Molecular Properties and Pharmacokinetic Behavior of Cetirizine, a Zwitterionic H_1-Receptor Antagonist

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The ionization and lipophilicity behavior of the antihistamine (H_1-receptor antagonist) cetirizine was investigated, showing the drug to exist almost exclusively as a zwitterion in the pH region 3.5−7.5. In this pH range, its octanol/water lipophilicity is constant and low compared to cationic antihistamines (log D = log P^Z = 1.5), whereas its H-bonding capacity is relatively large (Δlog $P^Z \geq 3.1$). Conformational, electronic, and lipophilicity potential calculations revealed that zwitterionic cetirizine experiences partial intramolecular charge neutralization in folded conformers of lower polarity. Pharmacokinetic investigations have shown the drug to be highly bound to blood proteins, mainly serum albumin, and to have a low brain uptake, explaining its lack of sedative effects. As such, cetirizine does not differ from "second-generation" antihistamines. In contrast, its very low apparent volume of distribution in humans (0.4 L kg^{-1}, smaller than that of exchangeable water) implies a low affinity for lean tissues such as the myocardium and is compatible with the absence of cardiotoxicity of the drug. The zwitterionic nature and modest lipophilicity of cetirizine may account for this pharmacokinetic behavior. The suggestion is offered that cetirizine and analogous zwitterions, whose physicochemical, pharmacokinetic, and pharmacodynamic properties differ from those of "first-" and "second-generation" drugs in this class, could be considered as "third-generation" antihistamines.

Introduction

Classical H_1-receptor antagonists (usually referred to as antihistamines) are highly lipophilic drugs possessing a single strongly basic center, thus existing at physiological pH mostly as lipophilic cations. For decades, a major disadvantage of antihistamines such as chlorpheniramine and hydroxyzine has been the occurrence of marked central effects such as somnolence due to facile brain penetration and in some cases also antagonism at other sites such as cholinergic receptors. A significant breakthrough in the treatment of allergies was therefore the discovery of terfenadine, astemizole, and analogues as a second generation of highly potent antihistamines devoid of noteworthy central nervous system (CNS) effects at normal doses while retaining the general structure of highly lipophilic basic drugs.[1,2] However, some of these drugs are now the object of close attention due to a nonnegligible cardiotoxicity at high doses, especially in the presence of substrates that compete for the same sites of metabolism.[3−6] This toxicity may be related to the high affinity of many highly lipophilic basic drugs for lean tissues.[7]

There is thus a need for antihistamines combining low CNS effects with a reduced potential for cardiotoxicity and drug−drug interactions. Novel antihistamines displaying both a basic amino and an acidic carboxylic group are now in clinical use or in development, the first marketed drug in this class being cetirizine, a potent H_1-receptor antagonist having additional synergistic pharmacodynamic properties.[2,8,9] The compound is also a metabolite of hydroxyzine formed by alcohol oxidation. Structurally, it is believed to exist predominantly as a zwitterion at physiological pH. As such, cetirizine displays a molecular structure markedly different from that of the more traditional H_1-receptor antagonists. The drug is expected to have favorable physicochemical properties, such as a relatively low lipophilicity, which would be postulated to result in an improved pharmacokinetic behavior compared to that of hydroxyzine. The literature contains some data on the pK_a values and lipophilicity parameters (log P_{oct}, log P_{alk}, and Δlog $P_{oct-alk}$) of cetirizine.[10,11] However, some of these data are questionable and need to be carefully reexamined.

The present study was undertaken to address three questions: (1) What physicochemical properties result from the molecular structure of cetirizine? (2) What are some important aspects of its pharmacokinetic behavior? (3) Can this pharmacokinetic behavior be explained in physicochemical terms? To address these questions, we examined key physicochemical properties of cetirizine, using high-precision techniques to determine its acid−base behavior and pH-partitioning profile in different solvent systems. The results were remarkably distinct from those of classical antihistamines and could be explained by intramolecular interactions using computational approaches.

Some important pharmacokinetic parameters of cetirizine were also determined, namely, its binding to plasma proteins, its brain extraction ratio, and its brain

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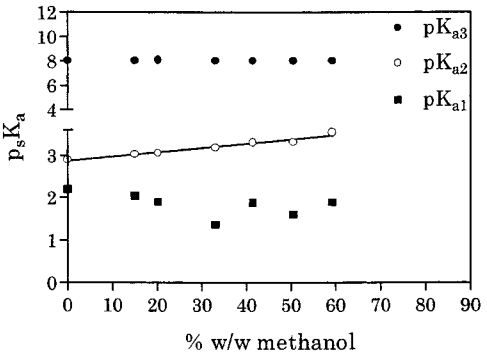

Figure 1. Influence of methanol concentration on the three macro-pK_a values of cetirizine.

efflux rate. These results, as well as its apparent volume of distribution taken from the literature, revealed a favorable distribution profile of cetirizine in the body and were interpreted in terms of its zwitterionic structure and resulting physicochemical properties.

Results and Discussion

Acid−Base Equilibria, Micro-pK_a Values, and Tautomeric Equilibrium Constant (K_Z) of Cetirizine. Previous studies[10] reported three macro-pK_a values for cetirizine (1.52, 2.92, and 8.27), the lowest of which was attributed to the carboxylic group. To reexamine this assignment, and particularly that of the carboxylic group, pK_a values were measured by potentiometric titration in methanol/water mixtures. Organic cosolvents of dielectric constant lower than that of water (e.g., methanol) weaken both acidic and basic groups, thus raising the pK_a of an acidic group and lowering that of a basic group.[12] However, not all ionization equilibria are affected to the same extent by a change in the dielectric constant of the solvent. Reactions leading to increased total charge (i.e., the dissociation of acids) are markedly influenced by the cosolvent, whereas the deprotonation of bases, where both sides of the dissociation equation display the same total charge, is influenced less.

Figure 1 shows the variation in pK_a values as a function of methanol concentration. Only pK_{a2} was affected to a marked extent by the presence of methanol, and the positive slope of the pK_{a2} curve allows its unambiguous attribution to the carboxylic group. The three pK_a values of cetirizine at 0% methanol are reported in Table 1.

