International Journal of Pharmaceutics, 16 (1983) 215–223
Elsevier

Binding of naproxen to human albumin. Interaction with palmitic acid

M.V. Calvo and A. Dominguez-Gil

Practical Pharmacy Department, Faculty of Pharmacy, University of Salamanca, Salamanca (Spain)

(Received December 1st, 1982)
(Accepted February 23rd, 1983)

Summary

A study was carried out on the binding of naproxen to human albumin using a molecular filtration technique. Free-drug levels were determined by spectrofluorometry.

Naproxen binds to human albumin in high proportions — the fraction of free-drug ranging between 0.02 and 0.1 for a concentration range of between 20 and 500 μg/ml.

The binding percentage is not modified by variations in the pH (5–7.8) and albumin concentration (1–7 g%). According to the graphical treatments of Scatchard, Klotz and Scott, two classes of binding sites of naproxen to human albumin were determined.

Palmitic acid causes a displacement of the anti-inflammatory agent in its binding to albumin, the fraction of free-drug increasing with the concentration of fatty acid.

Introduction

As is the case with other non-steroid anti-inflammatory agents, naproxen is bound to plasma proteins in high proportions. For a concentration close to 50 μg/ml, the fraction of free-drug is less than 0.01 (Runkel et al., 1974). Binding to plasma protein may limit the availability of the drug to the receptors, biotransformation and excretion mechanisms; it may act as a physiological solubilizer in its distribution and may even behave as a reservoir of the drug within the organism.

Albumin is an important vehicle for numerous endogenous substances, such as hormone, bilirubin and free fatty acids. The latter are bound to albumin in high proportions and show affinity constants higher than those of most drugs (Ashbrook

et al., 1975). It is therefore possible that a competitive type interaction could take place between free fatty acids and various drugs for the binding sites of plasma protein. Such an interaction has already been reported in 'in vitro' studies on phenylbutazone, salicylates, diphenylhydantoin (Rudman et al., 1971), and in 'in vivo' studies on warfarin and diphenylhydantoin (Gugler et al., 1974).

The purpose of the present work is to show the possible displacement that free-fatty acids can induce in the binding of naproxen to plasma proteins.

Materials and Methods

Materials

Naproxen (Syntex, Palo Alto, CA); human albumin in 20% aqueous solution (Behring); sodium palmitate (Sigma).

Determination of plasma protein binding

The binding of naproxen to human albumin was studied, analyzing the influence of different factors.

(a) Albumin concentration: solutions of naproxen at 30 μg/ml were used in solutions of human albumin ranging between 0.1 and 7.2 g%.

(b) pH of the solution: different solutions of naproxen at 50 μg/ml were used in solutions of albumin (4%) with pH values ranging between 5 and 7.8.

(c) Drug concentration: solutions of naproxen ranging between 15 and 500 μg/ml in 4.0% albumin solutions were used.

(d) Free fatty acids: a concentration of naproxen at 50 μg/ml was used in different solutions of palmitic acid/albumin with molar relationships ranging between 1 and 7. Also, different solutions of naproxen were prepared at concentrations between 15 and 500 μg/ml in solutions of palmitic acid/albumin at a molar relationship of 4.

The determination of the binding capacity of naproxen to plasma proteins was carried out by ultrafiltration, after the anti-inflammatory agent had been in contact with human albumin for 5 h at 37°C. Filtration was performed using a polycarbonate cell of 3 ml capacity, equipped with a molecular filtration Millipore membrane, type PTGC 0.13 10, capable of retaining molecules with molecular weights greater than 10^4. The operation was carried out with continuous shaking under a nitrogen pressure of 40 psig.

The naproxen concentration in the protein solution and in the ultrafiltrate was determined by a spectrofluorometric technique, since the compound has the property of showing natural fluorescence in an aqueous medium (Anttila, 1977). The spectrofluorometer used was a Carl-Zeiss Mod. LX-501; excitation and emission wavelengths were 330 and 360 nm, respectively.

Protein concentration was measured by the biuret method and that of free-fatty acids according to the method of Duncombe (1964).

