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Binding of ketoprofen enantiomers in various human albumin preparations

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Abstract

Published data conflict with respect to the enantioselective protein binding parameters of R(−) and S(+) ketoprofen. We studied whether differences in experimental conditions used and/or presence of interfering compounds could provide a possible explanation for these discrepancies. Equilibrium dialysis, supported by ultrafiltration (67 mM Sörensen phosphate buffer pH 7.4, 580 μM HSA, 37°C) allowed the characteristics of the binding sites to be determined according to Scatchard's analysis. (R) and (S)-ketoprofen concentrations were measured by HPLC. The free (R)-ketoprofen/ free (S)-ketoprofen (F_R/F_S) concentration ratio was calculated. The effect of octanoic acid (OA) found in currently marketed intravenous HSA solutions, and hippuric acid (HA), on F_R/F_S concentration ratio was considered. Two classes of binding sites were characterized for both enantiomers. The free (S)-ketoprofen concentrations remained equal to those of the (R)-antipode at low concentrations of racemate (2–35 μg ml^{-1}) indicating non-stereoselective albumin binding over the therapeutic range. From 35 μg ml^{-1}, the free (S)-ketoprofen concentrations were slighty greater than those of its antipode. Both OA and HA induced an increase of the free fraction of the enantiomers by a two-fold to a 15-fold order of magnitude. OA, but not HA, showed a more pronounced effect for the (S)-form leading to a marked decrease in F_R/F_S concentration ratio (0.61). Differences in HSA preparations used and/or the presence of interfering compounds may explain the variability in the reported protein binding characteristics of ketoprofen enantiomers.

Keywords: Ketoprofen enantiomers; Human serum albumin; Octanoic acid; Hippuric acid; Free fatty acids; Pharmacokinetics

1. Introduction

Ketoprofen (Fig. 1), 2-(3-benzoylphenyl) propionic acid, is a chiral non-steroidal anti-inflammatory drug (NSAID) of the 2 arylpropionate family. It is currently marketed as a racemate, an

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equimolar mixture of two enantiomers, R(−) and S(+). (S)-ketoprofen exhibits most of the anti-inflammatory activity whereas the (R)-enantiomer was found to possess analgesic properties independent of prostaglandin synthesis inhibition [1]. Thus, (R)-ketoprofen is regarded as a promising analgesic devoid of the racemate ulcerogenic side-effects [1].

In contrast to other arylpropionate derivatives, ketoprofen enantiomers undergo only limited chiral inversion (9–12%) in healthy subjects [2]. Nevertheless, ketoprofen disposition was reported to be enantioselective, especially in patients with renal failure and/or hypoalbulinaemia [2–4]. This may be related, at least in part, to stereoselective binding of ketoprofen to human serum albumin (HSA). In fact, plasma protein binding appears to be a primary determinant of distribution and clearance for compounds with a high percentage of binding to HSA and a low hepatic extraction, such as ketoprofen [5]. Unfortunately, published data on the protein binding parameters of (R)- and (S)-ketoprofen are conflicting. Depending on investigators, the protein binding of (R)-ketoprofen appeared higher, lower or equal to that of its antipode [6]. Since the experimental conditions, including the presence of interfering compounds may explain these discrepant results, we studied the binding characteristics of ketoprofen enantiomers in vitro.

2. Materials and methods

2.1. Chemicals

Racemic ketoprofen, essentially fatty acid free HSA (FAF HSA) (A1887 batch no. 14H9319), octanoic acid (OA) (C5038 batch no. 126H3416), or caprylic acid, and hippuric acid (HA) (batch no. 9024723) were obtained from Sigma Chemical (St Louis, MI) and Merck (Lyon, France). Human serum albumin for intravenous use in humans (iv HSA) was supplied by the French Laboratory of Biotechnologies (20% intravenous solution, batch no. 20991040, BLF, Les Ulis, France). All chemicals and solvents were of analytical reagent or high performance liquid chromatographic (HPLC) grade. Deionized water, purified in a Milli-Q™ system (Millipore, Bedford, MA) was used throughout the study.

