of the important determinants of the elimination of these drugs could be established. Together with knowledge of protein binding and the distribution of the drug into synovial fluid (if it can be predicted from the concentration of drug in plasma) the most appropriate dose of a particular NSAID could be determined to achieve maximum inhibition of the cyclo-oxygenase enzyme.

In conclusion, the studies of fenclofenac and naproxen indicate that variability in the pharmacokinetics of these drugs contribute only a small amount to the variability in clinical response and in absence of any concentration related toxicity, the doses of these drugs may be increased with the expectation that on average a greater response will be achieved. In the clinical setting the measurement of plasma concentration would appear to be unnecessary. The average slopes relating dose and clinical effect tend to be

shallow and these studies suggest that the doses used clinically are close to those necessary to achieve a maximum response.

These studies were conducted in patients with rheumatoid arthritis who were otherwise healthy and caution should be exercised in patients who are less healthy. The clearance of fenclofenac appeared to be reduced in older patients, and there was a significant negative correlation between naproxen clearance and age. This has been noted by others (Upton et al, 1984; McVerry et al, 1986). In the light of the reports of fatal hepatic toxicity in the elderly associated with the use of benoxaprofen (Taggart & Alderice, 1982), these observations suggest that particular care should be exercised in the use of these drugs in the elderly, especially females.

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APPENDICES

APPENDIX I

An example of the form used to record rheumatological measures for the fenclofenac and naproxen studies.

Assessment Form

Name:_____ Medication No:_____ Date:_____

Visit: (please tick)	Pre-single dose study ☐	First treatment Start ☐ Finish ☐	Second treatment Start ☐ Finish ☐	Third treatment Start ☐ Finish ☐	End final washout ☐

1. Ritchie articular index. ☐

2. Duration of morning stiffness ☐ minutes.

3. Grip strength.

Right hand		Left hand	
1st try	2nd try	1st try	2nd try

4. Global pain
   a) Visual analogue (ask patient to complete)

No Pain ——— Worst Pain Ever

   b) Verbal rating scale

None ☐ Mild ☐ Moderate ☐ Severe ☐

5. Assessment of therapeutic effect (end of treatment periods only).

	None	Fair	Good	Very Good
Patient	☐	☐	☐	☐
Doctor	☐	☐	☐	☐

6. Side-effects— "has the treatment upset you in any way?" (end of treatment periods only).
   If no complaints tick here ☐ otherwise complete below.

Symptom	Date of Onset Day	Month	Year	Number of days symptoms occurred since last visit	SEVERITY Mild	Moderate	Severe	RELATIONSHIP TO TEST DRUG Probably Related	Probably Not Related*	Unknown	EFFECT ON STUDY None	Study Med. interrupted	Study Med. discontinued	REQUIRED TREATMENT Yes**	No
									*					**	
									*					**	
									*					**	
									*					**	

*Probably not related—specify probable cause below **Required treatment: specify drugs used, special studies or consultations.

APPENDIX II

The equations describing the concentration-time profiles of drug in plasma or synovial fluid were determined from the differential equations by the method of Laplace Transforms (Gibaldi & Perrier, 1975).

GLOSSARY

General variables and constants

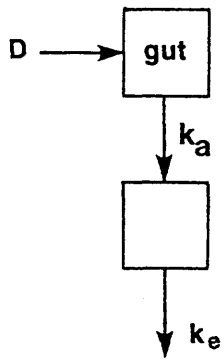
D	Dose (amount)
F	Availability
k_a	First order absorption rate constant
R	Zero order input rate constant
t	Time after the dose
T_{lag}	Time after the dose before drug is detected in plasma
T	Time from T_{lag} to the maximum concentration
t'	Time after T
X	Amount
dX/dt	Rate of drug amount
C	Concentration
V	Volume
CL	Clearance
k	First order rate constant

