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Binding of non-steroidal anti-inflammatory drugs to human serum albumin

M.T. Montero, J. Estelrich and O. Valls

Unitat de Fisico-Química, Departament de Farmàcia, Facultat de Farmàcia, Universitat de Barcelona, 08028 Barcelona (Spain)

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Summary

Binding of four nonsteroidal anti-inflammatory drugs (ibufenac, ibuprofen, flurbiprofen and butibufen sodium) to human serum albumin has been determined in vitro by equilibrium dialysis. Despite their similar chemical structures, the binding parameters obtained were different. Whereas flurbiprofen and ibuprofen presented nearly a single primary binding site, butibufen sodium and ibufenac afforded three such sites. The number of secondary binding sites have also been determined; the protein concentration had no effect on the binding parameters.

Introduction

A large variety of small molecules and drugs are transported in the circulation bound to plasma proteins. This binding of drugs to plasma proteins is an important parameter in pharmacokinetic studies, since the extent and affinity of such binding influences the distribution of the drug in the body (Vallner, 1977, Tillement et al., 1980).

A number of qualitative and quantitative techniques have been used to study the interaction between drugs and macromolecules (Meyer and Guttman, 1968; Weder and Bickel, 1970, Halfman and Nishida, 1972; Kurz et al., 1977), equilibrium dialysis being the most classical procedure employed to estimate serum protein binding of drugs.

Nonsteroidal anti-inflammatory drugs (NSAIDs) as a group are among the most widely used medications. The greatest pharmacological activity is found in some substituted phenylalkanoic acids. In this paper, we describe various aspects of the binding of four NSAID arylalkanoic acid derivatives: ibufenac (IBF), 4-isobutylacetic acid; ibuprofen (IBP), 2-(4-isobutylphenyl)propionic acid; flurbiprofen (FBP), 2-(2-fluoro-4-biphenyl)propionic acid; butibufen (BUT), 2-(4-isobutylphenyl)butyric acid; to human serum albumin (HSA) (Fig. 1). Since they are structurally related it was considered of interest to determine whether these compounds have similar binding properties.

Correspondence: M.T. Montero, Unitat de Fisico-Química, Departament de Farmàcia, Facultat de Farmàcia, Universitat de Barcelona, 08028 Barcelona, Spain.

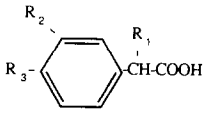

Fig. 1. Chemical structures of ibufenac, ibuprofen, flurbiprofen and butibufen.

NSAIDs	R_1	R_2	R_3
Ibufenac	-H	-H	$-CH_2-CH-(CH_3)_2$
Ibuprofen	$-CH_3$	-H	$-CH_2-CH-(CH_3)_2$
Flurbiprofen	$-CH_3$	-F	$-C_6H_5$
Butibufen	$-CH_2-CH_3$	-H	$-CH_2-CH-(CH_3)_2$

There are several experimental facts that show a dependence between binding parameters and protein concentration. For this reason, in order to demonstrate a possible dependence of the binding on protein concentration, ligand concentrations were maintained constant while the protein concentration was varied in a series of experiments.

It is important not only to calculate the affinity of drugs for albumin but also to classify their binding sites so that drug interaction can be predicted. The determination of individual binding sites for a drug bound to HSA simplifies comparisons with binding of other drugs. Two binding sites have been established on HSA: site I or the warfarin site, and site II or the diazepam site (Sudlow et al., 1975). Drug binding site I contains the single tryptophan residue of HSA. The effect of each drug on the intrinsic fluorescence of albumin thus allows one to determine whether these NSAIDs are site I or II drugs.

Materials and Methods

Human serum albumin, fraction V (lot no. 115F-9330) was obtained from Sigma (St. Louis, MO).

Albumin concentrations were measured spectrophotometrically using a Hewlett-Packard A-8531 diode array spectrophotometer. A molecular weight of 66 500 and a molar absorption coefficient of 32 430 $M^{-1}\,cm^{-1}$ at 278 nm were used.

All chemicals were analytical reagent grade. Water was double-distilled, the final stage being carried out in the presence of potassium permanganate in a borosilicate apparatus and then purified through a Milli-Q system (Millipore, U.S.A.).

The NSAIDs were gifts and used as supplied by the following manufacturers: IBF and FBP, Liade Lab. (Madrid); IBP, Lab. Casen (Zaragoza); BUT, ESSEX-España (Madrid). Owing to the low solubility of butibufen it was used as the sodium salt.

All experiments were performed in phosphate buffer (0.067 M) at pH 7.4 and 0.15 ionic strength.

Equilibrium dialysis

Experiments were carried out at 37 °C for 4 h under constant stirring at 20 rpm. using the Dianorm system (Diachema, Switzerland), with 2.5-ml dialysis cells. Dialysis membranes (molecular weight cut-off 12 000) were conditioned prior to use according to the manufacturer and were used immediately after preparation.

Preliminary distribution studies showed that equilibrium between both sides of the dialysis membrane was achieved within approx. 3.5 h and that significant binding of the NSAIDs to the dialysis membrane or cell walls of the apparatus did not occur.

