ORIGINAL ARTICLE

Blood distribution of levocetirizine, a new non-sedating histamine H_1-receptor antagonist, in humans

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ABSTRACT

The aim of the present study was to determine (1) the extent of levocetirizine binding to human blood cells, plasma and individual plasma proteins; (2) the parameters for levocetirizine binding to individual plasma proteins both at their physiological concentrations and, for human serum albumin (HSA), at a lower saturating concentration; and (3) to simulate levocetirizine distribution in human blood using the information obtained at physiological haematocrit (*H*) for blood cells and at physiological concentrations for individual plasma proteins. The nature of the main binding sites of HSA, i.e. site I (warfarin) and site II (diazepam), preferentially involved in levocetirizine binding was also investigated.

Over the range of therapeutic concentrations and multiples thereof, levocetirizine is extensively bound to blood components, the free fraction remaining constant (6.45%) and the fraction bound to blood cells and to plasma proteins accounting for 27.43 and 66.11%, respectively. The binding of levocetirizine to HSA in the presence of physiological concentrations of non-esterified fatty acids (NEFAs) is the main interaction of levocetirizine in blood (50.68% of overall blood binding). This interaction is fatty acid sensitive, with decreasing concentrations of NEFA increasing the amount of bound drug and vice versa. Levocetirizine is also bound to α_1-acid-glycoprotein and high-density lipoproteins (5.17 and 6.89% of overall blood binding, respectively). The displacement of levocetirizine by diazepam is consistent with the binding of this drug to HSA at site II, as diazepam is a specific marker for this site. The binding of levocetirizine to HSA at site II being characterized by a low association constant, other drugs sharing the same site with high association constants cannot displace levocetirizine except at very high plasma concentrations. In any case, at therapeutic concentrations of levocetirizine and at physiological protein concentrations, the observation that none of the levocetirizine binding proteins is saturated suggests that very little or no variation of the free fraction will occur although a different distribution of its bound forms is possible.

INTRODUCTION

Cetirizine dihydrochloride (available under the trademark Zyrtec, UCB Pharma, Brussels, Belgium) is an antihistamine of the second generation approved worldwide for the relief of symptoms of seasonal and perennial allergic rhinitis and chronic idiopathic urticaria. The daily therapeutic dose is 10 mg by the oral route. Cetirizine is a racemate, the *R*-enantiomer being levocetirizine dihydrochloride. The binding characteristics of cetirizine and

its enantiomers to human H_1-histamine receptors expressed in Chinese hamster ovarian cells have been investigated: competition experiments with [^{3}H]-mepyramine showed that cetirizine, levocetirizine (the *R*-enantiomer) and the *S*-enantiomer bound with high affinity and stereoselectivity to human H_1-histamine receptors (K_i values of 6, 3 and 100 nM, respectively); levocetirizine also dissociated from the receptors with a half-life of 120 min whilst that of the *S*-enantiomer was only 7 min [1]. As the *R*-enantiomer proved to be the more active enantiomer (eutomer), all the studies required for its development were carried out, including determination of pharmacokinetic parameters in addition to studies demonstrating the lack of chiral inversion when administered to animals and humans. Area under the curve (AUC) and maximum plasma concentration (C_{max}) of levocetirizine are higher than those of the distomer (mean AUC of eutomer/mean AUC of distomer = 2.14; mean C_{max} of eutomer/mean C_{max} of distomer = 1.75). Moreover, levocetirizine has a longer half-life (7.8 vs. 5.5 h), a lower non-renal clearance (9.70 vs. 28.70 mL/min) and a smaller apparent volume of distribution (0.41 vs. 0.60 L/kg) than the distomer [2]. The approval of levocetirizine for the treatment of allergic disease has recently been granted in 16 European countries (under the trademark Xyzal).