Above pH 3 the macrodissociation equilibria of cetirizine can be treated as a function of pK_{a2} and pK_{a3} only, since the ionization of the first basic site (pK_{a1}) is negligible. However, when investigating the microdissociation equilibria of cetirizine above pH 3, it is necessary to know at least one micro-pK_a or the tautomeric constant K_Z, defined as the ratio of the concentration of the zwitterionic and neutral forms ([Z]/[N]). The deductive method was used to obtain the value of the micro-pK_a^{BZ} (see Figure 2) starting from the cetirizine analogue hydroxyzine. The other micro-pK_a values and the tautomeric constant K_Z (Table 1) were then calculated according to Adam's equations.[13] The pK_a values of hydroxyzine (pK_{a1} = 1.75 and pK_{a2} = 7.49) were determined by potentiometry at 25 °C. According to the deductive approach, the pK_{a2} of hydroxyzine should be

Table 1. Micro- and Macroscopic pK_a Values of Cetirizine in Water at 25 °C

	values determined	
	by potentiometry	through pK_a^{BN}
macro pK_a		
pK_{a1}	2.19 (±0.06)[a]	
pK_{a2}	2.93 (±0.03)[a]	
pK_{a3}	8.00 (±0.02)[a]	
micro pK_a		
pK_a^{AZ}		2.93
pK_a^{BZ}		8.00
pK_a^{BN}	7.49[b]	
pK_a^{AN}		3.41
log K_Z		4.56

[a] Measured values with the best standardization procedure (phosphate). The values in parentheses are the standard deviations of three measurements. [b] The value is taken from pK_{a2} of hydroxyzine and considered as the microscopic dissociation constant of the cationic/neutral equilibrium.

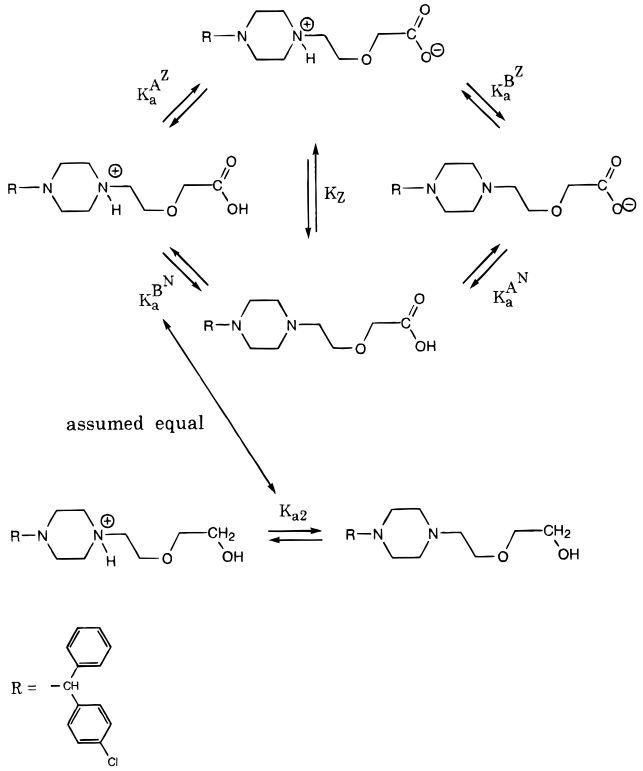

Figure 2. Microdissociation equilibria of cetirizine above pH 3 and the deductive approach to evaluate microdissociation constants using hydroxyzine.

similar to the micro-pK_a^{BN} of cetirizine, i.e., the microdissociation constant of the basic group when the acidic group is not ionized (Figure 2). The micro-pK_a value obtained must be considered as an approximation since the −CH_2OH group of hydroxyzine does not have the same electronic properties as the −COOH group of cetirizine.

The very high value of K_Z (K_a^{AZ}/K_a^{BN} = 36 000) means that the zwitterion is in very large excess over the neutral uncharged form and is the only species present in any significant amount at isoelectric pH. Under such conditions, the pK_a^{AZ} of cetirizine is identical to its macro-pK_{a2} and its pK_a^{BZ} to its macro-pK_{a3} (Table 1). Thus, the large difference between two pK_a values of cetirizine (ΔpK_a = pK_{a3} − pK_{a2} = 5.07) implies that the two macro-pK_a values are sufficient to describe its acid−

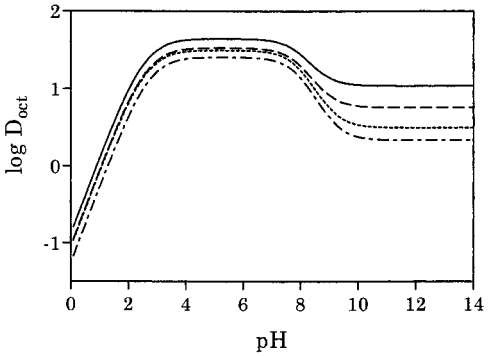

Figure 3. Distribution profile of cetirizine in the octanol/water system obtained by the potentiometric method. The concentrations of cetirizine and volume ratio of the two phases (V_{oct}/V_w) were as follows: (—) 2.64 mM, $V_{oct}/V_w = 0.03$; (– – –) 1.30 mM, $V_{oct}/V_w = 0.12$; (•••) 2.44 mM, $V_{oct}/V_w = 0.92$; (– • –) 1.35 mM, $V_{oct}/V_w = 0.92$.

base behavior at pH > 3 and that its protolysis equilibria can be treated as an *unbranched* system.[13]

pH-Dependent Lipophilicity Profiles of Cetirizine and Hydroxyzine in *n*-Octanol/Water. The pH-dependent lipophilicity profiles of cetirizine and hydroxyzine were determined by the pH-metric method. Emulsion problems made the measurements for cetirizine difficult at high pH values, high solute concentrations, and large volumes of octanol, suggesting an enhanced tensioactive ability of the anionic form of cetirizine compared to the zwitterionic or the dication as demonstrated by surface tension measurements (results not shown).

Figure 3 shows the octanol/water distribution profiles of cetirizine as a function of the relative volumes of the two phases and of the starting concentration of the solute in water, as determined by the pH-metric method. The plateaus around isoelectric pH vary within 0.2 log *P* unit. Above pH 10, where the log P_{oct} of the anionic form should be measured, larger differences appear. Since all measurements were performed at a constant ionic strength (0.15 mol L^{-1} KCl), the observed fluctuations in the log *P* values cannot be due to ion pairing[14] but may be caused by the tensioactive ability of the anionic form of cetirizine.