The total and free concentrations of NSAIDs were determined by spectrophotometry at the following analytical wavelengths: 266 nm for IBP, IBF and BUT and 248 nm for FBP. The molar absorption coefficients calculated and the total concentration of NSAIDs and HSA used are listed in Table 1.

TABLE 1

Total concentrations of NSAIDs and HSA and molar absorption coefficients used

Compound	Concentration		ϵ (M^{-1} cm^{-1})
	[NSAIDs] ($\times 10^{-4}$) (M)	[HSA] ($\times 10^{-4}$) (M)	
Ibufenac	0.023–0.640	0.49–7.67	295
Ibuprofen	0.075–4.193	0.57–5.97	323
Flurbiprofen	0.133–4.850	0.60–5.35	19 800
Butibufen sodium	0.027–0.455	0.55–7.53	373

The binding parameters – number of binding sites and association constants – were determined from plots of r/D_f vs r, where r is the molar ratio of bound drug and serum albumin concentrations and D_f is the molar concentration of free drug (Scatchard, 1949).

Results and Discussion

Scatchard plots of the binding data are shown in Figs 2–5. Each data point represents the average of two determinations from an equilibrium dialysis experiment. The Scatchard plots were non-linear in all cases, suggesting a multiple class binding. The calculated binding constants and number of binding sites corresponding to primary $K_1 n_1$ and secondary sites $K_2 n_2$ on the protein are summarized in Table 2.

The concentrations of NSAIDs used do not cover a very broad range. This is partly due to both the relatively low sensitivity of the analytical technique and the low solubility of the compounds (except in the case of BUT).

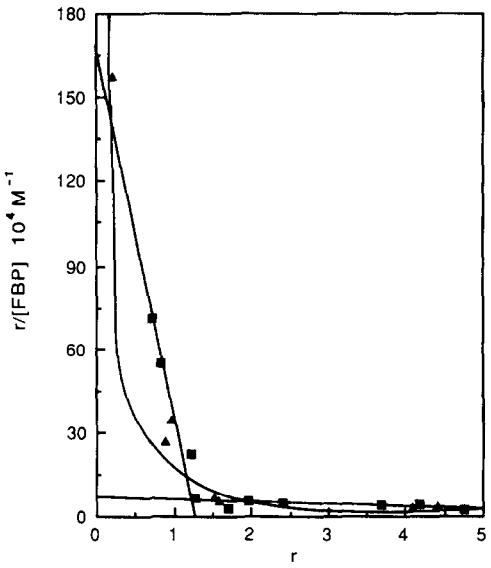

Fig. 3. Scatchard plots for the binding at several concentrations of flurbiprofen to 6.01×10^{-5} M (■) and 1.76×10^{-4} M (▲) human serum albumin at pH 7.4 and 37 °C using equilibrium dialysis. Points, experimental; curve, theoretical.

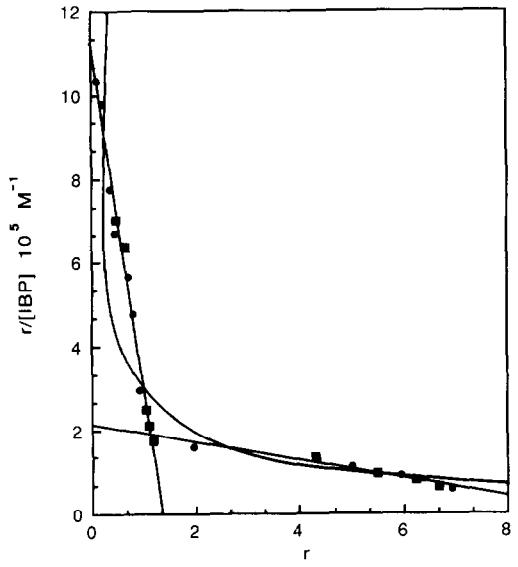

Fig. 2. Scatchard plots for binding at several concentrations of ibuprofen to 7.06×10^{-5} M (■) and 5.84×10^{-4} M (●) human serum albumin at pH 7.4 and 37 °C using equilibrium dialysis. Points, experimental; curve, theoretical.

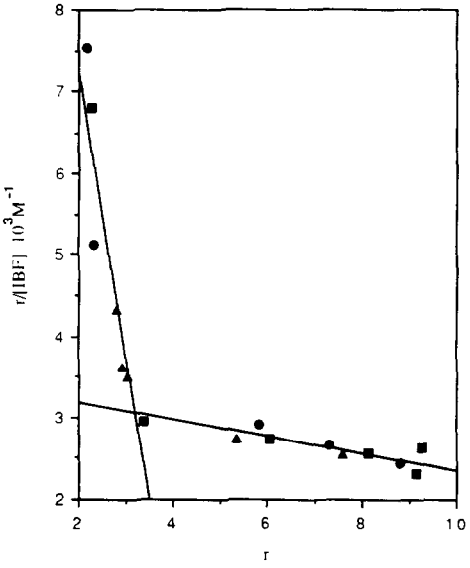

Fig. 4. Scatchard plots for the binding at several concentrations of ibufenac to 5.56×10^{-5} M (■), 1.73×10^{-4} M (▲) and 7.10×10^{-4} M (●) human serum albumin at pH 7.4 and 37 °C using equilibrium dialysis.