[^{14}C]-Levocetirizine (see *Figure 1*), administered orally as a single 5-mg dose (daily therapeutic dose) to four healthy male volunteers, was rapidly and extensively absorbed: the mean T_{max} of total radioactivity in whole blood and plasma was 0.75 h and the percentage of administered radioactivity recovered in urine and faeces at 168 h after the dose was 85.4 and 12.9%, respectively. The recovery of radioactivity was close to 100% [3]. No mechanistic studies have been carried out on the absorption of levocetirizine. As it is an enantiomer of cetirizine, the distribution of the ionic species of levocetirizine as a function of pH is the same as for cetirizine [4]. At a pH between 3.5 and 7.5, cetirizine and levocetirizine nearly exist exclusively as zwitterions, although it would appear that zwitterionic cetirizine experiences partial intramolecular charge neutralization in folded conformers of lower polarity [4]. It is therefore probable that cetirizine and levocetirizine are absorbed mainly by passive diffusion [5]. The absolute bioavailability of levocetirizine has not been assessed. As 85.4% of a radiolabelled oral dose was recovered in urine, it is apparent that at least this fraction of the dose was absorbed. In addition, 77% of the dose was recovered in urine as unchanged levocetirizine 48 h after dosing [3], which is equivalent to 96% of the radioactivity excreted over the same sampling period. This is indicative of a high absolute oral bioavailability for levocetirizine. Radioactivity has been measured both in whole blood and plasma after administration of [^{14}C]-levocetirizine [3]. The whole blood/plasma ratio of total radioactivity at time points up to 12 h ranged between 0.51 and 0.68. Considering that the mean value of haematocrit (H) in the male adult is 0.47 (0.40–0.54) [6], the data obtained therefore suggest that levocetirizine and/or its metabolites were not – or only very poorly – associated with blood cells. The binding of [^{14}C]-levocetirizine to human plasma proteins was studied both in vitro and ex vivo [3]. In vitro studies showed that, at concentrations ranging from 0.2 to 1 μg/mL (0.4–2.2 μM), i.e. close or much higher than the mean C_{max} value obtained after the 5-mg dose (0.27 μg/mL, 0.58 μM), the percentage bound was concentration-independent (94.89–95.5%). Plasma protein binding was also studied ex vivo in plasma samples collected at 1, 6 and 24 h post-dose and the results obtained were very similar to those obtained in vitro, e.g. 96.1% binding at 1 h.

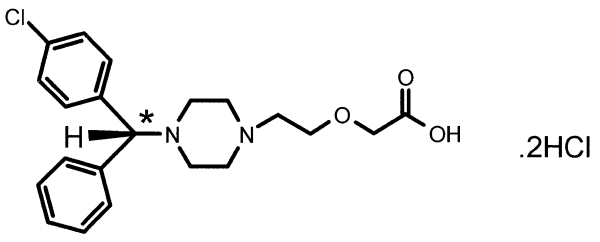

Figure 1 Structure of [^{14}C]-levocetirizine dihydrochloride.

The aim of the present study was to determine (1) the levocetirizine binding to blood cells, plasma and individual plasma proteins and (2) the parameters of levocetirizine binding to individual plasma proteins both at their physiological concentrations (total binding capacity NK_a where $N = n \times$ protein concentration) and, for human serum albumin (HSA), at a lower, saturating concentration (number of levocetirizine molecules (n) per molecule of protein, association constant K_a) and (3) to simulate levocetirizine distribution in human blood using the information obtained at physiological H for blood cells and at physiological concentrations for the individual plasma proteins. The nature of the main HSA binding sites, i.e. warfarin and diazepam [7,8], preferentially involved in levocetirizine binding was also investigated, the warfarin and diazepam sites being referred in the present paper as site I and site II, respectively [7].

MATERIALS AND METHODS

Chemicals

[^{14}C]-Levocetirizine dihydrochloride or [methine-^{14}C]-ucb 28556 was supplied by Amersham (UK). The specific activity was 2.18 GBq/mmol (59 mCi/mmol). The radiochemical purity determined by high-performance liquid chromatography was 99.3% and the radiochemical purity determined by thin-layer chromatography was 99.0%. [^{14}C]-Levocetirizine solutions were prepared by dilution in Sörensen's buffer pH 7.4 (Na_2HPO_4 M/15 and KH_2PO_4 M/15), Warfarin (Sigma A-3430, batch no. 36H0943; St Louis, USA) and diazepam (Sigma D-0899, batch no. 105F0451) were used.

Whole blood

Blood cells were obtained by centrifugation of whole blood collected into heparinized tubes from three consenting healthy volunteers (one male and two females aged 30–48 years) at the Laboratory of Pharmacology, Faculté de Médecine, Créteil, France. Blood cells were washed three times with NaCl 9% solution, then adjusted to an H value of 0.45 with an isotonic saline glucose buffer [Sörensen's buffer (dialysis buffer, see below) + NaCl 50 mM + $MgCl_2$ 1 mM + glucose 5 mM, pH 7.4]. Whole blood was reconstituted from blood cells and plasma obtained from the same volunteer.