Determination of binding parameters

From the experimental results, the association constant and the possible classes of

binding sites to the albumin molecule were determined. These parameters were obtained by the methods of Scatchard, Klotz and Scott (Vallner et al., 1976).

The fraction of bound-drug was calculated according to the equation established by Goldstein et al. (1968).

Results and Discussion

Fig. 1 shows the binding percentages of naproxen in human plasma and in solutions of albumin at a concentration similar to that of plasma. Naproxen is bound in high proportions to the plasma proteins, though the degree of binding is governed by the drug concentration. Thus, at a concentration of 100 μg/ml, the degree of binding in plasma and albumin is 99 and 96%, respectively, while at a concentration of 500 μg/ml, the respective percentages were 93 and 87%. It may be seen from these results that although naproxen is principally bound to albumin, other proteins must also participate in the process, quite probably globulins.

Because of its affinity for human albumin, the effect of various factors in the interaction was studied.

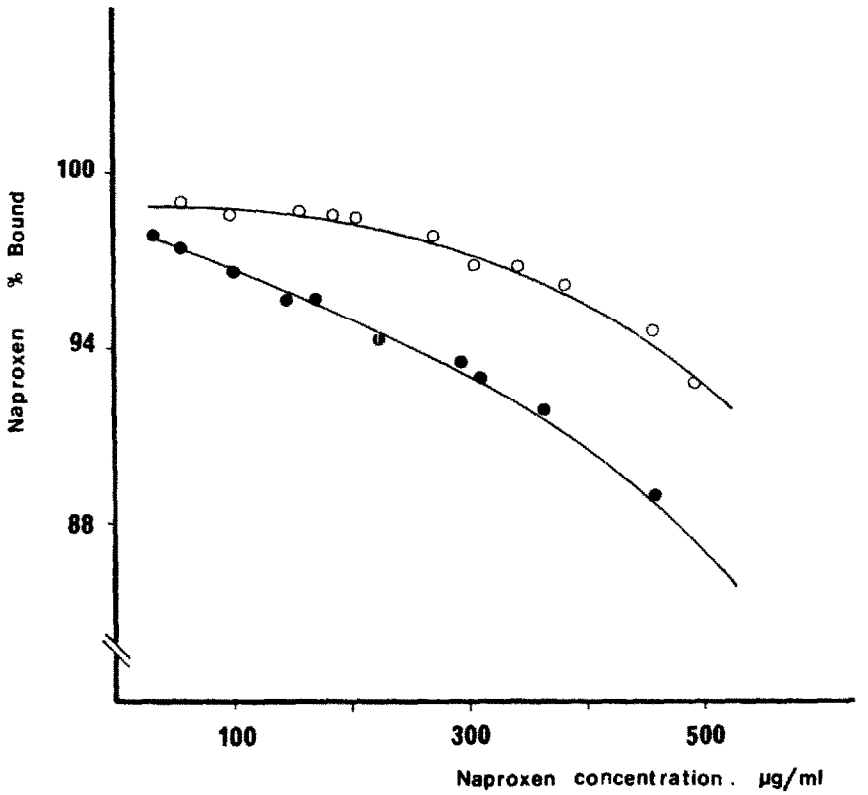

Fig. 1. Binding percentages of naproxen in human plasma and in solutions of human albumin with the same concentration as in serum (4 g%). Plasma (○); human albumin (●).

TABLE 1

PERCENTAGE OF NAPROXEN BOUND IN ALBUMIN SOLUTIONS OF DIFFERENT CONCENTRATIONS

Albumin (g%)	Naproxen concentration (μg/ml)			Percent bound
	Total	Free	Bound	
7.20	25.84	0.21	25.63	99.19
6.30	27.74	0.21	27.53	99.24
5.40	27.37	0.30	27.07	98.90
4.50	28.66	0.43	28.23	98.50
3.60	27.22	0.41	26.81	98.49
2.70	27.74	0.33	27.41	98.81
1.80	27.37	0.64	26.73	97.66
0.90	27.22	1.57	25.65	94.23
0.80	26.81	1.89	24.92	92.95
0.45	26.30	4.18	22.12	84.11
0.22	27.74	4.95	22.79	82.16
0.12	27.22	9.45	17.77	65 .8