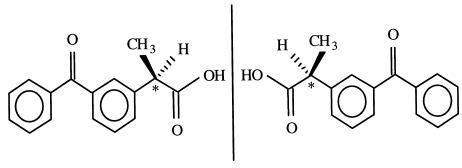

Fig. 1. Chemical structure of (R)- and (S)-ketoprofen.

2.2. Chromatographic conditions

Consistently with earlier studies, the free and total concentrations of (R)- and (S)-ketoprofen were measured by HPLC [7] in their corresponding compartments after dialysis or ultrafiltration. Briefly, the acidified samples were extracted in dichloromethane. After evaporation of the organic layer, the (S)- and (R)-ketoprofen were derivatized with a chiral amine (L-leucinamide) after addition of ethyl chloroformate as a coupling reagent and immediately assayed. The LOD was 5 ng ml^{-1} and the LLOQ was 10 ng ml^{-1}. At LLOQ, the within-run precisions were 13.5 and 15.2%, the between-run precisions were 19.3 and 18.1% for (R)- and (S)-ketoprofen, respectively. Due to the large degree of plasma binding, determination of the free enantiomeric concentrations at the 2 μg ml^{-1} required pooled samples (up to 10 ml) to be used as described by Vakily et al. [8].

The former diastereoisomeric amides were chromatographied at ambient temperature on a reversed-phase column (Kromasil™ C18 5 μm, Hypersil, Cheshire, UK). The technique used is well accepted and considered to be non-strereoselective [9]. The extent of derivatization is close to 100% [10]. The recovery for the internal standard reached 70%. The HPLC was performed with a *717 plus* automatic injector, a M510 pump (Waters™ Assoc., Milford MA), an UV-1000 Model Ultraviolet detector and a Datajet integrator (ThermoQuest™, San Jose CA). An UV6000LP SpectraSYSTEM® photodiode-array detector in combination with PC1000 and Spectacle software (ThermoQuest™, San Jose CA) achieved spectral resolution of peaks.

2.3. Determination of the HSA concentrations

The HSA concentrations and their stability in solutions were assayed on a Beckman® analyser (Synchron CX® 7 M, clinical system version 2.3.H, Albumin reagent, Beckman Instruments, Galway, Ireland). The bromocresol purple method provided high specificity in measurement of HSA.

2.4. Determination of protein binding by equilibrium dialysis

FAF HSA solutions (40 g l^{-1}) were freshly prepared in a Sörensen phosphate buffer (0.067 M, pH 7.4, 37°C) and immediately dialysed against an equal volume of the same buffer. The buffer solutions were spiked with racemic ketoprofen at concentrations ranging from 10 to 1200 μg ml^{-1}. Then, they were dialysed against 40 g l^{-1} FAF HSA solutions for determination of the binding parameters. A rotative Dianorm® equilibrium dialyser (Dianorm Geräte, München, Germany) was equipped with half-cell volumes of 1 ml. The dialysis Diachema membranes (Diachema A.G. Ruskliken, Switzerland) consisted of natural cellulose, they had a molecular-mass cut-off of 5000 Da. The equilibrium dialysers were placed in a temperature-controlled water bath at 37°C and the cells were rotated about their axis at a speed of 20 rpm for 4.5 h. Previous experiments showed that equilibrium dialysis was established without pertinent water-shift due to osmotic pressure within the period of time used [11]. By comparing initial to post-dialysis concentrations of HSA, it appeared that a significative water-shift, also called volume-shift, was observed from the fifth hour of dialysis. Thus, a 24-h dialysis experiment was conducted to determine the effect of the volume shift on the binding characteristics. The correctives measures on bound concentrations of (R)- and (S)-ketoprofen [12] were applied as follows:

$$B_{\text{correct}} = B_{\text{obs}}(P_{\text{o}}/P_{\text{end}})$$

where, B_{correct} is the concentration of bound drug after correction, B_{obs} is the concentrations of bound drug observed, P_{o} and P_{end} are the concentrations of protein measured before and after the experiment. The absence of drug adsorption on membrane or cell surface was previously verified by carrying out an experiment without protein [11].