Subscripts

1	Central compartment
2	Peripheral compartment
S	Synovial fluid
ss	Steady state
el	Elimination

In all equations t is $t-T_{lag}$

MODEL 1: One compartment model with first order absorption



Differential equation:

$$dX/dt = k_aD - k_eX \qquad (1)$$

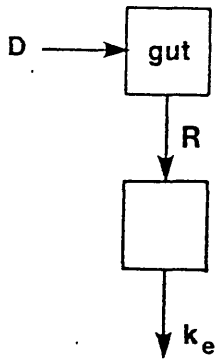
Solution:

$$C = \frac{k_aFD}{V(k_a-k_e)} (e^{-k_et} - e^{-k_at}) \qquad (2)$$

Estimated parameters: T_{lag}, k_a, V/F, CL/F

Derived parameters: $k_e = CL/V$

MODEL 2: One compartment model with zero order absorption



where R = FD/T

Differential equation:

$$dX/dt = R - k_e X \qquad (3)$$

Solution:

a) During absorption (t < T)

$$C = \frac{FD}{TVk_e}(1 - e^{-k_e t}) \qquad (4)$$

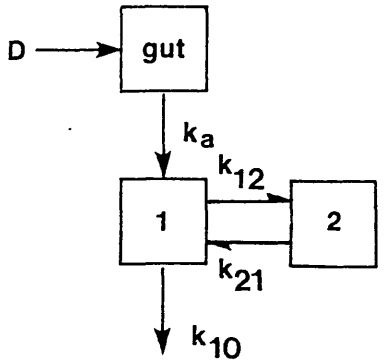
b) After absorption has stopped (t > T)

$$C = \frac{FD}{TVk_e}(1 - e^{-k_e T})\, e^{-k_e t'} \qquad (5)$$

Estimated parameters: T, CL/F, V/F

Derived parameter: $k_e = CL/V$

MODEL 3: Two compartment model with first order absorption



Differential equations:

$$dX_1/dt = k_aD + k_{21}X_2 - k_{12}X_1 - k_{10}X_1 \quad (6)$$

$$dX_2/dt = k_{12}X_1 - k_{21}X_2 \quad (7)$$

Solution:

$$C = Ae^{-\alpha t} + Be^{-\beta t} - (A + B)e^{-k_a t} \quad (8)$$

where

$$A = \frac{F.D.k_a(\alpha-k_{21})}{V_1(\alpha-\beta)(k_a-\alpha)}$$

$$B = \frac{F.D.k_a(\beta-k_{21})}{V_1(\alpha-\beta)(\beta-k_a)}$$

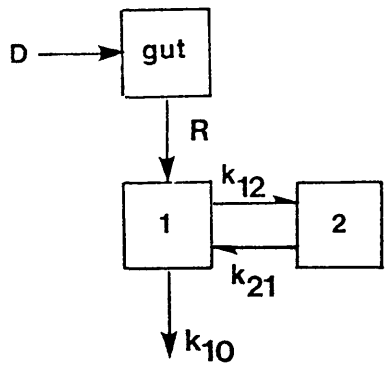
$$C = \frac{F.D.k_a(k_a-k_{21})}{V_1(\alpha-k_a)(k_a-\beta)}$$

α and β are complex rate constants which relate to k_{12}, k_{21} and k_{10}
$\alpha + \beta = k_{12} + k_{21} + k_{10}$

Estimated parameters: k_a, A, α , B, β

Derived parameters: $CL/F = \dfrac{D}{\frac{A}{\alpha} + \frac{B}{\beta}}$ $\quad V_1/F = \dfrac{D}{A + B}$

MODEL 4: Two compartment model with zero order absorption



Differential equations:

$$dX_1/dt = R + k_{21}X_2 - k_{12}X_1 - k_{10}X_1 \qquad (9)$$

$$dX_1/dt = k_{12}X_1 - k_{21}X_1 \qquad (10)$$

Solution:

a) During absorption ($t < T$)

$$C_1 = A(e^{-\alpha t} - 1) + B(e^{-\beta t} - 1) \qquad (11)$$

where $A = \dfrac{FD(k_{21}-\alpha)}{V_1 T\,\alpha(\alpha-\beta)}$ and $B = \dfrac{FD(\beta-k_{21})}{V_1 T\beta(\alpha-\beta)}$

b) After absorption has stopped ($t > T$)

$$C_1 = A(e^{-\alpha T} - 1)e^{-\alpha t'} + B(e^{-\beta T} - 1)e^{-\beta t'}) \qquad (12)$$