Albumin concentration

Table 1 shows the variations in the binding percentages of naproxen to human albumin for concentrations of the latter ranging between 7.2 and 0.1 g%. Only when the albumin concentration is below 1 g% may an important decrease be seen in the degree of binding of the drug. Jusko and Gretch (1976) report that in drugs with a high binding capacity to plasma proteins no changes are seen to take place in the degree of binding, even though considerable modifications are introduced in the protein concentration.

pH of the solution

Table 2 shows the binding percentages of naproxen in different buffered solutions

TABLE 2

BINDING PERCENTAGES OF NAPROXEN TO HUMAN ALBUMIN (4%) IN BUFFER SOLUTIONS AT DIFFERENT pH VALUES

pH	Concentration of naproxen (μg/ml)			Percent bound
	Total	Free	Bound	
5.0	54.39	2.72	51.67	95.50
6.0	61.13	2.29	58.84	96.25
6.2	57.49	1.28	56.21	97.77
6.7	59.05	1.28	57.77	97.83
7.0	54.39	1.28	53.11	97.65
7.5	56.46	1.21	55.25	97.86
7.8	54.39	1.14	53.25	97.90

of human albumin at 4% and at pH values ranging between 5 and 7.8. It may be seen that variations in the pH do not affect the interaction, because the electrostatic-like forces do not constitute the principal mechanism participating in the bond.

Drug concentration

Analysis was carried out of the binding of naproxen to human albumin at 4%, for drug concentrations ranging between 15 and 500 μg/ml. From the binding data we obtained the Klotz, Scott and Scatchard plots defining the binding kinetics of naproxen to plasma proteins, and these permit the calculation of the number of classes of binding sites, the number of binding sites and the binding constants in each class.

Table 3 shows the binding parameters of naproxen to human albumin obtained from the 3 aforementioned methods. The values are all very similar and naproxen may be said to have a primary class of binding sites to human albumin with a high affinity constant, $K_1 = 5\text{–}8 \times 10^7\ M^{-1}$ and a secondary binding site which also has a high affinity constant, $K_2 = 7\text{–}10 \times 10^6\ M^{-2}$.

Fig. 2 shows the fraction of bound-drug as a function of the total drug concentration, calculated with the affinity constants obtained with the Scott plot. It may be seen how the drug concentration influences its binding to the protein. On increasing the total drug concentration, the different binding sites become saturated and the

TABLE 3

BINDING PARAMETERS OF NAPROXEN IN SOLUTIONS OF HUMAN ALBUMIN AT 4% OBTAINED FROM THE 3 GRAPHICAL PROCEDURES. K (M^{-1}) IS THE ASSOCIATION CONSTANT AND "n" IS THE NUMBER OF BINDING SITES. (r represents the moles of drug bound per mole of protein, and D is the concentration (μM) of free-drug).

Parameter	Graphical value	$K_1 \times 10^7$	n_1	$K_2 \times 10^6$	n_2
1/r versus 1/(D) (Klotz)					
Intercept 1	0.82	8.15	1.23	6.68	5.99
Slope 1	0.01				
Intercept 2	0.17				
Slope 2	0.03				
r/(D) versus r (Scatchard)					
Intercept 1	80.63	4.84	1.67	9.32	4.57
Slope 1	48.35				
Intercept 2	42.57				
Slope 2	9.32				
D/r versus D (Scott)					
Intercept 1	0.01	6.58	1.38	10.00	4.35
Slope 1	0.72				
Intercept 2	0.02				
Slope 2	0.23				