The results of the three series of dialysis experiments were analysed according to the Scatchard's equation assuming two independent classes of binding sites,

$$B/P = n_i K_i F/1 + K_i F$$

where K_i is the association constant, n_i is the number of binding sites for sites of the ith class, and P is the albumin concentration. Accordingly, B and F were the molar concentrations of bound and free drug. The binding parameters were estimated by minimisation of the quadratic residues with a MicroPharm® least squares regression analysis program (version 3.5, LogInserm, Créteil, France), on a Compaq 4704 computer. The model (two classes) and the weighting (1, $1/y$ or $1/y^2$) were chosen according to minimisation of Akaike's criterion [13].

2.5. F_R/F_S concentration ratio of ketoprofen enantiomers in the racemate

Ultrafiltration experiments were performed in support of the free (R)-ketoprofen/ free (S)-ketoprofen concentration ratio obtained by equilibrium dialysis. A 67 mM Sörensen phosphate buffer solution (pH 7.4, 37°C) containing 40 g l^{-1} FAF HSA was spiked with concentrations of racemic ketoprofen ranging from 2 to 1200 μg ml^{-1}. This was instilled in the sample reservoir of a Centrifree® Micropartition System (Amicon Division, Danvers, CT) and centrifuged at $1680 \times g$ for 10 and 40 min (37°C) [14]. The corrections of the bound concentrations of (R)- and (S)-ketoprofen were applied as above.

Furthermore, equilibrium dialysis experiments were carried out in quadruplicate with therapeutic concentrations of racemic ketoprofen (2 μg ml^{-1}) and FAF HSA (40 g l^{-1} or 1 g l^{-1}) previously reported to show discrepant stereoselective binding results [6]. The rotative Dianorm® equilibrium dialyser (Dianorm Geräte, München, Germany) was equipped with half-cell volumes of 5 ml. The

Table 1
Characteristics of the binding sites of ketoprofen enantiomers according to the Scatchard's equation (racemic mixture in FFA HSA 40 g l^{-1})

Experiments (duration-number of points)	Ketoprofen	n_1	K_1 (μM^{-1})	n_2	K_2 (μM^{-1})
Equilibrium dialysis					
	S(+)	0.885 ± 0.115	0.461 ± 0.076	3.610 ± 0.903	0.006 ± 0.003
(4.5 h-36)	R(−)	0.871 ± 0.034	0.471 ± 0.051	3.753 ± 1.350	0.007 ± 0.004
	S(+)	1.055	0.521	3.253	0.005
(24 h-13)	R(−)	1.101	0.593	2.367	0.006
Ultrafiltration					
	S(+)	1.296	0.526	3.060	0.005
(10 min-12)	R(−)	1.243	0.558	2.751	0.008
	S(+)	1.002	0.572	2.689	0.009
(40 min-14)	R(−)	0.947	0.599	2.355	0.014

free (R)-ketoprofen/ free (S)-ketoprofen concentration ratios were determined as described as above.

2.6. Influence of OA and HA as potential displacers

The influence of OA and HA as potential displacers of ketoprofen enantiomers was studied with additional dialysis experiments in non-stereoselective binding conditions (10 μg ml^{-1} of racemic ketoprofen, 40 g l^{-1} of FAF HSA) [12,15] with and without OA or HA. The FAF HSA solutions (40 g l^{-1}) were spiked with either 13 mg of OA or 4.5 mg of HA per g of HSA. The concentrations of OA corresponded to those found in the currently marketed iv HSA preparations employed in humans and the concentrations of hippurate observed in plasma of uraemic patients [15]. The iv HSA is currently obtained from blood, plasma, or serum of healthy human donors by fractionation according to the Cohn cold ethanol process. Pharmaceutical solutions are then pasteurized for 10 h at 60°C to inactivate human immunodeficiency and hepatitis viruses. During this process, sodium octanoate is usually added to prevent HSA denaturation. The free (R)-ketoprofen/ free (S)-ketoprofen concentration ratio in iv HSA solutions was also determined by equilibrium dialysis. Thus, a 67 mM Sörensen phosphate buffer solution (pH 7.4, 37°C) containing 40 g l^{-1} iv HSA was spiked with 10 μg ml^{-1} of racemic ketoprofen.