Plasma and individual human plasma proteins

Human plasma obtained from volunteers and from Bio Media (Boussens, France) was stored at −20 °C until use. Concentrations were measured by the Biuret method (Sigma ref. 541-2) for total proteins, by the bromocresol purple method (Sigma ref. 625-2) for HSA, by immunodiffusion (Dade Behring ref. Osli 03) for α_1-acid-glycoprotein (AAG) and using the NEFA C kit (Wako Chemicals GmbH ref. 994-75409) for non-esterified fatty acids (NEFAs).

HSA [(Sigma A-1887, batch No. 14H9319) without free fatty acids (molar ratio NEFA/HSA = 5.10^{-5})] and HSA with NEFA (Sigma A-6909, batch no. 127F-9400, molar ratio NEFA/HSA = 1.25) were dissolved in dialysis buffer [(2.36 g KH_2PO_4 + 19.2 g Na_2HPO_4, $12H_2O$)/L, pH 7.4] at 52 or 2.64 g/L. HSA concentration was measured using the same method as that used in plasma.

AAG (Sigma G-9885, batch no. 125H9329) and immunoglobulin G (GG) (Sigma G-4386, batch no. 106F9315) were dissolved in dialysis buffer prepared at 1.00 and 12 g/L, respectively. AGG concentrations were measured by the same method as that used for plasma and GG concentrations by the Lowry method (Folin-Ciocalteu's phenol reagent Merck, ref. 1.09001).

The three lipoproteins were obtained by sequential ultracentrifugal flotation at increasing density of the normolipidemic plasma obtained from a fourth healthy donor (male aged 63 years) at the Laboratory of Pharmacology [9]. The purity of each lipoprotein fraction was measured by the bromocresol purple method in order to determine possible contamination with HSA. Lipoprotein concentration was measured using the Lowry method by measuring the apolipoprotein concentration. Each fraction was diluted in dialysis buffer to obtain a final concentration of 0.5 g/L for very-low-density lipoproteins (VLDL), 3 g/L for low-density lipoproteins (LDL) and 3.5 g/L for high-density lipoprotein (HDL).

Binding to blood cells

A range of [^{14}C]-levocetirizine concentrations (0.12–6.42 μM) was incubated in a suspension of blood cells in buffer ($H = 0.36$–0.51) or in the homologous plasma ($H = 0.40$–0.46). The samples in glass vials were incubated (at 37 °C for 30 min) with gentle orbital shaking in a Brunswick water bath. Aliquots of whole suspensions were taken for liquid scintillation counting. After centrifugation at 1500 ***g*** in a Jouan CR 412 centrifuge for 10 min at 4 °C, aliquots of supernatant were counted. After conversion of counts to concentration, the drug concentration in blood cells (C_E) was calculated by the following equation:

$$C_E = [C_t - (C_p)(1 - H)]/H \quad (1)$$

where C_t is the total concentration of the suspension, C_p the concentration in the supernatant and H the haematocrit.

Binding to plasma and individual plasma proteins: equilibrium dialysis studies

Microvolumes (< 2% of the final volume) of levocetirizine in buffer were added to plasma or protein solutions to obtain the desired concentrations (0.12–5.27 μM). Binding was measured by equilibrium dialysis at 37 °C using a Dianorm® apparatus with 2 × 250 μL capacity cells. A Spectrapor 2 membrane tubing with a cut-off 12–14 kDa (Spectrum Medical Industries) was inserted between the two chambers of the cell.

As previously observed, the pH of frozen plasma samples was approximately 8 [10]. Concentrated lactic

acid was then added to the thawed plasma to adjust the pH to 7.35–7.40. Preliminary studies showed that equilibrium was achieved within 2 h with respect to the free drug fraction. The concentration in each compartment was measured at equilibrium by liquid scintillation counting (Packard, Tricarb 460 CD). After conversion of counts to concentrations, the drug bound to protein (B) was calculated by the following equation:

$$B = nPK_aF = NK_aF \quad (2)$$

where K_a is the affinity constant, P the protein concentration and $N = nP$ the concentration of binding sites. Bound (B) and free (F) concentrations were then analysed by an iterative non-linear regression program using the least-squares criterion [11].