Estimated parameters: T_{lag}, T, α, β, k_{21}, V_1

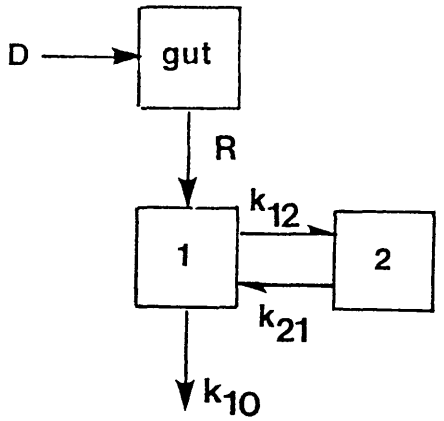
Derived parameters:

$$CL = V_1/k_{21}$$

$$V_2 = V_1 k_{12}/k_{21}$$

$$V_{ss} = V_1 + V_2$$

MODEL 5: Two compartment model with zero order absorption - central and peripheral compartment concentrations



Differential equations:

dX_1/dt see equation (9)

dX_2/dt see equation (10)

Solution:

a) During absorption ($t < T$)

C_1 see equation (11)

$$C_2 = C(e^{-\alpha t} - 1) + D(e^{-\beta t} - 1) \qquad (13)$$

where $C = \dfrac{FDk_{12}}{V_2 T\alpha(\alpha-\beta)}$ and $D = \dfrac{FDk_{12}}{V_2 T\beta(\beta-\alpha)}$

b) After absorption has stopped ($t > T$)

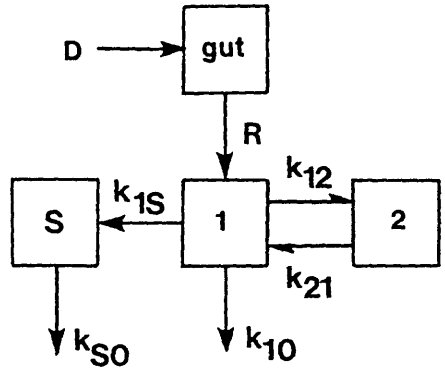
C_1 see equation 12

$$C_2 = C(e^{-\alpha T} - 1)e^{-\alpha t'} + D(e^{-\beta T} - 1)e^{-\beta t'} \qquad (14)$$

Estimated parameters: T_{lag}, T, α , β , k_{21}, V_1, V_2

Derived parameters: See Model 4

MODEL 6: Two compartment model with zero order absorption and a synovial fluid compartment.



Differential equations:

$$dX_1/dt = R + k_{21}X_2 - k_{12}X_1 - k_{10}X_1 \quad (15)$$

$$dX_2/dt = k_{12}X_1 - k_{21}X_2 \quad (16)$$

$$dX_2/dt = k_{1S} - k_{S0}X_S \quad (17)$$

Solution:

a) During absorption ($t < T$)

C_1 see equation (11)

$$C_S = \frac{AV_1K_{1S}}{(k_{S0}-\alpha)V_S}(e^{-\alpha t} - e^{-k_{S0}t}) + \frac{BV_1k_{1S}}{(k_{S0}-\beta)V_S}(e^{-\beta t} - e^{-k_{S0}t}) \quad (18)$$

b) At the end of absorption ($t = T$)

$$C_S = C_S(T)$$

where A and B are as defined previously for equation (11)

c) After absorption has stopped ($t > T$)

C_1 see equation (12)

$$C_s = \frac{AV_1k_{1S}}{(k_{SO}-\alpha)V_S}(e^{-\alpha T} - 1)(e^{-\alpha t'} - e^{-k_{SO}t'}) \qquad (19)$$

$$+ \frac{BV_1k_{1S}}{(k_{SO}-\beta)V_S}(e^{-\beta T} - 1)(e^{-\beta t'} - e^{-k_{SO}t'}) + C_S(T)e^{-k_{SO}t'}$$

stimated parameters: T_{lag}, T, α, β, k_{21}, V_1, k_{SO}, k_{1S}/V_S

erived parameters: see Model 4.

Multiple dosing: steady state

Any equation which describes the time course of a drug after a single dose can be directly converted to a multiple dose equation by multiplying each exponential term containing 't' by the multiple dosing function (Gibaldi & Perrier, 1975):

$$\frac{1 - e^{nk\tau}}{1 - e^{-k\tau}} \tag{20}$$

where n is the number of doses, τ is the dosing interval and k is the first order rate constant.