To confirm these results, the lipophilicity profile of cetirizine was reexamined by CPC (centrifugal partition chromatography). A plateau in the bell-shaped curve of Figure 4 is observable between pH 3.5 and 7.5. The high K_Z value implies that the log D_{oct} measured around the isoelectric pH (5.46) corresponds to the log P_{oct} of the zwitterion. The log *D* value above pH 11 characterizes the log P_{oct} of the anionic form. The log P_{oct} of the cationic form cannot be deduced from this distribution profile because this species is never alone in solution. Thus, the log P_{oct} of the cationic form was calculated by nonlinear regression analysis of experimental log *D*/pH data, using eq 4 (see Experimental and Methodological Section). In this fitting procedure, the pK_a values were kept constant while the log *P* values were optimized. As shown in Table 2, the pH-metric method and CPC yielded similar log *P* values for the zwitterionic and cationic forms. This convergence suggests that the emulsion problem caused by cetirizine anion does not perturb the pH-metric determination of log P^Z and log P^C in the octanol/water system.

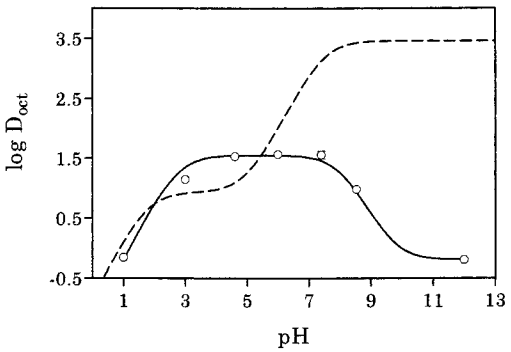

Figure 4. Distribution profiles in the octanol/water system of cetirizine (solid line) and hydroxyzine (broken line) determined by CPC and the pH-metric method, respectively. The curve for cetirizine was obtained by fitting the experimental data to eq 4.

Table 2. Lipophilicity Parameters (log *P*, log *D*) and H-Bonding Parameters (Δlog *P*, Δlog *D*) of Hydroxyzine and Cetirizine

parameter	hydroxyzine	cetirizine	
log P_{oct}^N	3.50 (±0.10)[b]		*a*
log P_{oct}^Z		1.55 (±0.17)[b]	1.50 (±0.07)[c]
log P_{oct}^C	0.93 (±0.11)[b]	1.12 (±0.14)[b]	1.01 (±0.14)[c]
log P_{oct}^A			−0.19 (±0.18)[c]
log P_{dod}^N	1.25 (±0.01)[b]		*a*
Δlog $P_{oct-dod}^N$	2.3		*a*
log $D_{oct}^{7.4}$	3.1		1.5
log $D_{dod}^{7.4}$	0.9		−1.6
Δlog $D_{oct-dod}^{7.4}$	2.2		3.1

[a] Values not experimentally accessible because of the large value of K_Z. [b] Values obtained by potentiometric method (standard errors are given in parentheses). [c] Values obtained by nonlinear regression analysis of log D_{oct}/pH data measured by CPC ($n = 17$, $r^2 = 0.98$, $s = 0.11$; 95% confidence intervals are given in parentheses).

The lipophilicity profiles of cetirizine and hydroxyzine (Figure 4) show major differences, since the latter at pH 7.4 has a 100-fold higher lipophilicity than the former. This phenomenon, which results from the predominance of the neutral species at pH around and above the pK_a, explains the tendency of classical antihistamines (of which hydroxyzine is an example) to accumulate in lean tissues.[7]

pH-Dependent Lipophilicity Profiles in *n*-Dodecane/Water and Hydrogen-Bonding Capacity of Cetirizine and Hydroxyzine. The relationship between lipophilicity parameters and distribution in the body is well-exemplified. One of the most successful parameters to predict brain uptake is the hydrogen-bonding capacity of solutes, determined as the difference between log P_{oct} and log P_{alk} (Δlog $P_{oct-alk}$). It has been demonstrated that the lower this parameter, the higher the brain uptake.[15]

To compare the H-bonding capacity of the nonsedative cetirizine and the sedative hydroxyzine, their lipophilicity profile in dodecane/water was determined (Figure 5). The distribution profile of hydroxyzine was performed by the potentiometric method, whereas that one of cetirizine was determined by CPC since the pH-metric method is not applicable to log *D* values lower than −1. Cetirizine produced a bell-shaped curve having a plateau which cannot be explained by the pK_a values and which does not fit any partitioning model. This unexpected behavior in dodecane/water might be due to the abnormal partitioning of cetirizine at high pH values

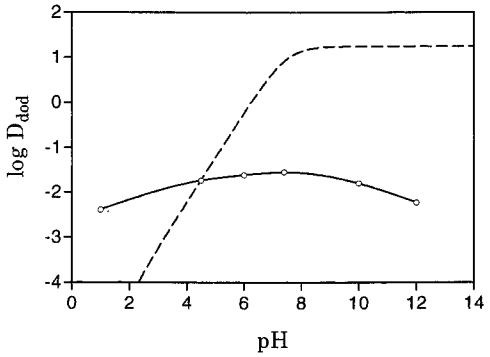

Figure 5. Distribution profiles of cetirizine (solid line) and hydroxyzine (broken line) in the dodecane/water system as measured by CPC and the pH-metric method, respectively.

caused by the surfactant character of its anionic form. Nevertheless, log *D* values around the isoelectric pH give a good estimate of log D^{max} and hence log P_{dod} of the zwitterion (−1.6).

A Δlog $P_{oct-alk}$ value of 3.87 was reported for the neutral noncharged form of cetirizine.[11] This value, which suggests a high H-bonding capacity, was deduced from experimental log *D* values measured at pH 7.4 (log $D^{7.4}$), using an equation which considered only the log *P* of the neutral noncharged species, when in fact this form is now shown to be the only one that does not exist in water at any pH. The difference Δlog $D_{oct-alk}^{7.4}$ was taken as the physicochemical parameter expressing the actual H-bonding capacity at physiological pH and most likely to be related to pharmacokinetic parameters.

As shown in Table 2, hydroxyzine has a relatively small Δlog $D_{oct-alk}^{7.4}$ value (2.2), whereas that for cetirizine is larger (3.1) and indicates a higher H-bonding capacity. Since cetirizine at pH 7.4 is predominantly in its zwitterionic state, its Δlog $D_{oct-alk}$ value in Table 2 is in fact the Δlog $P_{oct-alk}$ of the zwitterion. According to the brain uptake model of Young et al.,[16] sedating antihistamines have a moderate value of Δlog $P_{oct-alk}$ (<2), whereas that of nonsedating antihistamines is generally greater than 3.