2.7. Statistical analysis

The results are expressed as means ± standard deviation. Statistical differences between the means were assessed using Student's t-test at a $\alpha = 0.05$ level of significance. StatPhar® version 1.9 (Faculty of Pharmaceutical Sciences, Limoges, France) was used for statistical analysis.

3. Results

3.1. Parameters of the binding sites: n_i and K_i

The binding data for the Scatchard's analysis are presented in Table 1 and Fig. 2. The dialysis data were analysed assuming two independent classes of binding sites with a non-linear least-squares curves fitting procedure. According to the Scatchard's equation, n_1 were the numbers of high affinity binding sites, and n_2 the number of low affinity binding sites per μM of FFA HSA. K_1 were the high-affinity constants and K_2, the low-affinity constants. The binding parameters of the two enantiomers in racemate were quite similar (Table 1) indicating lack of enantioselectivity of primary binding sites and lack or poor enantioselectivity for the secondary ones. Furthermore, the binding characteristics estimated by equilibrium dialysis at 24 h or by ultrafiltration with correction of the volume shift were consistent with those estimated at 4.5 h (Table 1).

3.2. F_R/F_S concentration ratio of ketoprofen enantiomers in the racemate

The results from ultrafiltration and dialysis experiments are presented in Fig. 3 and Table 2. Using FAF HSA, equilibrium dialysis and ultrafiltration generated quite similar data (Fig. 3). The free (S)-ketoprofen concentrations remained equal to those of the (R)-antipode at low concentrations of the racemate (2–35 μg ml^{-1}) indicating non-stereoselective albumin binding in the therapeutic range (Fig. 3). Above 35 μg ml^{-1} the free (S)-ketoprofen concentrations were slightly, but significantly greater than those of its antipode indicating weak stereoselective binding ($P < 0.001$). The mean F_R/F_S concentration ratio for ultrafiltration and equilibrium dialysis were 0.93 ± 0.04 and 0.93 ± 0.05, respectively (Fig. 3).

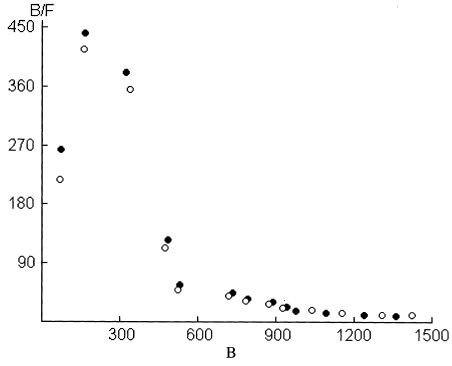

Fig. 2. Scatchard plot of S (○) and R (●) ketoprofen obtained from equilibrium dialysis by using a 40 g l^{-1} fatty acid free HSA-Sörensen solution: B and F are the concentrations of bound and free drug (μM), respectively.

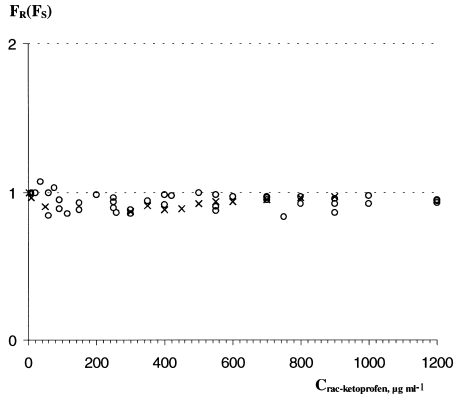

Fig. 3. Free (R)-ketoprofen/ free (S)-ketoprofen (F_R/F_S) concentration ratio obtained from equilibrium dialysis (o) and ultrafiltration (x) by using a 40 g l^{-1} fatty acid free HSA-Sörensen solution.