At steady state, n can be set to infinity and the multiple dosing function simplifies to:

$$\frac{1}{1 - e^{-k\tau}} \tag{21}$$

1. One compartment model with zero order absorption (Model 2)

$$C = \frac{FD\ (1 - e^{-k_e T})e^{-kt'}}{VTk_e(1 - e^{-k_e \tau})} \tag{22}$$

2. Two compartment model with zero order absorption (Model 4)

$$C = \frac{FD}{V_1 T(\alpha - \beta)} \left[\frac{(k_{21} - \alpha)(e^{-\alpha T} - 1)e^{-\alpha t'}}{(1 - e^{-\alpha \tau})} + \frac{(\beta - k_{21})(e^{-\beta T} - 1)e^{-\beta t}}{(1 - e^{-\beta \tau})} \right] \tag{23}$$

APPENDIX III

For a drug which is bound to two independent sites in plasma, the equation relating free and bound concentrations, assuming the law of mass action is:

$$Cb = \frac{nP_1\ Cu}{K_{d1} + Cu} + \frac{nP_2\ Cu}{K_{d2} + Cu} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Since:

$$Cb = C - Cu \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$C = Cu + \frac{nP_1\ Cu}{K_{d1} + Cu} + \frac{nP_2\ Cu}{K_{d2} + Cu} \quad \ldots\ldots\ldots\ldots\ldots (3)$$

Rearrangement of this equation with Cu as the dependent variable and C as the independent variable, gives a cubic equation:

$$Cu^3 + (C - K_{d1} - K_{d2} - nP_1 - nP_2)\ Cu^2$$
$$+ (C.K_{d1} + C.K_{d2} - K_{d1}.K_{d2} - nP_1.K_{d2} - nP_2.K_{d1})\ Cu$$
$$- C.K_{d1}.K_{d2} = \emptyset \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

which cannot be solved explicitly. Rearrangement of Equation 1 in terms of Cu, gives a quadratic equation which can be solved:

$$A.Cu^2 + B.Cu + C = \emptyset \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

and Cu is given by the positive root:

$$Cu = \frac{-B + \sqrt{B^2 - 4AC}}{2A} \quad \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

where:

$$A = nP_1 + nP_2 - Cb$$

$$B = nP_1.K_{d2} + nP_2.K_{d1} - Cb.K_{d1} - Cb.K_{d2}$$

$$C = - Cb.K_{d1}.K_{d2}$$

The program used to determine the free concentration in plasma from total drug concentration using the binding parameters determined from fitting binding data to the Langmuir isotherm for two classes of binding sites.

```
1 DIM CTOT(50),FR(50),FREE(50)
2 F1$="££££.££££":F2$="£.£££££"
5 LIMIT=8
10 PRINT "Type 1"
20 INPUT "No.of sites... ";N1
30 INPUT "Kd1..... ";KD1
40 PRINT
50 PRINT "Type 2"
60 INPUT "No.of sites... ";N2
70 INPUT "Kd2..... ";KD2
75 I=0
80 I=I+1:PRINT:IF I> 50 THEN 300
90 INPUT "Ctotal..... ";CT:CTOT(I)=CT
105 ITER=1
110 DELTA=.05:F=0:F1=-1
120 F=F+DELTA
130 F2=N1*F/(KD1+CT*F) + N2*F/(KD2+CT*F) + F - 1
140 IF F1< 0 AND F2 >= 0 THEN 200
150 F1=F2:GOTO 120
200 ITER=ITER+1:IF ITER=LIMIT THEN 250
210 F=F-DELTA:DELTA=DELTA/10:GOTO 120
250 CF=F*CT:FR(I)=F:FREE(I)=CF
260 PRINT "Free fraction = ";F
270 A$="Y":INPUT "Repeat...[Y] ";A$:IF A$<>"N" AND A$<>"n" THEN 80
280 PRINT
290 PRINT
300 PRINT "Summary":PRINT "======="
310 PRINT "          Class 1          Class 2"
315 PRINT "          =======          ======="
320 PRINT "No.       ";:PRINT USING F1$;N1;:PRINT "          ";:PRINT USING F1$;
N2
330 PRINT "Kd        ";:PRINT USING F1$;KD1;:PRINT "          ";:PRINT USING F1$
;KD2
340 PRINT
350 PRINT "   Total conc.     Free conc.     Free fraction"
360 PRINT "   ===========     ==========     ============="
370 FOR J=1 TO I
380 PRINT J;:PRINT "   ";:PRINT USING F1$;CTOT(J);:PRINT "    ";:PRINT USING F1$
;FREE(J);:PRINT "         ";:PRINT USING F2$;FR(J)
390 NEXT
400 INPUT "Switch ON printer and type key";A$
410 LPRINT "Summary":LPRINT "======="
415 LPRINT "          Class 1          Class 2"
420 LPRINT "          =======          ======="
425 LPRINT "No.       ";:LPRINT USING F1$;N1;:LPRINT "          ";:LPRINT USING
F1$;N2
430 LPRINT "Kd        ";:LPRINT USING F1$;KD1;:LPRINT "          ";:LPRINT USING
 F1$;KD2
435 LPRINT
440 LPRINT "   Total conc.     Free conc.     Free fraction"
445 LPRINT "   ===========     ==========     ============="
450 FOR J=1 TO I
455 LPRINT J;:LPRINT "   ";:LPRINT USING F1$;CTOT(J);:LPRINT "    ";:LPRINT USI
NG F1$;FREE(J);:LPRINT "         ";:LPRINT USING F2$;
FR(J)
460 NEXT
```