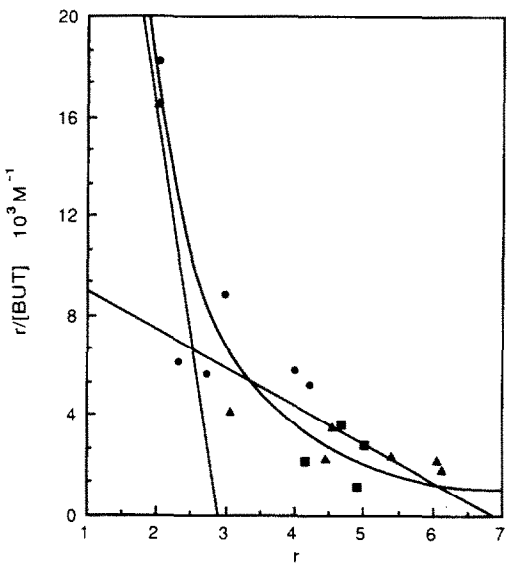

Fig. 5. Scatchard plots for the binding at several concentrations of butibufen sodium to 6.16×10^{-5} M (■), 1.76×10^{-4} M (▲) and 7.12×10^{-4} M (●) human serum albumin at pH 7.4 and 37°C using equilibrium dialysis. Points, experimental; curve, theoretical.

Binding of flurbiprofen and ibuprofen to human serum albumin

Although FBP and IBP (Figs 2 and 3) bind to HSA at about one primary binding site, the K_1 value was higher for FBP than for IBP.

IBP binds strongly to a single primary binding site with an association constant of 7.93×10^5 M^{-1} and 9–10 secondary sites with an association constant of 2.22×10^4 M^{-1}. Based on the above results and bearing in mind that the maximum therapeutic levels of IBP in humans are within the range 20–40 μg/ml and that the physiological concentration of albumin is 35.0–45.8 μg/ml, we can establish that IBP will be bound predominantly to a single binding site on the protein. Consequently, the number of free binding sites will be high and hence other drugs are unlikely to produce any competitive inhibition on the albumin-IBP interaction.

FBP presents a primary affinity constant of 1.38×10^6 M^{-1} with 1.19 binding sites. These results are in good agreement with literature values (Kober and Sjöholm, 1980; Honoré and Broderson, 1984; Aarons et al., 1985; Girgis et al., 1985). On the other hand, the number of secondary sites was between 7 and 8 with an association constant of 0.898×10^4 M^{-1}.

TABLE 2

Binding constants for the binding of NSAIDs to HSA at 37°C and pH 7.4 using equilibrium dialysis

Compound	Primary binding site		Secondary binding site	
	n_1	K_1 (M^{-1})	n_2	K_2 (M^{-1})
Butibufen sodium	3.11	7.08×10^3	7.58	1.09×10^3
Ibufenac	3.04	4.04×10^3	37.67	0.86×10^2
Ibuprofen	1.13	7.93×10^5	9.67	2.22×10^4
Flurbiprofen	1.19	1.38×10^6	7.69	8.98×10^3

K_1 and K_2, association constants; n_1 and n_2, number of binding sites per molecule obtained from the plot $r/[D_f]$ against r.

Binding of ibufenac and butibufen sodium to human serum albumin

The graphical estimation of the binding parameters of IBF and BUT (Figs 4 and 5) indicated that the interaction of both NSAIDs with HSA occurred with three primary albumin-binding sites and an association constant of 10^{-3} M^{-1} was obtained. While the values of K_1 and n_1 were very similar as regards the affinity for the secondary binding sites of HSA, there was a significant difference between IBF and BUT.

On the one hand, BUT shows very similar values of K_1 and K_2 and therefore lacks specificity in the binding to HSA, under the experimental conditions used, with respect to differentiating between the high- and low-affinity binding sites. On the other, IBF binds weakly to 37–38 secondary sites with an association constant of 0.86×10^2 M^{-1}. Such a value as well as the large number of secondary sites is indicative of low-affinity binding of IBF to HSA when the primary sites are occupied.

Effect of protein concentration on binding

The binding of these NSAIDs was also studied at different protein concentrations. Agreement among the binding parameters estimated for different protein concentrations (Figs 2–5) indicates

that the binding is independent of HSA concentration within the range studied.

Identification of NSAID-binding sites on the protein

It is well established that IBP and FBP are site II drugs (Sudlow et al., 1976; Sjöholm et al., 1979; Kober and Sjöholm, 1980). The use of fluorescence spectroscopy in studying the binding properties of these NSAIDs has confirmed that IBP and FBP are not as effective as IBF and BUT at quenching the intrinsic fluorescence of albumin. Thus, we can conclude that they do not share the same site as warfarin in the albumin molecule, which precludes the use of albumin fluorescence to study the binding.

Further experiments on competitive displacement between these drugs should be performed in order to ascertain whether IBF and BUT bind to the characterized site II for IBP and FBP in HSA.

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