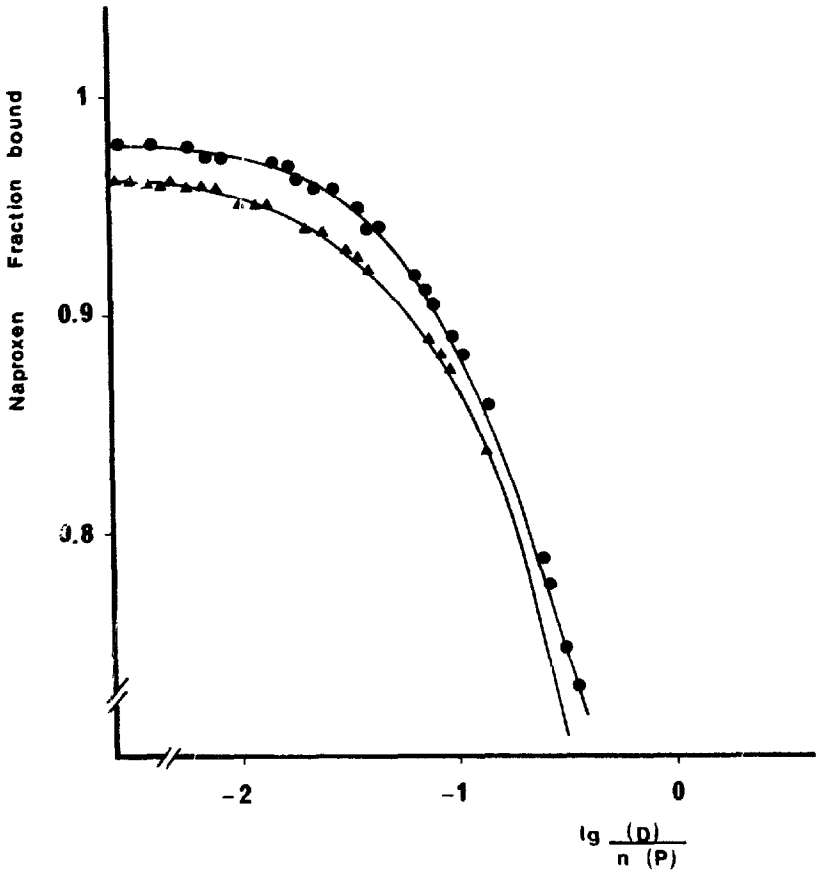

Fig. 2. Fraction of naproxen bound to human albumin at 4% as a function of the total drug concentration, calculated for the two association constants obtained by the graphical treatment of Scott. K_1 (●), K_2 (▲).

fraction of bound-drug decreases; such a decrease is greater for the affinity constant with the highest value.

Free-fatty acids

Fig. 3 shows the percentages of free-drug as a function of the concentration of palmitic acid, for a naproxen concentration of 50 μg/ml. This percentage increases with the concentration of fatty acid, though only when the palmitic acid/albumin relationship is high can displacement be of importance. Similar results have been obtained with other drugs which bind to albumin in high proportions (Spector and Santos, 1973).

Fig. 4 shows the binding percentages of naproxen to human albumin obtained in the absence and in the presence of palmitic acid at a molar relationship with albumin of 4. Fig. 5 shows the binding results according to Scatchard's plot. These results indicate that palmitic acid displaces naproxen from its binding sites with albumin. In the presence of palmitic acid, the affinity constant for the primary binding sites has a value of approximately 50% of that obtained in its absence, while

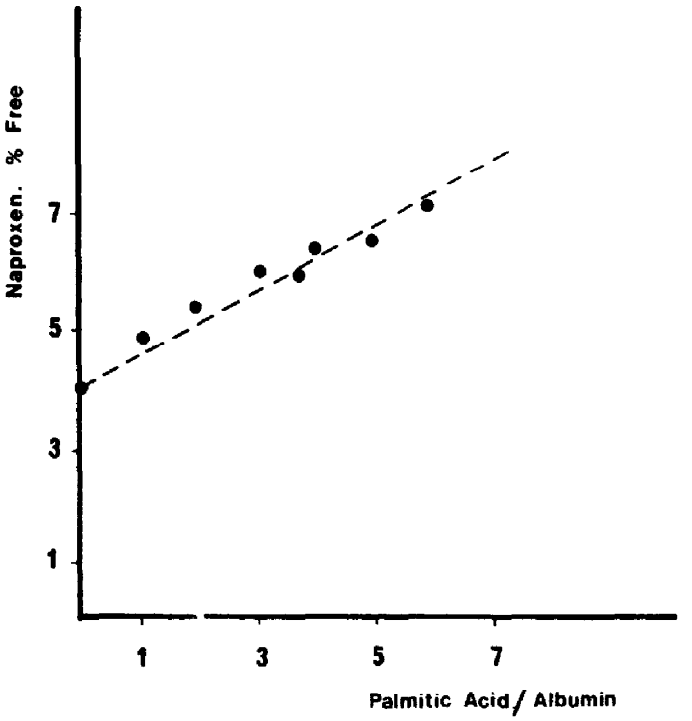

Fig. 3. Percentage of naproxen unbound to human albumin as a function of the molar relationship between palmitic acid and albumin. Albumin 1.159×10^{-3} mM; naproxen concentration 50 μg/ml.