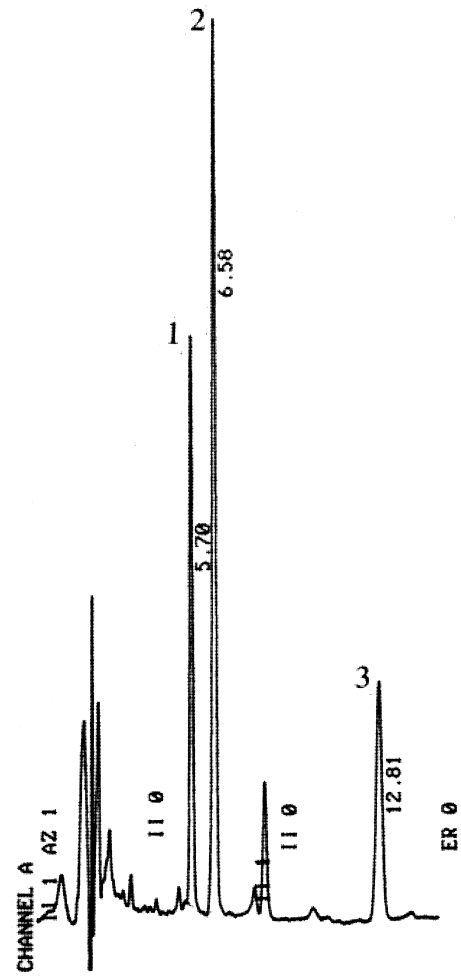

Fig. 4. Profile of the free enantiomeric concentrations of 10 μg ml^{-1} racemic ketoprofen in FAF HSA solution (40 g l^{-1}) together with OA. Peaks: 1, R-ketoprofen; 2, S-ketoprofen; 3, IS.

3.3. Effects of HA and OA on the F_R/F_S concentration ratio

The results from dialysis experiments are summarised in Table 2 and Fig. 4. Addition of HA did not result in any significant alteration of the

Table 2
In vitro binding of R(−) and S(+) ketoprofen to fatty acid free (FAF) and therapeutic BLF (iv HAS) solutions by using 2 and 10 μg ml^{-1} concentrations of racemic ketoprofen[a]

Experiments (number of replica)	HAS (g l^{-1})	Ketoprofen (μg ml^{-1})	Interfering compouds (mg g^{-1} HSA)	Free concentrations		Statistics	F_R/F_S ratio
				R(−)	S(+)		
Ultrafiltration (10)							
	FAF (40)	2	–	1.01 ± 0.15	0.96 ± 0.13	NS	1.05 ± 0.11
Dialysis (4)							
	FAF (40)	2	–	1.92 ± 1.24	1.76 ± 1.18	NS	1.10 ± 0.03
	FAF (1)	2	–	133.00 ± 6.00	133.25 ± 8.30	NS	1.00 ± 0.03
	FAF (40)	10	–	9.52 ± 0.30	9.29 ± 0.23	NS	1.02 ± 0.01
Dialysis (3)							
	FAF (40)	10	OA(13)	80.47 ± 2.89	132.60 ± 5.50	$P<0.001$	0.61 ± 0.001
	FAF (40)	10	HA (4.5)	16.49 ± 0.54	15.59 ± 0.81	NS	1.06 ± 0.02
	ivHSA	10	–	106.90 ± 6.48	181.94 ± 10.12	$P<0.001$	0.59 ± 0.01

[a] Free concentration values represent the means ± S.D. NS, non-significant (Student's paired t-test, $P>0.05$).

F_R/F_S concentration ratio as compared with that obtained with FFA HSA solutions. There was, however, a twofold increase in the free concentrations of both ketoprofen enantiomers. Of note, HA underwent extraction, derivatization and was chromatographied in the same conditions as ketoprofen enantiomers. We observed a difference in the retention time of 15.6 s between HA and (R)-ketoprofen.

Addition of OA to the FAF HSA solution, without the pasteurization step of the ivHSA solution, resulted in a nine-fold and a 15-fold increase in the free concentrations of (R)- and (S)-ketoprofen enantiomers, respectively. Subsequently, the free (S)-ketoprofen concentration was 1.65-fold that of its optical antipode and the F_R/F_S concentration ratio averaged 0.61. Thus, the addition of OA resulted in a marked decrease in the F_R/F_S concentration ratio ($P < 0.001$). Similar findings were observed with ivHSA solutions suggesting that such an alteration might be related to the presence of OA in ivHSA preparation. A similar, but non-stereoselective, increase of the free concentrations of ketoprofen enantiomers was observed when 2 μg ml^{-1} of the racemate were dialysed against 1 g l^{-1} fatty acid free HSA solution.