APPENDIX IV

To run the 1980 version of NONMEM (Beal & Sheiner, 1980) a control file must be provided by the user which is specific for the structural model and the data set to be analysed. The 'PRED', a Fortran subroutine, contains the function for the structural model, together with the derivatives of the function (G array) with respect to each of the parameters (θ). The G functions define the inter-subject error structure (normal or log normal). The intra-subject error structure (additive or proportional) is defined in the H function.

Examples of control files and 'preds' are given for linear and E_{max} concentration-response models and protein binding models.

a) The 'pred' and control file for a linear model used to describe dose or concentration-response data in Chapters 5 and 6.

```
(0001)      SUBROUTINE PRED(ICALL,NEWIND,THETA,DATREC,INDXS,F,G,H)
(0002)C     LIN 2 PARAM
(0003)C
(0004)C     PARAM INTERCEPT & SLOPE
(0005)C
(0006)      DIMENSION DATREC(3),THETA(2),G(2),INDXS(1),H(1)
(0007)      DOUBLE PRECISION THETA,F,G,H
(0008)      C=DATREC(9)
(0009)      F=THETA(1)+THETA(2)*C
(0010)      G(1)=1.0
(0011)      G(2)=C
(0012)      G(1)=G(1)*THETA(1)
(0013)      G(2)=G(2)*THETA(2)
(0014)      H(1)=1.0
(0015)      RETURN
(0016)      END
(0017)**END**
```

```
(0001)DATA    AKLIB1.NAP12   .....................Data file name
(0002)****
(0003)PROB    CONC EFFECT    .....................Problem name
(0004)DATA      1   0  71   9      ...............Data set structure
(0005)ITEM      1   8   0   0   1     ............Positions of data items
(0006)LABL    SUBJ    DOSE    ORDR    CONC    EFF1    EFF2    EFF3    EFF4    FR
EE                                        ........Labels for data items
(0007)FORM
(0008)(9F7.1)
(0009)STRC      2   2   !   0   0   1   0   1   0
(0010)THTA      9.0  -0.001    ..................Initial estimates of θ's
(0011)DIAG     3.0    0.005    ..................Initial estimates of η's
(0012)DIAG     2.0             ..................Initial estimate of ϵ
(0013)ESTM      02000   !   5   0   0   0   0 ....Estimation parameters
(0014)COVR      0               ..................Covariance matrix
(0015)TABL      2               ..................Instructions for tables
(0016)SCAT      0   9           ..................Instructions for scatterplots
(0017)SCAT      9   8
(0018)SCAT      9  10
(0019)SCAT     10  11
(0020)SCAT     10  12
(0021)SCAT      9  11
(0022)SCAT      8  11
(0023)SCAT      8  12
(0024)SCAT      9  12
(0025)SCAT     10   8
(0026)**END**
```

