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DISEASE AND DRUG-INDUCED CHANGES IN NAPROXEN BINDING TO PLASMA

F.A.Ismail, N. Khalafallah and Said A. Khalil
Department of Pharmaceutics, Faculty of Pharmacy,
University of Alexandria, Alexandria Egypt

ABSTRACT

Plasma binding data were generated for the nonsteroidal antiinflammatory drug, naproxen, by equilibrium dialysis. Plasma samples were obtained from 7 healthy volunteers, from 43 uremic patients and from a blood bank. Drug in plasma was equilibrated with buffer across a suitable membrane. Buffer and plasma compartments were analysed by HPLC for free and total naproxen concentrations. Creatinine, urea and albumin plasma levels were determined by suitable methods. Binding of naproxen to healthy plasma exceeded 99% at concentrations attained in therapy. Free naproxen fraction was consistantly higher in uremic plasma with binding values ranging from 89-99%. The correlations examined indicated a dependence of naproxen free fraction on the degree of renal impairment,

indicated by creatinine and urea plasma levels. Binding of naproxen was independent of albumin concentration at plasma albumin levels higher than 15 g/1. Apart from disease, plasma binding of naproxen was also perturbed, but to a lesser degree, by some other nonsteroidal antiinflammatory drugs such as flufenamic acid and aspirin.

The results of the present study indicate that plasma binding of naproxen is impaired in patients suffering from chronic renal failure of different genesis.

INTRODUCTION

Total plasma drug concentrations are usually relied upon in dosage adjustment while, in fact, it is the drug in plasma water that elicits the pharmacologic and toxicologic effects and is manipulated pharmacokinetically by clearance organs. Difficulty in routine determination of free drug concentration is responsible for this discrepancy. Fortunately, the majority of drugs show linear plasma binding in therapeutic concentration such that total plasma drug levels provide a precise index of corresponding free levels. However, drugs are often used under conditions, such as disease and coadministration of other drugs, which perturb plasma binding, resulting in dissociation between free and total drug levels. Measurement of total drug, in such cases, could be misleading particularily for drugs which are extensively

bound to plasma proteins where small changes in percent binding can potentially lead to considerable variation in free drug levels. Studies indentifying conditions which perturb plasma binding and data quantitating changes in free fraction are required to enable physicians safe and efficaceous handling of such drugs in different patient populations.

Naproxen is a nonsteroidal antiinflammatory drug which is extensively bound to plasma proteins. The drug is frequently prescribed in Egypt to different patient populations suffering from inflammatory conditions of the joints among other ailements. The present study reports disease and drug induced changes in naproxen plasma binding.

MATERIALS AND METHODS

The drugs used were kind gifts from their suppliers: naproxen, Syntex Laboratories, palo Alto, Calif., USA; diflunisal, Merck Sharp & Dohme research Lab., NJ, USA; aspirin, Bayer, Leverkusen, West Germany; flufenamic acid, Park Davis & Co., Pontypool, Mon., UK; ibuprofen, Kahira pharmaceutical & Chemical Industrial Co., Egypt, under licence from Boots Company PLC., Nottingham, UK; ketoprofen, Alexandria Pharmaceutical Co., Alexandria, Egypt, under licence of Rhone Poulenc, Paris, France;

fenoprofen calcium, Eli Lilly and Company, Indianapolis, USA; Fentiazac, Wyeth Laboratories Taplow, Maidenhead, Berks, USA; bumadizone calcium, BYK Gulden pharmazeutika, Konstanz West Germany, and diclofenac sodium, Swisspharma S.A.A., Egypt.

Plasma Sources- plasma samples were obtained after consent from seven healthy volunteers and from 43 patients with chronic renal insufficiency attending weekly dialysis sessions in a nearby clinic. Blood samples (5-10 ml) were withdrawn from the healthy volunteers, and from the uremic patients prior to commencing the dialysis session, in heparinized tubes. The blood was centrifuged and the plasma was frozen. One plasma bag (250 ml) was purchased from a blood bank.