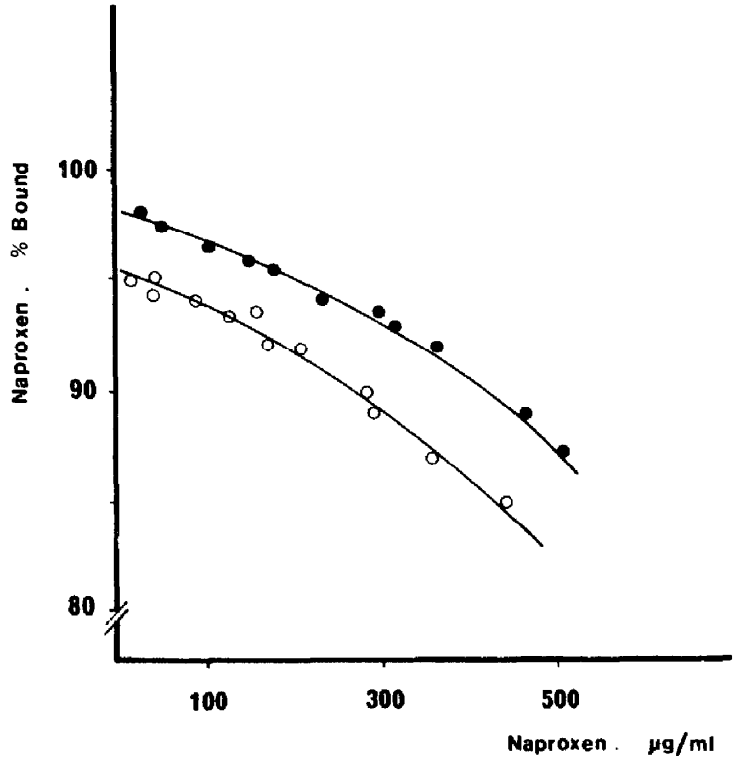

Fig. 4. Binding of naproxen to human albumin (4%) in the absence (●) and presence (○) of palmitic acid. Molar relationship between palmitic acid and albumin was 4.