Relationship between the Lipophilicity of Cetirizine and Its Transport Rate Constants in a Triphasic System. To understand the pharmacokinetic behavior of cetirizine and its pH-dependent partitioning, transfer rate constants were measured in a triphasic system known as Koch's flask.[17] A set of neutral, ionized, and zwitterionic solutes covering a log *D* range from −1.5 to 1.5 were used to calibrate the system (Table 3). A bilinear relationship was found between the log of the transfer rate constants (log k_1 and log k_{-1}) and log *D* (Figure 6). The rate constant log k_1 is known to be thermodynamically controlled and to increase linearly with lipophilicity.[14,18,19] With further increases in lipophilicity, the diffusion of the solute becomes rate-limiting and a plateau is reached when kinetic control replaces thermodynamic control. The reverse occurs for the rate constant log k_{-1}.

The absolute values of the transfer rate constants of cetirizine (Table 3) at different pH values fit well the bilinear relationships of Figure 6, indicating that the kinetics of partitioning of cetirizine does not differ from that of other zwitterionic, neutral, or charged solutes and that its rates of transfer are not controlled by

Table 3. Rate Constants of Transfer (k in min^{-1}) in a Triphasic System and log *D* Values of Various Solutes at Different pH

molecules	pH	log *D*	log k_1	log k_{-1}
	Oxicams			
piroxicam	3.0	1.43	−0.69	−2.42
piroxicam	1.0	0.63	−1.00	−1.93
N-methylpiroxicam	7.4	0.20	−1.22	−1.73
N-methylpiroxicam	3.0	0.14	−1.19	−1.63
O-methylpiroxicam	1.0	0.07	−1.32	−1.69
piroxicam	7.4	−0.08	−1.53	−1.75
N-methylpiroxicam	1.0	−0.12	−1.51	−1.69
	Peptides			
Trp-Phe	7.4	−0.52	−1.94	−1.74
Phe-Phe	7.4	−1.02	−2.29	−1.57
Leu-Phe	7.4	−1.13	−2.55	−1.77
Trp-Tyr	7.4	−1.38	−2.73	−1.72
	Neutral Molecules			
phenol	7.4	1.54	−0.68	−2.5
N-methylbenzylamide	7.4	0.86	−0.78	−1.94
pyridine	7.4	0.57	−0.83	−1.70
	Other Zwitterionic Molecules			
labetalol	8.8	1.03	−0.89	−2.22
labetalol	4.6	−0.80	−2.07	−1.58
acrivastine	12.0	0.86	−0.89	−2.00
acrivastine	9.4	0.24	−1.15	−1.7
acrivastine	6.8	−0.03	−1.31	−1.59
acrivastine	3.0	−0.48	−1.68	−1.59
acrivastine	1.5	−0.93	−2.19	−1.56
cetirizine	7.4	1.40	−0.83	−2.56
cetirizine	4.6	1.20	−0.81	−2.27
cetirizine	9.0	0.75	−0.95	−2.00
cetirizine	11.0	0.23	−1.39	−1.92

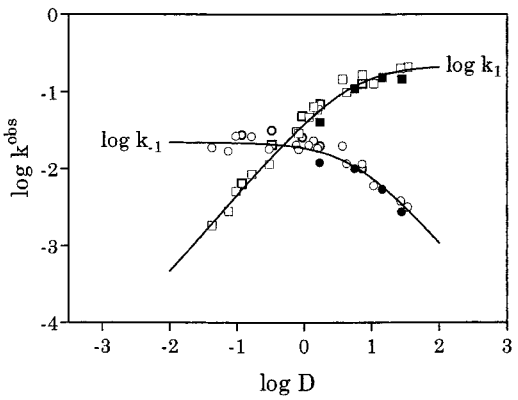

Figure 6. Bilinear relationships between transfer rate constants and log D_{oct} values in octanol/water systems for cetirizine (■, log k_1; ●, log k_{-1}) and a number of calibration compounds (□, log k_1; ○, log k_{-1}).

additional factors such as specific conformational effects. Also, the transport rate constants of cetirizine afforded an independent measure of log D_{oct} values (1.2 ± 0.2 at the isoelectric pH and 0.23 ± 0.1 at pH 11) which were in reasonable agreement with those obtained by potentiometry and by CPC (Figures 3 and 4, Table 2).

Molecular Electrostatic Potential, Lipophilicity Range, and Intramolecular Interactions. Using quenched molecular dynamics (QMD) as a conformational search strategy, 52 conformers were identified for zwitterionic cetirizine. The 28 conformers identified for the neutral form were not investigated further, being of no relevance in solution. The two main classes of conformers of zwitterionic cetirizine are folded and extended ones. These differed markedly in their polarity, as seen in their molecular electrostatic potential (MEP). The MEPs of two representative conformers