Binding Study- Naproxen binding to plasma was determined by equilibrium dialysis in plexiglass cells maintained in a water bath at 37. Plasma was spiked with drug. The Samples (0.8 ml) were then dialysed against an equal volume of isotonic phosphate buffer, PH 7.4, prepared from Na_2HPO_4 (8mM);NaH_2PO_4(1.8mM) and NaCl(77mM). The equilibrium time required was 18 hours.

Using this assembly, binding of naproxen to healthy and uremic plasma was investigated at an initial naproxen plasma level of 100 ug/ml. The possible effect of

heparin, present in the blood collection tubes, on the binding data was also investigated by comparing naproxen binding data determined in serum and heparinized plasma obtained from two healthy volunteers and two uremic patients. In addition, naproxen binding profile, and the effects of plasma dilution and of other antiinflammatory drugs on naproxen binding was studied using blood bank plasma.

HPLC Determination of Free and Total Naproxen- Buffer and plasma compartments were analysed for free and total naproxen by an HPLC method[1].Both compartments were analysed to exclude the effect of drug membrane binding, if any, on the binding results. Diflunisal was used as internal standard, 2 and 50 ug being added to buffer (0.4 ml) and plasma samples (0.5 ml) respectively as well as to corresponding standards. Plasma proteins were precipitated by adding one ml of acetonitrile (acentonitrile for chromatography, E. Merck, Darmstadt) followed by centrifugation. Ten ul of buffer samples and plasma supernatents were injected onto a reversed phase column (Perkin-Elmer, RP-8 column). The mobile phase, consisting of 55% methanol (Methanol for chromatography, E.Merck, Darmstadt) in phosphate buffer, 0.05 M, PH 5.5, was pumped at a flow rate of 1.2 ml/min. The eluent was detected at 264 nm. Retention times were 6.5 and 8.5 minutes for naproxen and i.s. respectively. Recovered

standards were prepared by spiking control (drug free) plasma and buffer with naproxen to cover a concentration range of 1-100 ug/ml. Inter-day coefficients of variation determined in plasma at four concentrations were 19.32% (n=3) at 10 ug/ml, 9.92% (n=10) at 20 ug/ml, 11.35% (n=10) at 50 ug/ml and 5.24% (n=7) at 100 ug/ml. Similarly, inter-day coefficients of variation determined in buffer at three concentration levels were 17.30% (n=4) at 1 ug/ml, 11.65% (n=10) at 2 ug/ml and 4.25% (n=10) at 4 ug/ml. The presence in the analysed samples of the other nonsteroidal antiinflammatory drugs used did not interfere with naproxen, nor with i.s., peaks.

Determination of Creatinine,Albumin and Urea Plasma Level

These determinations were carried out for the same plasma samples used in the binding study. Creatinine was assayed colourimetrically by reacting with picric acid, and albumin was determined colourimetrically by reacting with bromocresol green[2].The chemicals used in the creatinine assay were picric acid, Reidel-de-Haen, A:G. Germany; sulphuric acid, B.D.H. chemicals, Ltd Poole England; sodium hydroxide, Prolabo, Paris; sodium tungstate, analytical grade, U.S.S.R., and creatinine hydrochloride, analytical grade. The chemicals used in albumin assay were bromocresol green, R.A.L. Prolabo, Paris; succinic acid, Fluka AG, chemische Fabrik, CH-9470 Bucks SE, East Germany; Tween 80, B.P., and human serum

albumin of placental origin 20% solution, Institute Merieux S.A., Lyon, France. Interday coefficients of variability for creatinine assay, determined from standards measured on three separate days, were low (0.92% and 0.34% at creatinine plasma levels of 2.3 and 4.5 mg/100 ml). Similarly, coefficients of variablility for albumin assay ranged from 0.71-1.56% over a plasma concentration range of 20-50 g/I. Urea was determined using a urea kit, S 180, BioMerieux laboratory reagents and products, Marcy L'Etoile/ 69260 charbonnieres, les Bains, France.