Characterization of HSA binding sites

Levocetirizine binding to HSA (40 μM) was studied in the absence and presence of warfarin and diazepam, markers for HSA binding sites. The binding data (association constant K_a and number of levocetirizine molecules bound per molecule of HSA) without inhibitors were obtained first using a Scatchard plot without fixing n and then by fixing n as an integer value ($n = 1$) in order to determine the number of classes of binding sites and the respective values of the association constant (site-oriented method) [12–14].

The binding data in the presence of the inhibitors (300 μM for both warfarin and diazepam) were analysed according to classical inhibition models [15,16]: the sites are common when inhibition is competitive but are not the same when non-competitive or uncompetitive. The binding data in the presence of the inhibitors were also analysed using the site-oriented method [12–14].

RESULTS

Binding to blood cells

Levocetirizine (0.21–6.42 μM) was extensively bound to blood cells suspended in glucose saline buffer ($H = 0.45$) (binding between 75 and 85%). The binding was ascribed to a non-saturable process characterized by a mean NK_a value of 5.20 ± 0.36 (mean ± SD, $n_a = 9$). In whole blood, the binding of levocetirizine to blood cells was significantly decreased (binding between 57 and 16%, $H = 0.45$) and the binding capacity was reduced (mean $NK_a = 0.56 \pm 0.40$) (see *Table I*). Levocetirizine distribution in blood cells is quantitatively limited in the presence of plasma proteins, which indicates that the plasma proteins bind levocetirizine to an extent reducing diffusion into blood cells.

Binding to plasma and individual plasma proteins

Over the concentration range of 0.21–4.98 μM, levocetirizine binding to normal plasma was high (mean binding = 91.21 ± 0.66%) and this non-saturable binding was characterized by a mean value of 10.39 ± 1.00 for the total binding capacity NK_a (see *Table I*). The binding parameters of levocetirizine to individual plasma proteins are also presented in *Table I*. Levocetirizine is mainly bound to HSA and to a lesser extent to HDL and AAG. Binding to GG and the two other lipoproteins (LDL and VLDL) is low. As binding to the proteins was non-saturable, no association constant or binding site concentration could be derived from these data. As for plasma, a total binding capacity NK_a relating to the free drug concentration was therefore estimated. It can be observed that the increase in NEFA concentration significantly reduced the binding of levocetirizine to HSA.

Table I Parameters of [^{14}C]-levocetirizine binding to blood cells, plasma and individual plasma proteins (mean ± SD).

Blood cells, plasma or individual plasma proteins at physiological concentrations	Range of levocetirizine concentrations (μM)	Percentage bound	NK_a total binding capacity	Number of assays (n_a)
Blood cells in plasma ($H = 0.45$)	0.23–6.89	56.87–15.64	0.56 ± 0.40	9[a]
Blood cells in buffer ($H = 0.45$)[b]	0.21–6.42	84.79–74.75	5.20 ± 0.36	9[a]
Plasma	0.21–4.98	91.21 ± 0.66	10.39 ± 1.00	9[a]
HSA (52 g/L or 788 μM) (NEFA/HSA = 5.10^{-5})	0.23–5.27	97.56 ± 0.10	39.95 ± 0.69	3
HSA (52 g/L) (NEFA/HSA = 1.25)[b]	0.21–4.72	88.95 ± 0.39	7.86 ± 0.09	3
AAG (1.00 g/L or 25 μM)[b]	0.14–3.48	44.71 ± 3.74	0.80 ± 0.06	3
GG (12 g/L or 75 μM)[b]	0.12–2.89	12.92 ± 2.32	0.14 ± 0.02	3
VLDL (0.5 g/L or 50 nM)[b]	0.12–2.77	8.51 ± 2.48	0.09 ± 0.003	3
LDL (3 g/L or 1 μM)[b]	0.14–3.18	22.74 ± 1.95	0.30 ± 0.02	3
HDL (3.5 g/L or 11.67 μM)[b]	0.16–3.67	51.58 ± 1.11	1.07 ± 0.03	3

[a]Three assays performed per subject.

[b]Results used for the simulation of the blood distribution of levocetirizine.