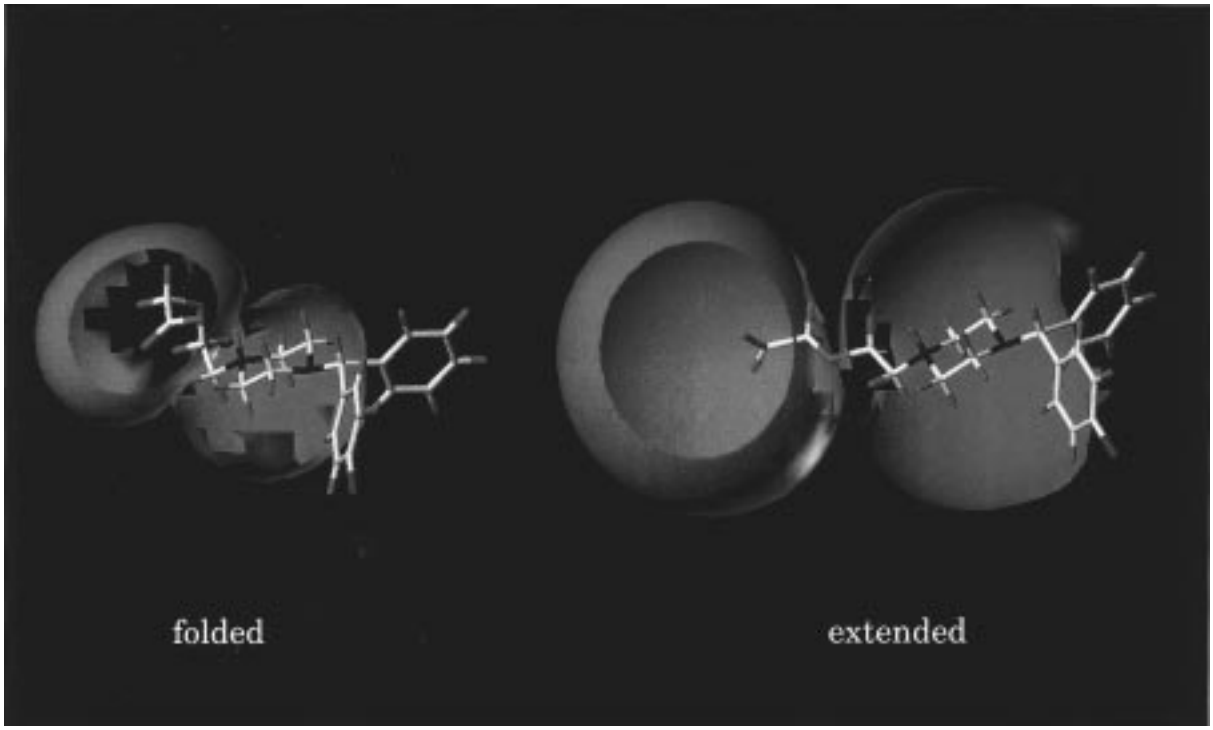

Figure 7. 3D-MEPs of two representative conformers of cetirizine in zwitterionic form. Two isoenergetic contour levels are represented: red for a value of −25 kcal mol^{-1} of the negative potential and blue for a value of 25 kcal mol^{-1} of the positive potential.

(Figure 7) show the high dipolarity of the extended conformer and the markedly less polar nature of the folded conformer due to partial intramolecular neutralization.

The relationship of gas-phase calculations to conformational behavior in solution could be questioned. For example, such calculations can exaggerate the energy of an internal ionic bond in folded conformers. Calculations performed with simpler zwitterionic tautomers using solvation models[20] implemented in the AMSOL5.4 package[21] have revealed that folded conformations remain of low energy. Because it is very difficult to perform conformational analyses in a biphasic solvent system such as octanol/water, the selection of gas-phase conformations by QMD and the distribution of their properties afford useful insights into the behavior of flexible compounds in solution.[22−25]

The virtual log *P* value of each zwitterionic conformer was calculated using the molecular lipophilicity potential (MLP). The results for two representative conformers (Figure 8) suggest a somewhat more extended polar region (red) in the extended conformer and a somewhat expanded nonpolar region (blue) in the folded conformer. To assess better these differences in lipophilicity, we calculated the virtual log *P* value of each conformer. Virtual log *P* values ranged from 0.3 to 1.3, with the extended conformers having the lower log *P* values and the folded conformers the higher ones. This value of 1.3 is close to the experimental log P^Z of 1.5 (see Table 2), indicating that folded conformers of zwitterionic cetirizine dominate its partitioning behavior.

Plasma Protein and Serum Binding. No loss of radioactivity due to nonspecific adsorption to dialysis cells was observed (84−113% recovery). Equilibrium was reached in less than 2 h, allowing the duration of incubations to be set to 3 h. HSA, AAG, and lipoproteins significantly bound cetirizine (Table 4). Some illustrative data are shown in Figures 9 (saturable binding to HSA) and 10 (nonsaturable binding to HDL). The in vitro binding data were satisfactorily fitted to a model simulating all serum binding contributions,[26] indicating that human serum albumin was the main protein carrier of cetirizine in blood. This fit was performed using the parameters derived from FFA-containing HSA since these gave a total serum binding closer to the observed value (see below). This observation also indicated that FFA in serum inhibited cetirizine binding to HSA. Lipoproteins and α_1-acid glycoprotein also bound cetirizine, but their contribution appears negligible (5% or less of total serum binding). These binding properties of cetirizine cannot be compared with those of other antihistamines, for which, to the best of our knowledge, only total serum binding data exist (see later, Table 5). Cetirizine binding to whole serum was high (88−89%), confirming simulations (91%).[26] Varying concentrations from 0.2 to 2.0 μg mL^{-1} did not affect binding (data not shown).

The binding to albumin was saturable, was of moderate affinity ($K_a \approx 10$ mM^{-1}), and involved two binding sites ($n = 1.8$). Commonly, the binding of acidic drugs is to HSA at the warfarin or diazepam binding site.[27] To define the binding site(s) of cetirizine on HSA, markers of the two sites were also used (results not shown). In both cases, the competitive model gave the best fit (best correlation, smallest residual sum of squares), indicating that cetirizine can bind to the warfarin and diazepam sites on HSA. To refine the results, the data obtained in the absence or presence of inhibitor were analyzed according to a two-site model with *n* fixed to unity for each site. The results indicate that cetirizine binding to HSA can be decomposed into a higher affinity component ($K = 13.8$ mM^{-1}) and a