RESULTS

Free and total plasma naproxen concentrations determined by direct HPLC analysis of buffer and plasma compartments are given in Figure 1. The range of total equilibrium naproxen concentration examined(50-100 ug/ml) covered concentrations usually attained in therapy based on recommended daily doses of naproxen[3], and corresponding steady-state peak and trough plasma levels[4] . Free naproxen concentrations in plasma from healthy volunteers were very low(0.5 ug/ml,mean of seven plasma samples). The extent of binding in these samples exceeded 99% in agreement with previous reports of naproxen binding to human plasma in this concentration range[4,5]. Binding of naproxen to blood bank plasma was

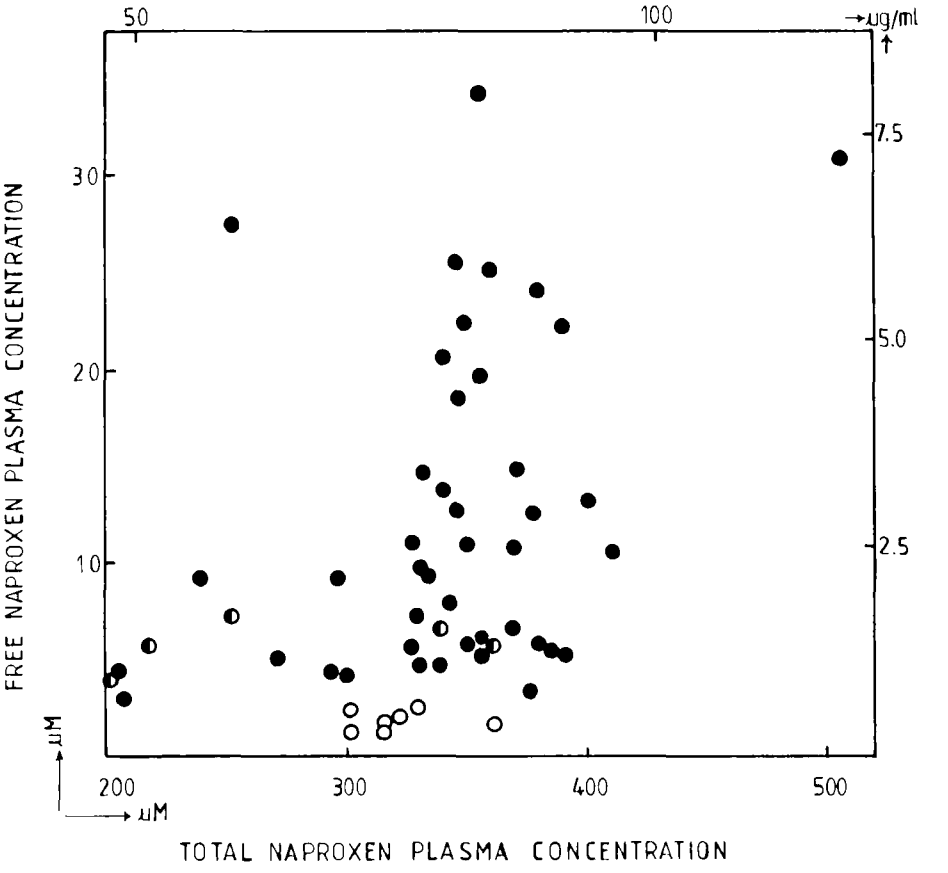

FIGURE 1

Total and free naproxen concentration determined in healthy plasma ○ (7 volunteers), in uremic plasma ● (43 patients) and in blood bank plasma ◐ .

slightly lower (Fig.1). Variation in albumin concentration from donors may account for this decreased binding. Measurement of naproxen binding in plasma from uremic patients showed much higher free naproxen concentration averaging 3.0 ug/ml (mean of 43 patients). The extent of binding dropped below 95% in ten of the samples examined. The lowest binding percent recorded for these

TABLE I
Binding of Naproxen to Serum and Heparinized Plasma, Obtained from Healthy Volunteers and from Uremic Patients, determined at 37 at a Total Naproxen Concentration of 100 ug/ml

Source of Serum/plasma	Percent free naproxen[1] in serum	in heparinized plasma
Healthy volunteers		
1	0.95(0.26)[2]	0.94 (0.07)
2	0.96(0.29)	1.20
Uremic patients		
1	1.33[3]	1.22 (0.39)
2	1.86 (0.91)	2.08 (0.25)

1- Values are the mean of two binding runs.
2- Standard deviation
3- Sample volume did not permit duplication.

samples was 89.1% Only one of the 43 samples examined reached a binding extent of 99%. This sample had the lowest urea level among the uremic plasma samples examined. The presence of heparin in the blood collection tubes did not influence naproxen binding to healthy and uremic plasma (Table I).