Simulation of blood distribution of levocetirizine

Using the previously estimated binding parameters of levocetirizine to the different blood fractions (blood cells suspended in buffer at a physiological H and individual plasma proteins in buffer at the physiological plasma concentration), the distribution of levocetirizine in blood at therapeutic concentrations and multiples thereof (0.12–6.42 μM) was simulated using the mathematical treatment previously described by Urien et al. [17]. This simulation gives values of binding different from those measured with each individual blood fraction, as it takes into account the interactions among the different blood fractions simultaneously present in blood. According to the results obtained by the simulation, levocetirizine is extensively bound in blood, as the simulated free fraction (fu) was only 6.45%; it is principally bound to HSA and blood cells bind 27.4% of the total drug (see *Table II* and *Figure 2*).

Table II Simulation of the distribution of levocetirizine in human blood (parameters used for simulation are reported in Table I).

Blood components	Percentage of levocetirizine (0.12–6.42 μM)
–	6.45[a]
–	93.55[b]
Blood cells	27.43
HSA + NEFA	50.68
AAG	5.17
GG	0.88
VLDL	0.56
LDL	1.92
HDL	6.89

[a]Free (fu).

[b]Total bound.

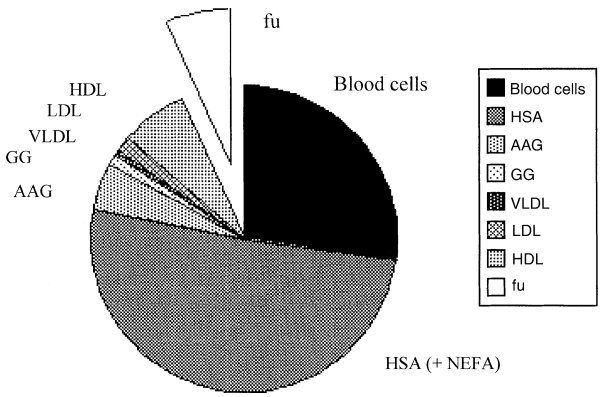

Figure 2 Simulation of the distribution of levocetirizine in human blood.

Characterization of the HSA binding site of levocetirizine

When the concentrations of [^{14}C]-levocetirizine increased from 0.76 to 153 μM, the binding of [^{14}C]-levocetirizine to HSA (40 μM) decreased from 58 to 33% (see *Table III*). The analysis of this saturable binding with the Scatchard plot permitted the calculation of the number of molecules of levocetirizine bound to a molecule of HSA ($n = 2.35 \pm 0.14$) and the value for the association constant K_a of 11.02 ± 2.40 mM^{-1}, indicating a moderate affinity (see *Table IV*).

Two or more levocetirizine molecules bound to a molecule of HSA are not necessarily bound to the same class of binding sites; interactions with the main HSA binding site markers, i.e warfarin (site I) and diazepam (site II), were used to define the binding sites of levocetirizine on HSA. When the concentrations of [^{14}C]-levocetirizine increased from 0.66 to 141 μM, the

Table III Mean (±SD, $n_a = 6$) binding of [^{14}C]-levocetirizine to HSA (2.64 g/L or 40 μM) in the absence and presence of warfarin or diazepam.

[^{14}C]-Levocetirizine alone		[^{14}C]-Levocetirizine + warfarin (300 μM)		[^{14}C]-Levocetirizine + diazepam (300 μM)	
Concentration (μM)	Percentage bound to HSA	Concentration (μM)	Percentage bound to HSA	Concentration (μM)	Percentage bound to HSA
0.76 ± 0.02	57.72 ± 5.59	0.66 ± 0.06	51.38 ± 4.15	0.56 ± 0.05	23.43 ± 2.22
1.45 ± 0.08	56.16 ± 6.62	1.44 ± 0.10	53.45 ± 1.78	1.13 ± 0.10	23.74 ± 1.18
2.77 ± 0.22	56.79 ± 5.95	2.74 ± 0.06	52.03 ± 3.65	2.21 ± 0.17	24.06 ± 1.23
5.72 ± 0.19	57.15 ± 3.56	5.32 ± 0.21	50.40 ± 4.49	4.56 ± 0.18	23.24 ± 2.15
11.20 ± 0.10	54.77 ± 4.12	10.70 ± 0.50	48.83 ± 2.59	9.33 ± 0.69	23.24 ± 1.90
21.20 ± 2.10	49.91 ± 5.11	20.90 ± 0.30	46.59 ± 2.32	17.80 ± 0.70	21.76 ± 1.18
40.30 ± 2.60	46.02 ± 3.31	40.00 ± 5.50	43.10 ± 1.92	36.20 ± 1.50	21.52 ± 1.84
76.40 ± 2.30	38.81 ± 2.87	76.30 ± 1.50	34.89 ± 1.21	66.70 ± 9.80	20.19 ± 0.75
116.00 ± 3.00	36.04 ± 1.56	113.00 ± 4.00	30.59 ± 0.71	106.00 ± 3.00	19.47 ± 0.82
153.00 ± 9.00	32.61 ± 1.62	141.00 ± 14.00	28.66 ± 1.37	139.00 ± 2.00	19.01 ± 1.40