b) The 'pred' and control file for an Emax model used to analyse dose or concentration-response data

```
(0001)      SUBROUTINE PRED(ICALL.NEWIND.THETA.DATREC.INDXS.F.G.H)
(0002)C     EMAX 3 PARAM
(0003)C
(0004)C     PARAM CMAX. T50 3 C0
(0005)C
(0006)      DIMENSION DATREC(3).THETA(3).G(3).INDXS(1).H(1)
(0007)      DOUBLE PRECISION THETA.F.F1.G.H
(0008)      T=DATREC(9)
(0009)      F1=THETA(1)*T/(THETA(2)+T)
(0010)      F=F1+THETA(3)
(0011)      G(1)=T/(THETA(2)+T)
(0012)      G(2)=(THETA(2)+T)*(THETA(2)+T)
(0013)      G(2)=-THETA(1)*T/G(2)
(0014)      G(3)=1.0
(0015)      G(1)=G(1)*THETA(1)
(0016)      G(2)=G(2)*THETA(2)
(0017)      G(3)=G(3)*THETA(3)
(0018)      H(1)=1.0
(0019)      RETURN
(0020)      END
(0021)**END**
```

```
(0001)DATA    AKLIB1.NAP1
(0002)****
(0003)PROB    CONC EFFECT
(0004)DATA       1   0  71   9
(0005)ITEM       1   8   0   0   1
(0006)LABL    SUBJ    DOSE    ORDR    CONC    EFF1    EFF2    EFF3    EFF4    FR
EE
(0007)FORM
(0008)(9F7.1)
(0009)STRC       3   3   1   0   0   1   0   1   0
(0010)THTA       -5.0    50.0     10.0
(0011)DIAG        10.0     10.0     10.0
(0012)DIAG         5.0
(0013)ESTM       02000   4   5   0   0   0   0
(0014)COVR       0
(0015)TABL       2
(0016)SCAT       0   9
(0017)SCAT       9   8
(0018)SCAT       9  10
(0019)SCAT      10  11
(0020)SCAT      10  12
(0021)SCAT       9  11
(0022)SCAT       9  12
(0023)SCAT      10   8
(0024)SCAT       8  11
(0025)SCAT       8  12
(0026)**END**
```

c) The 'pred' and control file for a Langmuir isotherm rearranged in terms of free concentration used to analyse naproxen protein binding data.

```
      SUBROUTINE PRED(ICALL,NE,IND,THETA,DATREC,INDXS,F,G,H)
C
C
C          BINDING PRED
C            N1 & N2 PROP. TO ALB.
C
      DOUBLE PRECISION THETA,H,G,F
      DOUBLE PRECISION XN1,XK1,XN2,XK2,A,B,C,DD,DFDA,DFDB,DFDC
      DIMENSION THETA(4),DATREC(4),H(1),G(4),INDXS(1)
      XB=DATREC(2)
      ALB=DATREC(4)
      XN1=THETA(1) + THETA(5)*ALB
      XK1=THETA(2)
      XN2=THETA(3) + THETA(6)*ALB
      XK2=THETA(4)
      A=XN1+XN2-XB
      B=XN1*XK2 + XN2*XK1 - XK1*XB - XK2*XB
      C=- XK1*XK2*XB
      DD=B*B-4.*A*C
      DD=SQRT(DD)
      F=-B/(2.*A) + DD/(2.*A)
      DFDA=-F/A- C/(A*DD)
      DFDB= -1./(2.*A)-B/(2.*A*DD)
      DFDC=-1./DD
      G(1)=DFDA+DFDB*XK2
      G(2)=DFDB*(XN2-XB)-DFDC*XK2*XB
      G(3)=DFDA+DFDB*XK1
      G(4)=DFDB*(XN1-XB) -DFDC*XK1*XB
      H(1)=F
      RETURN
      END
```

```
DATA    FDUN.BIND
MSFO    FDUN.MSF2
****
PROB    BINDING DATA WITH N1,N2 PROP. TO ALB
DATA      1   0 156   4
ITEM      1   3   C  10   1
INDX      0   0   0   0   0   0   0   0
LABL    SUBJ     BND      FREE     ALB
FORM
(4F10.4)
STRC      6   4   1   1   1   1   0   1   0
THTA      0.000   C.070 000.000   6.900   2.000  12.000
LOWR      0.000   0.050  00.000   4.000    1.5     8.0
UPPR    000.000   0.200 000.000   9.000    5.     15.
DIAG      5.000   0.025 500.000   2.000
DIAG      0.100
ESTM      0 500   3   1   0   1   1
COVR      1
TABL      1   1
TABL      3   1   0   2   0   3   0
SCAT      1   3
SCAT      2   6
SCAT      3   6
SCAT      4   6
SCAT      5   6
SCAT      2   7
SCAT      3   7
SCAT      4   7
SCAT      5   7
```

PUBLICATIONS

Dunagan, F.M.; Kelman, A.W.; McGill, P.E. & Whiting B. (1985). Estimation of the NSAID concentration-effect relationship in rheumatoid arthritis. British Journal of Clinical Pharmacology **19**, 548P.

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PRESENTATIONS AT SCIENTIFIC MEETINGS

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