Creatinine, urea and albumin levels determined for the different 51 plasma samples are given in Table II.

Judging by these levels, the seven volunteers represented a healthy population in terms of kidney function. As expected, the uremic plasma samples showed a wide-ranged elevated creatinine and urea levels indicating renal impairment of varying severity. Albumin levels in the patient population, however, were similar

TABLE II
Creatinine, Urea and Albumin Plasma Levels Determined in Healthy Volunteers and Uremic Patients.

Plasma constituent	Plasma levels in Normal volunteers[1](n=7) range	mean	Uremic patients(n=43) range	mean
Creatinine, mg/100 ml	0.77-1.38	1.18	1.78-23.47	13.79 n =27[2]
Urea, mg/100 ml	16.04-32.41	25.16	29.63-348.15	162.85 n = 40
Albumin, g/l	24.92-46.22	37.41	22.81-55.70	33.38 n = 40

1- Corresponding levels in blood bank plasma were: creatinine 0.88 mg/100 ml, urea 18.87 mg/100 ml and albumin 16.22 g/l.
2- Plasma volume was insufficient to carry out all three determinations in some of the samples collected.

to those of the normal volunteers. No correction of binding data, determined in uremic plasma, was necessary for albumin concentration.

Plasma creatinine and urea concentrations were correlated with percent free naproxen in normal and uremic plasma (Figure 2). A positive and statistically significant correlation resulted in both cases, in contrast to plasma albumin level which showed poor correlation with naproxen binding. The effect of albumin concentration on naproxen binding was further examined using blood bank plasma diluted to cover a wider range of albumin concentration than was found in the normal and uremic plasma samples (Figure 3). The figure also includes data reported in literature for the effect of albumin concentration on naproxen binding. Good agreement