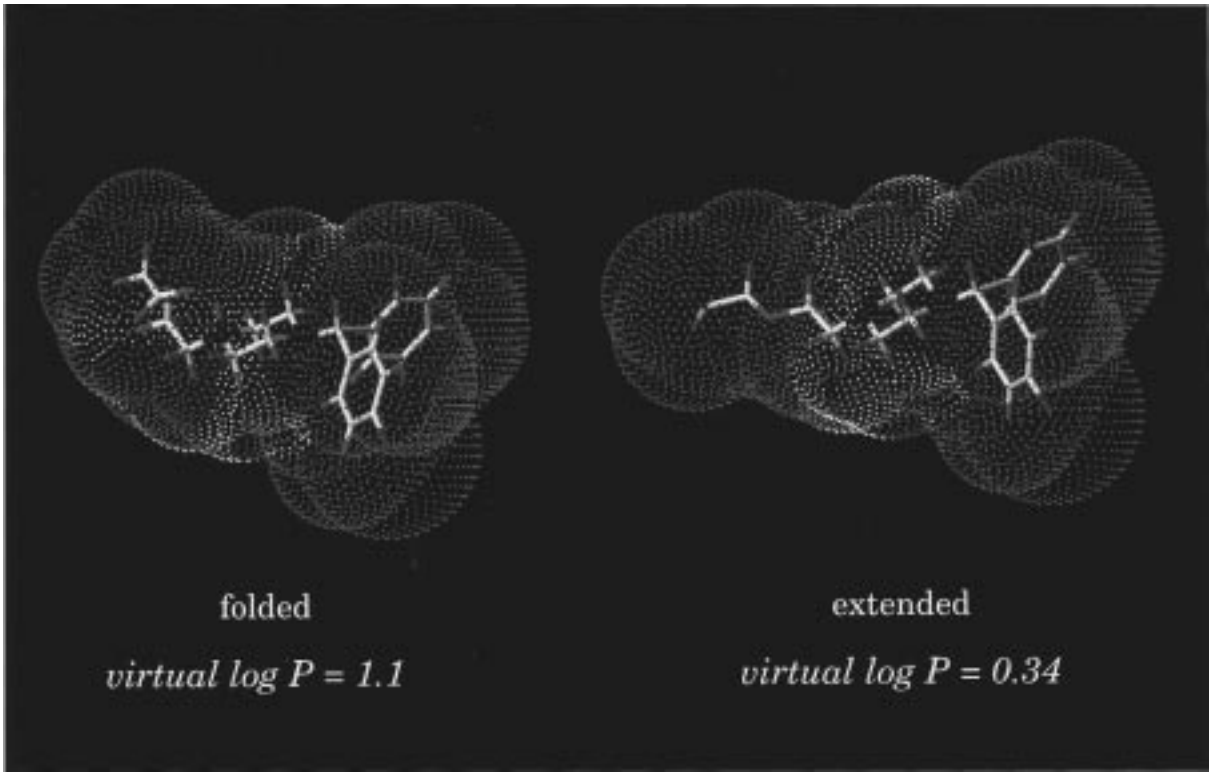

Figure 8. MLP of two representative conformers of cetirizine in zwitterionic form, represented on the solvent-accessible surface area. The polar and hydrophobic parts of the surface are visualized by red and blue dots, respectively. Back-calculation afforded the virtual log *P* value of the two conformers.

Table 4. Cetirizine Binding Parameters to Isolated Serum Proteins[a]

protein	n	K_a	nK_a
HSA	1.78 (±0.14)	9.78 (±1.30)	
HSA with FFA	2.24 (±0.44)	5.42 (±1.33)	
AAG	1.74 (±0.67)	5.12 (±2.45)	
γ-globulins			not measurable
HDL			75.1 (±0.6)
LDL			2.21 (±9)
VLDL			289 (±24)

[a] The n and K_a parameters (number of sites and association constant, respectively) are given for a saturable binding (K_a and nK_a are in mM^{-1}). When binding was not saturable, only the nK_a product parameter could be estimated. Values in parentheses are the standard deviation of three measurements.

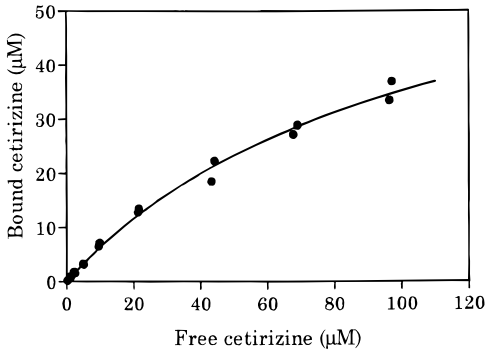

Figure 9. Saturable binding of cetirizine to human serum albumin at pH 7.4 and 37 °C.

lower affinity component ($K = 5.1\ mM^{-1}$). Diazepam decreased the high-affinity component, whereas warfarin decreased the low-affinity component (results not shown), suggesting that cetirizine binding to HSA is primarily to the diazepam site and secondarily to the warfarin site.

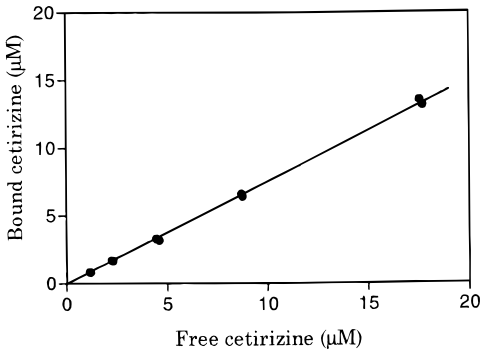

Figure 10. Nonsaturable binding of cetirizine to HDL at pH 7.4 and 37 °C.

Given the binding constants and the number of sites ($n = 2$), the binding capacity of HSA toward cetirizine is relatively high ($2 \times 233 = 466\ \mu g\ mL^{-1}$), a value to be compared with the therapeutic plasma concentrations of the drug (around 300 ng mL^{-1} under steady-state conditions at a daily dose of 10 mg). Cetirizine saturation of serum binding sites is thus very unlikely to occur, and as noted above serum binding was unchanged for cetirizine in the concentration range 0.2−2 $\mu g\ mL^{-1}$. Similarly, interactions at serum binding sites are unlikely.

Cerebral Distribution. Unwanted side effects such as sedation and drowsiness in patients treated with histamine H_1-antagonists result from a high brain uptake of the drug and/or a high receptor occupancy in the frontal cortex. In the rat, brain uptake of cetirizine was independent of concentration and low, i.e., 19.4% ± 0.4% of total drug injected via a serum bolus in the common carotid artery using Oldendorf's method.[28] Mepyramine, a classical antihistaminic agent, exhibits

Table 5. Pharmacokinetic Parameters of Some H_1 Antagonists in Humans[2,31−35]

	$t_{1/2\beta}$[a]	B[b]	V_D[c]
First-Generation Antihistamines			
bromopheniramine	24.9 ± 9.3	72	2.5−10
chlorpheniramine	22 ± 6.0	69−72	5.9 ± 0.9
diphenhydramine	31.05	85−98	3.3
hydroxyzine	20.0 ± 4.0		16.0−30.5
triprolidine	30	90	8.7 ± 7.8
Second-Generation Antihistamines			
astemizole	26	96	48
azelastine	25		14.5
loratadine	12 ± 4	97	120
mizolastine	12.9 ± 4.5	98	1.4 ±0.4
terfenadine	16−23	98	2.2−2.9
Zwitterionic Antihistamines			
acrivastine	1.7 ± 0.2	50	0.64 ± 0.13
carebastine	13−16	98	2.1−2.4
cetirizine	10	88−90	0.4
fexofenadine	18.3 ± 2.0	65	5.6 ± 0.7

[a] $t_{1/2\beta}$, elimination half-life, in h. [b] B, drug bound in plasma, in %. [c] V_D, volume apparent of distribution, in L kg^{-1}.