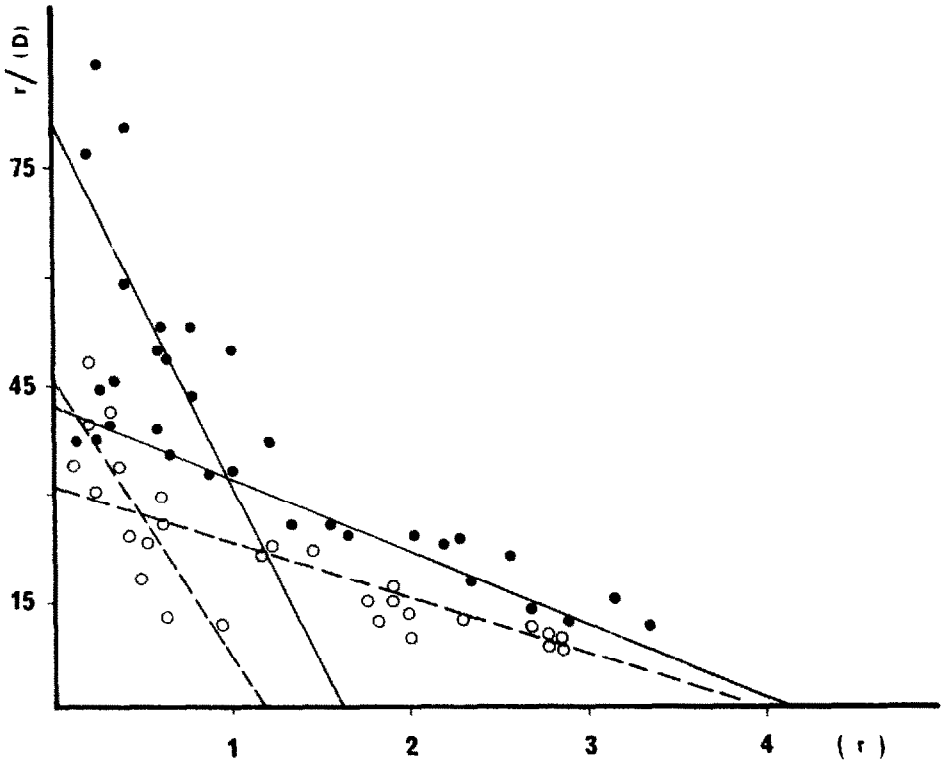

Fig. 5. Scatchard plot of the binding data of naproxen to human albumin at 4%, in the absence (●———●) and presence (○———○) of palmitic acid. Molar relationship between palmitic acid an albumin was 4.

the association constant for the secondary binding sites and the number of binding sites in each class are not significantly modified. Thus, the displacement of naproxen by palmitic acid may be said to take place principally in the primary binding sites to albumin.

Such an interaction is of great interest from the pharmacokinetic point of view, since in various clinical situations, such as diabetes mellitus, acute myocardial infarction, hyperthyroidism, etc (Morehouse et al., 1963; Kurien and Oliver, 1966; Rich et al., 1959), the serum levels of free-fatty acids are seen to be raised for lengthy periods; this could cause an increase in the fraction of free-drug and hence lead to greater access to the peripheral tissues and a possible increase in its pharmacological activity.

Acknowledgements

We would like to thank Mr. Skinner, for his help in translating this paper into English.

References

Anttila, M., Fluorometric determination of naproxen in serum. J. Pharm. Sci., 66 (1977) 433–434.

Ashbrook, J.D., Spector, A.A., Santos, E.C. and Fletcher, J.E. Long chain fatty acid binding to human plasma albumin. J. Biol. Chem., 50 (1975) 2333–2338.

Duncombe, W.G., The colorimetric microdetermination of non-esterified fatty acids in plasma. Clin. Chim. Acta, 9 (1964) 122–125.

Goldstein, A., Aronow, L. and Kalman, S.M., Principles of Drug Action: the Basis of Pharmacology, John Wiley, New York, 1968, p. 161.

Gugler, R., Shoeman, D.W. and Azarnoff, D.L., Effect of in vivo elevation of free fatty acids on protein binding of drugs. Pharmacology 12 (1974) 160–165.

Jusko, W.J., and Gretch, M. Plasma and tissue protein binding of drugs in pharmacokinetics. Drug Metab. Rev. 5 (1976) 43–140.

Kurien, V.A., and Oliver, M.F. Serum free fatty acids after acute myocardial infarction and cerebral vascular occlusion. Lancet, ii (1966) 122.

Morehouse, J.A., Steinberg, J. and Rosen, N.J., Sex differences in serum free fatty acid levels in diabetic subjects. J. Clin. Endocr. Metab., 23 (1963) 1080.

Rich, C.E., Bierman, E.L. and Schwartz, I.L. Plasma non-esterified fatty acids in hyperthyroid states. J. Clin. Invest., 38 (1959) 275.

Rudman, D., Bixler, T.J. and Del Rio, A.C. Effect of free fatty acids on binding of drugs by bovine serum albumin, by human serum albumin and by rabbit serum. J. Pharmacol. Exp. Ther., 176 (1971) 261–272.

Runkel, R., Forchielli, E., Sevelius, H., Chaplin, M. and Segre, E., Nonlinear plasma level response to high doses of naproxen. Clin. Pharmacol. Ther., 15 (1974) 261–266.

Spector, A.A. and Santos, E.C., Influence of free fatty acid concentration on drug binding to plasma albumin. Ann. N.Y. Acad. Sci., 226 (1973) 247–258.

Vallner, J.J., Perrin, J.H. and Wold, S., Comparison of graphical and computerized methods for calculating binding parameters for two strongly bound drugs to human serum albumin. J. Pharmacol. Sci., 65 (1976) 1182–1187.