4. Discussion

HSA consists in a sequence of 585 amino acids containing 17 disulphide bridges. The three dimensional structure of HSA showed three homologous domains (domains I, II and III) that assemble to form a heart-shaped protein. Each domain (I, II, III) is composed of two subdomains (A and B) with common structural motifs. The principal binding sites of ligands to HSA are located in hydrophobic cavities in subdomains IIA and IIIA. The HSA binding site I, also called warfarin binding site, and the site II, also called indole–benzodiazepine binding site represent the binding sites for a large number of tightly bound drugs [16]. They are probably not pre-shaped, but formed according to the needs of the ligands in subdomains IIA (for site I) and IIIA (for site II) [17]. Furthermore, HSA undergoes two conformational transitions depending on the pH of the solutions [16]. The N–B transition occurs in weakly alkaline solutions and the N–F transition in the acidic pH range [16]. It is well known that the binding properties of site I and II are influenced by the N–B transition occurring within the physiological pH range [16]. In tissue capillaries, drug-HSA complexes may exhibit different characteristics [18]. NSAIDs are extensively bound to HSA, mainly in the sites I and II as determined by the site-specific probe displacement experiments. It is generally agreed that the ketoprofen enantiomers are bound to both sites I and II of HSA; they bind mainly to site II (high affinity and low number of sites) and secondarily to site I (low affinity and high number of sites) [15].

Although the experimental conditions used in our study were quite similar to those of Dubois et al. [12] with respect to HSA, ketoprofen concentrations and type of buffer, there are some differences in the results obtained. Contrasting with our findings, Dubois et al reported a 10-fold difference between the (R)- and the (S)-ketoprofen affinity constant (K_2) at the low affinity site. In our study, the association constants, K_i and the number of sites, n_i, of (R) and (S)-ketoprofen indicated lack of enantioselectivity for the primary site and lack or poor enantioselectivity for the secondary one. At therapeutic concentrations of racemic ketoprofen (2 μg ml^{-1}) where binding to site I is negligible [19], we did not observe any significant enantioselective binding whatever the methods used (ultrafiltration or equilibrium dialysis). This is in good agreement with the conclusions of Noctor et al. [20] assuming non enantioselective binding of ketoprofen and suprofen to their primary site of binding localised at site II.

We found that ketoprofen protein binding was weakly enantioselective at concentrations above 35 μg ml^{-1}. Our results agree with the F_R/F_S values and the lack of stereoselectivity previously reported by others in humans [6,21].

The F_R/F_S concentration ratio was determined by Dubois et al. on a spiked plasma sample of one healthy subject. This ratio was less than 1 over the therapeutic range studied [12], contrasting with our data and those of other authors [6,21]. These

discrepant results may be attributed to intersubject variability of binding of drugs to HSA natural mutants [22,23]. The changes in binding properties may play a role since the N–B conformational transition of HSA, takes place within the physiological pH range [16]. Furthermore, some endogenous compounds, which concentrations vary depending on physiopathological states, may play a role as potential displacers [12,15,24]. Among these endogenous compounds, OA and HA may be ketoprofen displacers from HSA binding sites. OA is a plasmatic non-esterified fatty acid (plasma reference concentration: 1.15 mg l^{-1}) used as a thermoprotective agent in currently iv HSA preparations for clinical use. The quantity of OA added is usually 13 mg g^{-1} of ivHSA. The ability of OA to alter the binding of NSAIDs to HSA has already been used for chiral chromatography on immobilized albumin HPLC column [25]. HA is the major constituent of the identified uraemic toxins with a concentration approaching 1 mM (4.5 mg g^{-1} HSA) in patients with severe renal failure [15]. Interestingly, both are probenecid-like inhibitors of the renal organic anion transport by which ketoprofen enantiomers are excreted [26,27]. OA inhibited the chiral inversion of ibuprofen in vitro [28]. In addition, some endogenous compounds may be responsible for analytical interferences especially at very low concentrations of NSAIDs enantiomers. Since HA is an organic acid, it undergoes the derivatization reaction on its carboxylic group and it is chromatographied in the same conditions as those for ketoprofen enantiomers. Its retention time difference with (R)-ketoprofen averaged 15.6 s only. Thus, HA, and many other organic acids, may be considered as potential sources of analytical interferences depending on the chromatographic conditions.