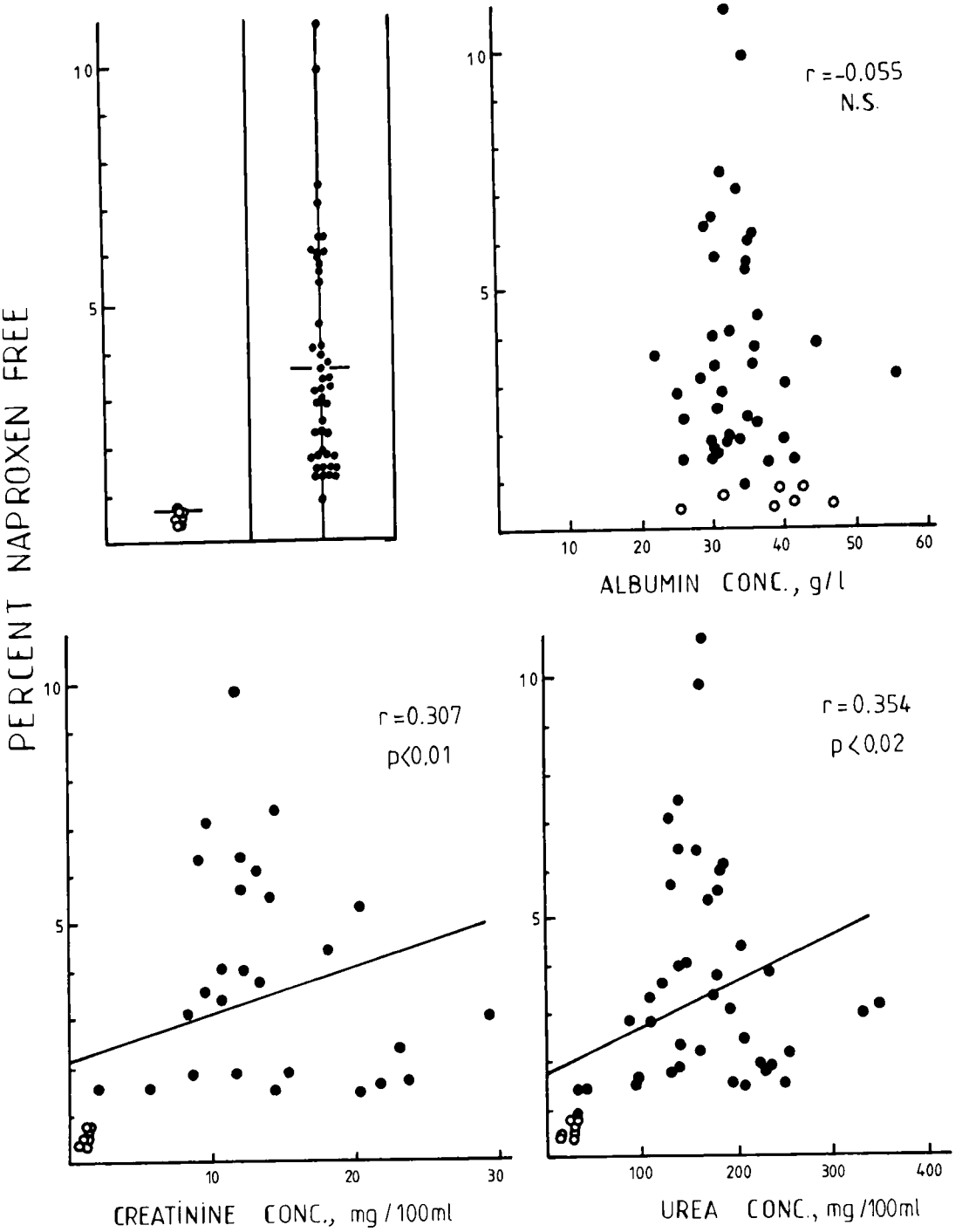

FIGURE 2

Percent naproxen free in healthy O , and uremic ● plasma. Binding was determined by equilibrium dialysis at an initial naproxen plasma concentration of 100 ug/ml.

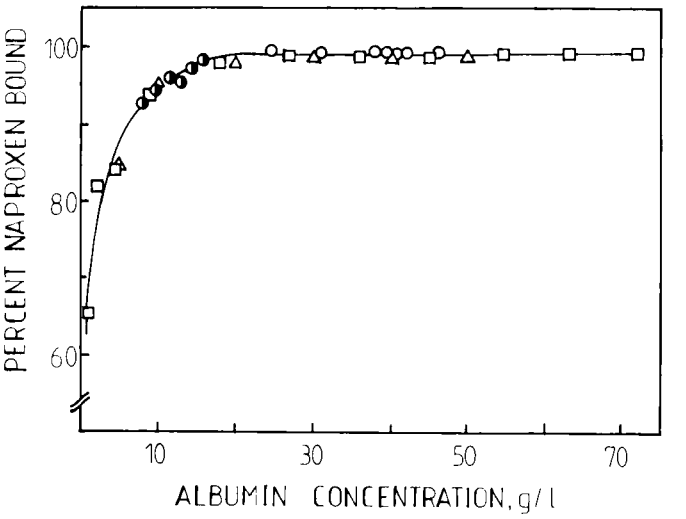

FIGURE 3

The effect of plasma albumin concentration on naproxen binding determined in blood bank plasma ◐ in healthy human plasma ○ and in human albumin solution (data from reference 12 □, and from reference 6 △). The initial naproxen concentration ranged from 20-120 ug/ml.

was observed between results of the different studies. Only at an albumin level below 15 g/1 was naproxen binding perturbed (Figure 3).