Table IV Mean (±SD, $n_a = 3$) binding parameters of [^{14}C]-levocetirizine to HSA (40 μM, NEFA/HSA = 1.25) obtained in the absence and, according to the different inhibition models, in the presence of warfarin or diazepam.

Binding parameter	[^{14}C]-Levocetirizine alone	[^{14}C]-Levocetirizine + warfarin (300 μM)			[^{14}C]-Levocetirizine + diazepam (300 μM)		
		Competitive	Uncompetitive	Non-competitive	Competitive	Uncompetitive	Non-competitive
N	2.35 ± 0.14	2.08 ± 0.08	2.38 ± 0.09	2.17 ± 0.08	2.37 ± 0.11	2.97 ± 0.39	2.56 ± 0.13
K_a (mM^{-1})	11.02 ± 2.40	13.39 ± 0.96	10.46 ± 0.73	12.46 ± 0.79	10.64 ± 0.87	7.05 ± 1.26	9.36 ± 0.78
RSS	154	377	331	339	314	1044	346
R	0.9961	0.9939	0.9946	0.9945	0.9937	0.9792	0.9931

RSS, residual sum of squares; *R*, correlation coefficient.

Table V Parameter estimate for levocetirizine binding to HSA (40 μM) according to one class of binding sites as obtained from the Scatchard's plot made with experimental data (without fixing *n*) and according to the two or three classes of binding sites model using the site-oriented method ($n = 1$).

Binding parameters	Without fixing *n*	Site-oriented method with $n = 1$	
		Two classes of binding sites model	Three classes of binding sites model
N	2.35 ± 0.14	1	1
K_a (mM^{-1})	11.02 ± 2.40	–	–
K_{a1} (mM^{-1})		14.45	20.32
K_{a2} (mM^{-1})		14.43	3.76
K_{a3} (mM^{-1})		–	3.77
RSS	154	289	242
R	0.9961	0.992	0.993

RSS, residual sum of squares; *R*, correlation coefficient.

binding of [^{14}C]-levocetirizine to HSA (40 μM) in the presence of warfarin (300 μM) decreased from 52 to 29% (see *Table III*), indicating that the binding and its decrease as a function of increasing concentrations of levocetirizine were very similar to those obtained in the absence of warfarin. When the concentrations of [^{14}C]-levocetirizine increased from 0.56 to 139 μM, the binding of [^{14}C]-levocetirizine to HSA (40 μM) in the presence of diazepam (300 μM) decreased from 24 to 19% (see *Table III*), indicating that the binding of levocetirizine was substantially modified by the presence of diazepam.

Analysis of the data with the different models of inhibition (competitive, non-competitive and uncompetitive) [15,16] showed that, in the presence of diazepam, the competitive model gave the best fit (highest correlation coefficient, lowest residual sum of squares) indicating that levocetirizine can bind to the diazepam site (see *Table IV*), whereas the model best describing the interaction between HSA and levocetirizine in the presence of warfarin was the uncompetitive inhibition model (see *Table IV*).

Table VI Association constant values of levocetirizine binding to HSA (40 μM) estimated assuming three classes of binding sites using the site-oriented method ($n = 1$) and their evaluation in presence of warfarin or diazepam.