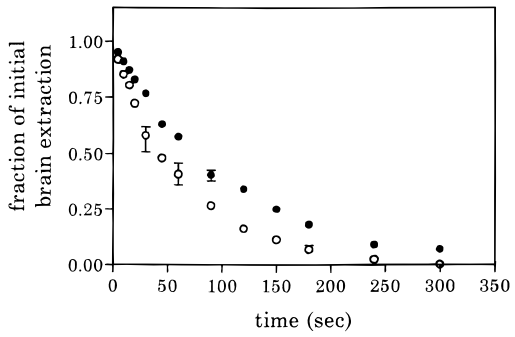

Figure 11. Time course of radioactivity in brain after bolus injection in the carotid artery of [^{14}C]cetirizine (○) or tritiated water (●) dissolved in serum.

high brain penetration with brain uptake indexes varying between 50% and 95% depending on concentration and on the presence of other basic drugs (e.g., diphenhydramine, diphenylpyraline, chlorpheniramine, and propranolol).[29] This suggests that these basic drugs enter the brain by a carrier-mediated system.

Kinetic experiments showed that the brain-to-blood transfer rate (efflux rate) of cetirizine was high (0.016 ± 0.002 s^{-1}) and faster than the brain-to-blood transfer rate of tritiated water (0.010 ± 0.002 s^{-1}) (Figure 11). Together with the low brain uptake of cetirizine, these data indicate that the drug is very weakly retained in brain tissues and returns rapidly from the brain into the blood. This is in keeping with the results of ex vivo studies by Snyder and co-workers,[30] who showed that, 1 h after intraperitoneal administration to rats, cetirizine did not occupy brain histamine H_1-receptor. The present results offer a kinetic complement to these earlier studies and explain that the low brain concentrations of cetirizine and its lack of sedative-hypnotic effects are due to a low uptake and rapid efflux from the brain. The passive or active nature of this efflux remains to be established, but this calls for extensive investigations.

Relation between Physicochemical and Pharmacokinetic Properties

Structure−Property Relations. The physicochemical investigations reported here reveal a number of molecular properties of cetirizine that contrast with those of classical antihistamines. The latter exist under physiological conditions as mixtures of a moderately lipophilic cation and a highly lipophilic neutral form, as exemplified here for hydroxyzine. In contrast, cetirizine exists practically exclusively as a zwitterion of moderate lipophilicity over a broad pH range encompassing the physiological range, and at no pH does it exist as a neutral form. In other words, and in sharp contrast to the lipophilic amines, the log D of cetirizine remains constant over almost 4 pH units. Zwitterions such as cetirizine can thus be considered to have a "buffered" lipophilicity.

Our study also shows that the lipophilicity of zwitterionic cetirizine is markedly conformation-dependent. Extended conformers have revealed a low lipophilicity (corresponding to a virtual log P down to 0.3), perhaps too low for a favorable pharmacokinetic behavior. However, a partial intramolecular neutralization raises the virtual log P of zwitterionic cetirizine by as much as 1 unit and could account for the experimental log P of 1.5 (Table 2).

Protein and Serum Binding. How do these molecular properties of cetirizine relate to its pharmacokinetic properties? A first group of pharmacokinetic results reported here are the binding characteristics of cetirizine to serum and serum proteins (Figures 9 and 10). The high serum binding of cetirizine (around 90%) compares well with the serum binding of most antihistamines (in the range 80−95%, except for a few compounds at 50−70%) (Table 5).[2,31−35] Thus, the zwitterionic versus cationic nature of antihistamines does not appear to be a determining factor in their global serum binding. What may be influenced by this structural difference is the nature of intermolecular forces governing protein binding, the selectivity toward the various serum proteins, and the HSA binding sites involved. More detailed binding studies with other antihistamines are needed before this problem can be solved.

Cerebral Distribution. Brain permeation and resulting side effects are a major therapeutic issue with antihistamines. The so-called first-generation antihistamines readily cross the blood−brain barrier and elicit sedation and somnolence.[2] A low H-bonding capacity appears as a key factor to explain accumulation in the brain.[11,15] Thus, hydroxyzine has a relatively low $\Delta\log D_{oct-alk}$[7.4] value (2.2) (Table 2). High incidence of sedation is not a characteristic of the so-called second-generation antihistamines, whose low brain accumulation is explained by a very high log D and a high H-bonding capacity.[11,15]

Cetirizine is known to have a low potential for sedation, and the present study confirms its fast efflux from and low residence in the brain. As far as central side effects are concerned, cetirizine does not differ from the "second-generation" antihistamines, and in fact all reviews classify it in this group. However, it is shown here that the physicochemical properties accounting for an absence of brain accumulation differ partly for "second-generation" antihistamines and cetirizine. What these drugs have in common is a relatively high H-bonding capacity, as assessed by $\Delta\log P_{oct-alk}$ of their neutral and zwitterionic forms, respectively.[15] Indeed the marked H-bonding capacity (and hence lack of

sedative effects) of cetirizine under physiological conditions is due to its zwitterionic form, an interesting concept for the design of novel antihistaminergic agents.

Pharmacokinetic Properties Postulated To Be Specific to Zwitterionic Antihistamines: Low Volume of Distribution and Limited Metabolic Oxidation. Up to this point in the discussion, the zwitterionic nature of cetirizine has not been found to be related to pharmacokinetic properties markedly different from those of "second-generation" antihistamines. The last part of the discussion focuses on two major and significant pharmacokinetic differences: namely, distribution in the body and extent of biotransformation.

As lipophilic basic drugs, classical antihistamines have a high potential for accumulating into lean tissues, e.g., the myocardium.[3,7] This property finds an expression in the apparent distribution volume (V_D in L kg^{-1}), which for first- and second-generation antihistamines is always larger than 1 L kg^{-1} and often much larger (Table 5).[2,31] For cetirizine, the corresponding value is 0.4 L kg^{-1}.[2,31] This difference is a significant one, given that the reference value of V_D is 0.6 L kg^{-1}, i.e., the volume of exchangeable water in the body.[31] Drugs with a V_D < 0.6 L kg^{-1} have a low and restricted distribution in tissues, whereas high apparent volumes of distribution result from a high affinity for and distribution into tissues. In fact, a low volume of distribution has been described as a determinant of safety for antihistamines,[5] and the present study suggests that a low V_D could be a direct consequence of the specific lipophilicity profile of zwitterions. Validation of this hypothesis awaits the publication of the V_D of other zwitterionic antihistamines.

A small volume of distribution should not hinder antihistamines from reaching their targets and elicit therapeutic effects, since the H_1 receptors involved in allergic reactions are located in the external membrane of cells in the blood stream or in nearby connective tissues. This should even favor the drug's action, since high blood concentrations resulting from this small volume of distribution imply high concentrations of the nearby receptor compartment. This is evidenced by the fact that cetirizine was bound to peripheral H_1 receptors following oral administration.[36] Negligible binding to brain H_1 receptors was also observed, in line with its poor tissue diffusion and low residence in the brain.