Binding of naproxen to blood bank plasma was linear (fraction bound > 0.98) within a total naproxen concentration range of 20-120 ug/ml, beyond which percent bound declined. Scatchard plot (figure 4) yielded binding constants values of 5.9×10^5 M^{-1} and 2.0×10^4 M^{-1} for K_1 and K_2 respectively. The number of primary and secondary binding sites were 1.16 (n_1) and 8.17 (n_2). Binding of naproxen to blood bank plasma in

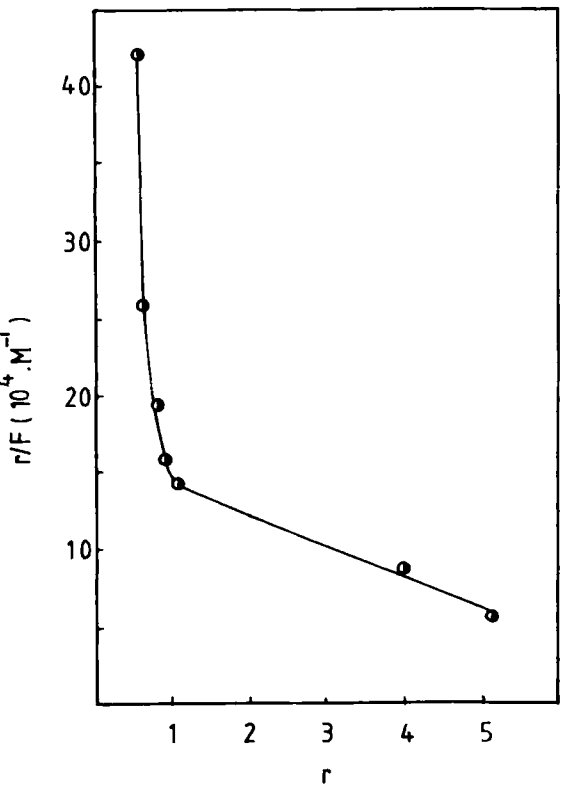

FIGURE 4

Scatchard plot for the binding of naproxen to human plasma at 37°.

absence and presence of some nonsteroidal antiinflammatory drugs, added in therapeutic concentrations, is shown in figure 5. Ibuprofen (50 ug/ml), as a potential competing ligand, showed no effect. The binding profiles of naproxen alone and in presence of ibuprofen were superimposed. Similarly, but not shown in figure 5, ketoprofen (5 ug/ml), fenoprofen calcium (25 ug/ml), fentiazac (0.8 ug/ml), bumadizone calcium (50 ug/ml),