Association constant	Levocetirizine	Levocetirizine + warfarin	Levocetirizine + diazepam
K_{a1} (mM^{-1})	20.32	22.49	2.46
K_{a2} (mM^{-1})	3.76	1.73	2.46
K_{a3} (mM^{-1})	3.77	1.73	2.46

Data obtained in the absence of the inhibitors were analysed according to a two or three classes of sites model assuming one molecule of levocetirizine fixed to each class of sites [12–14]. The results indicated that levocetirizine binding to one molecule of HSA can be broken down into one levocetirizine molecule bound to a site with higher affinity ($K_{a1} = 20.32$ mM^{-1}) and two levocetirizine molecules each bound to a site with lower affinity ($K_{a2} = 3.76$ mM^{-1}, $K_{a3} = 3.77$ mM^{-1}) (see *Table V*). The binding data obtained in the presence of the inhibitors (see *Table III*) were treated according to the three classes of sites model and confirmed that diazepam decreased the affinity of levocetirizine for the site of high affinity, whereas warfarin had only a minor impact on the affinity of levocetirizine for the two sites of low affinity (see *Table VI*).

DISCUSSION

Over the range of therapeutic concentrations and multiples thereof, levocetirizine is extensively bound in blood, its free fraction (fu) remaining constant (6.45%) and the fraction bound to blood cells and to plasma proteins accounting for 27.43 and 66.11%, respectively. Therefore, it can be deduced that the retention forces for levocetirizine are located in plasma, most likely because of its affinity for the circulating proteins. It would appear that blood cells do not have binding sites able to retain significant amounts of the drug. The methodology used

in this study did not allow the evaluation of binding to erythrocytes compared with that of leucocytes.

The results obtained show that the binding of levocetirizine to HSA in the presence of physiological concentrations of NEFA is the main interaction of levocetirizine in blood (50.68% of overall blood binding at a physiological H of 45%). This interaction is fatty acid sensitive, which means that decreasing concentrations of fatty acids increase the amount of bound drug and vice versa. As both partners of the interaction with HSA exhibit carboxylic acid groups, competition at this level is likely to occur. This is a characteristic of site II of HSA according to Sudlow et al. [18]. Moreover, the displacement of [^{14}C]-levocetirizine by diazepam is consistent with the binding of this drug to site II of HSA, as diazepam is a specific marker for site II. As a non-integer number of binding sites ($n = 2.35 \pm 0.14$) was found by the Scatchard model, the equilibrium binding data were fitted according to the site-oriented model as previously described [12–14]. The binding characteristics obtained are compatible with the presence of three classes of binding sites for levocetirizine on the HSA molecule. One is the site II already described ($K_{a1} = 20.32$ mM^{-1}) and the two others are characterized by very low association constants ($K_{a2} = 3.76$ mM^{-1}, $K_{a3} = 3.77$ mM^{-1}) and thus considered as non-specific.

The lipophilicity parameters of a number of antihistamines have been determined by Timmerman [19]. The log D for cetirizine, and consequently for levocetirizine in octanol at pH 7.4 is 1.04, which is a relatively low value. However, the drug is bound to HDL as much as to AAG.

It is clear that over the range of expected plasma concentrations of levocetirizine in humans [steady state concentrations in the range of 0.03–0.29 μg/mL (0.06–0.63 μM) after administration of the daily dose of 5 mg to healthy volunteers for 8 days (UCB, data on file)], the binding to plasma proteins remains constant and therefore plasma protein binding of levocetirizine in vivo in humans is a non-saturable process. The binding of levocetirizine to HSA at site II being characterized by a low association constant, other drugs sharing the same site with high association constants cannot displace levocetirizine except at very high plasma concentrations. In any case, at therapeutic concentrations of levocetirizine and at physiological protein concentrations, the observation that none of its binding proteins is saturated suggests that very little or no variation of the free fraction will occur although a different distribution of its bound forms is possible. The mean renal clearance of levocetirizine is 29.78 mL/min [2]. When corrected for plasma protein binding (91.21%, see *Table I*), the value for the unbound form of levocetirizine is 312.18mL/min. Therefore, levocetirizine is excreted both by glomerular filtration and active tubular secretion.

The binding of levocetirizine to human plasma proteins is slightly higher than that reported for cetirizine (88–90%) [7]. Cetirizine is also principally bound to HSA and to a lesser extent to AAG and HDL (mean value for the association constant K_a for HSA $= 9.78 \pm 0.01$ mM^{-1} and for $n = 1.78 \pm 0.14$) [7]. It is not possible to know whether the small differences observed in the values of the binding parameters K_a and n between cetirizine and levocetirizine are statistically significant because data were obtained from different studies in which the experimental conditions were not rigorously identical. Both levocetirizine and cetirizine bind to site II of HSA and this binding is decreased by increased concentrations of free fatty acids.

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