It is well known that the accumulation of free fatty acids results in an increase in the free fractions of many acidic drugs or endogenous compounds [29,30]. For OA, the existence of a common region for high affinity binding localised at site II has previously been demonstrated [31]. This may explain why the presence of OA resulted in a decrease in the association constants of both enantiomers of ibuprofen and naproxen [32]. It was suggested that the two ligands (R)- and (S)- bind to overlapping areas and that OA induces a change in shape of the binding site rather than acting by direct competition in the large area of site II [32]. The same mechanism may explain why OA increased the free fraction of both ketoprofen enantiomers in vitro.

We observed a marked decrease in the F_R/F_S concentration ratio of ketoprofen enantiomers after addition of OA. Similar findings were observed with ketorolac [25]. For ibuprofen and naproxen, the effect of this fatty acid on free energies of interaction was greater on the (R)- than on the (S)-forms [32]. On the other hand, OA did not alter the F_R/F_S concentration ratio of etodolac [32]. Of note, etodolac enantiomers are both bound to site I and only the (S)-form is strongly bound to site II [33]. Surprisingly, the addition of one of the main plasmatic FFA (i.e. oleic, palmitic, or stearic acids) to the protein solution resulted in an increase of the binding of (R)-etodolac, and a decrease in that of its antipode [33].

An artefactual production of free fatty acids, including FFA release associated with heparin [34] was also shown to alter F_R/F_S concentration ratio of some drugs. Thus, enantioselective protein binding determination in post-heparin plasma samples may be altered by the continued heparin lipase activity, in vitro, during binding studies. This artefact is reported to be particularly marked in haemodialysed patients who are frequently hypertriglyceridaemic and receive large doses of heparin [15]. As a result, the experimentation on serum was considered to be preferable for carrying out such experiments [35].

Similarly, some pathophysiological states, including partum [2,3], migraine headache [36] and pain [37] may cause an increase in FFAs and blood lipids, presumably related to a stress-induced adrenergic lipolysis [38]. This may occasionally affect the total (S)/total (R) concentration ratio. The recently described pain related alteration in the ibuprofen enantiomers disposition could be ascribed to this phenomenon [39]. The increased postprandial ratio of (S)/(R)-ibuprofen for C_{max} and AUC may also be related to a postprandial increase of circulating FFAs [40].

Finally, obesity, high dietary fat intake, insulin resistance, vigorous exercise, alcohol, caffeinated beverage, oral contraceptives and smoking [36] or infusion of currently marketed therapeutic HSA solutions await investigations as possibly altering factors of the F_R/F_S concentration ratio.

HA (1 mM) induced a similar increase of the unbound fraction for both enantiomers. Our results are in agreement with those reported by Sakai et al. [15]. The increase in FFAs and uraemic toxins with an indole ring observed in renal failure was associated with an increase in the free (S)-ketoprofen concentrations only [15].

5. Conclusion

Ultrafiltration and dialysis experiments generated similar data. The association constants, the number of sites and the F_R/F_S concentration ratio of (R) and (S)-ketoprofen indicated lack of enantioselectivity for the primary site (site II) and weak enantioselectivity for the secondary one (site I). The binding of ketoprofen to HSA appears to be non-stereoselective over the therapeutic concentration range when standard experimental conditions (67 mM Sörensen phosphate buffer pH 7.4, 580 μM HSA, 37°C) are used. OA, a FFA, but not HA, an organic acid, may contribute to explain the decrease in F_R/F_S concentration ratio occasionally reported in humans. Finally, the variety of HSA solutions used and the presence of endogenous or exogenous displacers, may explain divergent results with respect to the protein binding of ketoprofen enantiomers from one study to another.

Appendix A. Nomenclature

FFA	free fatty acid
F_R/F_S	free (R)-ketoprofen/ free (S)-ketoprofen
HA	hippuric acid
HAS	human serum albumin
FAF HAS	fatty acids free human serum albumin
iv HAS	human serum albumin for iv use in humans
OA	octanoic acid
NSAID	non-steroidal anti-inflammatory drug

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