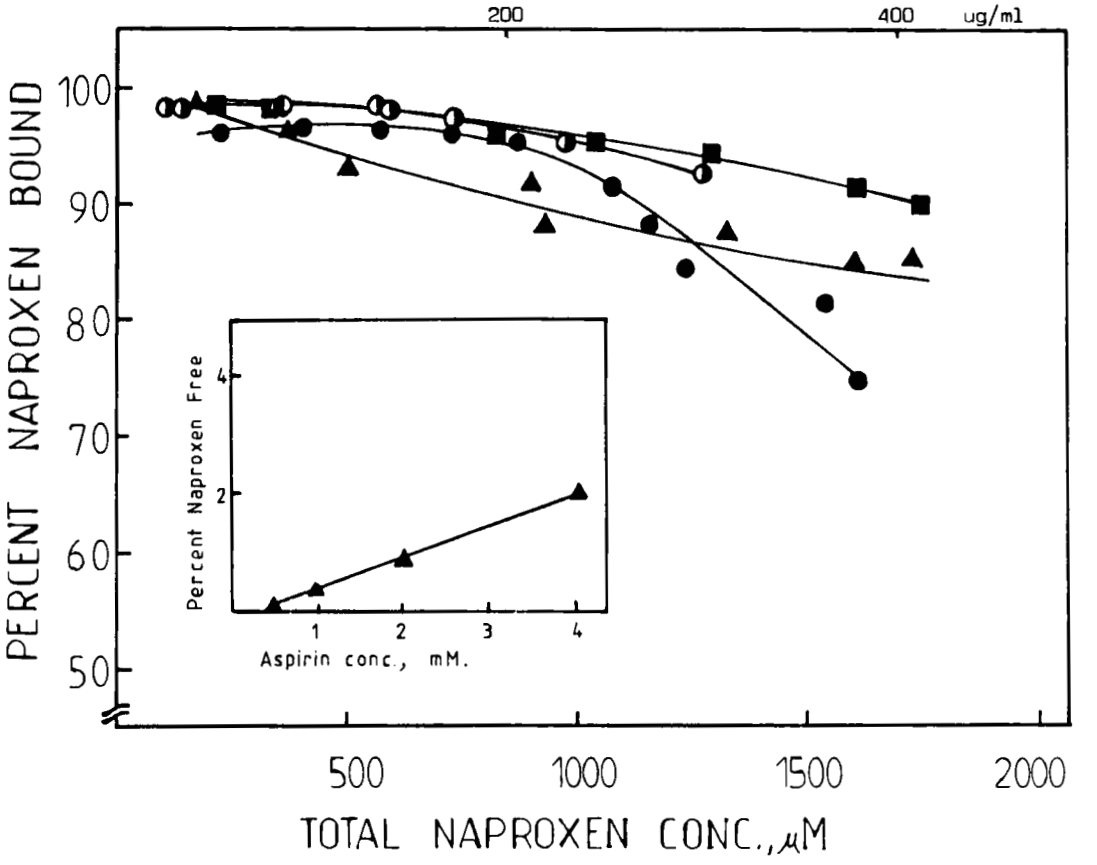

FIGURE 5

Binding of naproxen to blood bank plasma ● , in presence of flufenamic acid, (50 ug/ml) ◐ of aspirin, (720 ug/ml)▲ and of ibuprofen, (50 ug/ml) ■.

dicolofenac sodium (2 ug/ml), and aspirin (100 ug/ml) had no effect on naproxen binding at an initial naproxen plasma level of 100 ug/ml. Flufenamic acid (50 ug/ml), on the other hand, affected naproxen binding. The binding profile showed a decline in fraction bound particularly at naproxen concentration greater than 230 ug/ml.

Although aspirin at a level of 100 ug/ml did not perturb naproxen binding, the presence of increasing concentrations of aspirin caused a linear increase in naproxen free fraction (insert, figure 5). The binding profile of naproxen was displaced over the whole concentration range examined in presence of 4000 uM (∽ 700 ug/ml) of aspirin.

DISCUSSION

The present study has looked at free naproxen plasma concentrations resulting from equilibrating naproxen, in vitro with human plasma from different sources. Free naproxen concentrations in uremic plasma were consistantly high compared to control plasma. The results indicated a definite increase in naproxen free fraction in the patient population examined. These patients will probably respond to naproxen therapy at relatively lower total plasma concentrations then nonuremic patients.

The patient population examined covered a wide range of renal impairment as indicated by creatinine and urea levels determined for these patients in the same plasma samples used in the binding study. The correlations examined indicated that the severity. of renal impairment was a determinant factor in decreasing naproxen binding. Albumin plasma levels in the uremic patients were comparable to normal volunteers. This

provided an opportunity to study the effect of endogenous substances, such as creatinine and urea, on naproxen binding without further perturbation in binding resulting from a depleted albumin pool.

The scatter of data points in the correlations examined indicated that factors other than elevated creatinine and urea levels must have altered naproxen binding in some of the samples examined. Free fatty acids were not determined in the percent study and may have contributed[6]. A modified albumin pool may have been involved. Evidence of altered albumin in uremia has been reported[7]. Plasma albumin concentration, per se, did not influence naproxen binding unless the albumin pool was strongly depleted as indicated by examining naproxen binding in diluted plasma. This is not unusual for a drug such as naproxen with high binding to plasma.

Some concern has been expressed lately at the possible effect of heparin on drug binding through stimulating lipolysis; the resulting increase in the fatty acid may decrease drug binding[8]. The binding data obtained using serum and heparinized plasma harvested from the same blood sample in healthy volunteers and uremic patients, did not support this assumption under the conditions of the present study.

Displacement of naproxen by other antiinflammatory drugs had been reported[9-11], and was demonstrated in the

present study using blood bank plasma. However, judging by the degree of displacement in relation to drug and displacer concentration, the clinical consequences of these interactions are probably insiginifcant. Whether or not such interactions would be more pronounced in uremic patients requires exploring.

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