But while a low volume of distribution is compatible with, and even favorable to, antihistaminic action as discussed above, it certainly accounts for a highly restricted tissue penetration and hence a lack of demonstrated cardiotoxicity. In contrast to other antihistamines, the zwitterionic cetirizine is known to have a very low potential for cardiac toxicity.[31]

Another pharmacokinetic property that distinguishes cetirizine and analogous zwitterions from other H_1-receptor antagonists is the extent of their biotransformation in humans. All cationic antihistamines have in common an extensive and often complete elimination by oxidative biotransformation in patients,[2] creating a potential for significant interpatient variations and drug−drug interactions.[2] In contrast, all zwitterionic antihistamines such as acrivastine, cetirizine, levocabastine, and fexofenadine (the carboxylic acid metabolite of terfenadine) undergo limited biotransformation in humans and are excreted unchanged for the major part of a dose.[2,37] This is clearly a result of limited liver penetration and/or low affinity for cytochromes P450, and it would be interesting to establish whether a relation exists for these zwitterions between extent of biotransformation and lipophilicity, once their log P^Z values are published. The advantages of a highly restricted biotransformation in terms of interpatient variability and metabolic drug−drug interactions have been stressed.[5,31]

Conclusion

The present study reports the ionization and partitioning behavior of the antihistaminic drug cetirizine. The compound exists as a zwitterion in the broad pH region of 3.5−7.5, where it displays a constant log D of 1.5 and a relatively high H-bonding capacity. Partial intramolecular charge neutralization plays a marked role in modulating these properties, which can explain why cetirizine has a very low cerebral uptake as shown here, resulting in a low incidence of CNS effects such as sedation and somnolence. The rapid efflux of cetirizine from the brain accounts at least in part for its low cerebral uptake. The mechanism of this efflux (active transport?) and its relation to the zwitterionic nature of the drug will not be easy to unravel.

With its low incidence of central side effects, cetirizine resembles "second-generation" antihistamines. The latter, however, are highly lipophilic basic drugs, a property that seems to account for their lack of brain penetration but which results in a very high affinity for lean tissues[7] and hence a potential for cardiotoxicity. In contrast, cetirizine has a small volume of distribution in humans (0.4 L kg^{-1}, smaller than that of exchangeable water), despite a high affinity to serum albumin. The hypothesis is put forward that the zwitterionic nature and modest lipophilicity of cetirizine account for this pharmacokinetic behavior.

Thus, cetirizine has in common with "second-generation" antihistamines a low cerebral uptake but differs from them in a number of physicochemical (e.g., lipophilicity), pharmacokinetic (e.g., low affinity for lean tissues and low biotransformation), and pharmacodynamic (low cardiotoxicity) properties. The suggestion is therefore offered that cetirizine and analogous zwitterions should be considered as "third-generation" antihistamines.

Experimental and Methodological Section

Compounds. Cetirizine·2HCl and hydroxyzine·2HCl were kindly supplied by UCB Pharma (Braine-l'Alleud, Belgium). The following products were obtained from the indicated sources: piroxicam from Pfizer (Groton, CT, Cincinnati, OH), *N*-methylpiroxicam and *O*-methylpiroxicam from Prof. K. Takács-Novák (Semmelweis University of Medicine, Budapest, Hungary), labetalol from Prof. G. Cheymol (Hôpital St. Antoine, Paris, France), and the peptides WF, FF, LF, and WY from Bachem Feinchemikalien AG (Bubendorf, Switzerland). All other chemicals were of analytical grade and purchased from Fluka Chemika (Buchs, Switzerland).

Labeled [^{14}C]cetirizine (54.31 mCi/mmol or 2009 MBq/mmol, P071, batch 93201) was supplied by IsotopChim (Le Belvédère, F-04310 Ganagobie-Peyruis, France). The radiochemical purity of [^{14}C]cetirizine was greater than 98%, as checked by the

manufacturer using TLC and HPLC. The compound was stored at −20 °C until use.

The human proteins were human serum albumin (HSA) devoid of free fatty acids (FFA) (Sigma A-1887, molar ratio FFA:HSA = 0.04); human serum albumin (HSA-FFA) (Sigma A-6909, molar ratio FFA:HSA = 1.27), α_1-acid glycoprotein (AAG) (Behring, 99% pure), and γ-globulins (GG) (Sigma Cohn's fraction II, 99% pure). Lipoproteins (VLDL, LDL, and HDL fractions) were prepared in the site of investigation by sequential ultracentrifugation of serum at increasing density as previously described.[38]

Determination of Protonation Macroconstants. The dissociation constants of cetirizine and hydroxyzine were determined at 25 ± 0.1 °C by potentiometric titration using the Sirius PCA101 apparatus (Forest Row, East Sussex, U.K.), equipped with a semi-micro combination pH electrode (Orion), a temperature probe, an overhead stirrer, a precision dispenser, and a six-way valve for distributing reagents and titrants (0.5 mol L^{-1} HCl, 0.15 mol L^{-1} KCl, 0.5 mol L^{-1} KOH). All experiments were carried out under a slow argon flow to avoid CO_2 absorption. A weighted sample (2−10 mg) was supplied manually; the diluent and all the other reagents were added automatically. Bjerrum plots were used to calculate precise pK_a values.[39] The detailed experimental procedures and data analyses have been described elsewhere.[40] The electrode standardization for the water pK_a values was done in the recommended nonstandard way, namely, by titrating 0.023 mol L^{-1} K_2HPO_4 from pH 1.8 to 12.2 using 0.5 mol L^{-1} KOH as titrant. This procedure must be applied with the PCA instrument when determining pK_a values lower than 3 or higher than 11 and using low sample concentrations. Further details can be found elsewhere.[41]

To clarify the pK_a assignments for cetirizine, its protonation macroconstants were also determined by a mixed-solvent method.[42,43] Five distinct 15-mL semiaqueous solutions of 2.4−2.6 mmol L^{-1} cetirizine in 15.0−59.3% (w/w) methanol were acidified with HCl (0.5 mol L^{-1}) and titrated from pH 1.8 to 11.

Potentiometric Determination of Partition Coefficients. The log D_{oct} values of cetirizine and hydroxyzine were measured using the Sirius PCA 101 instrument. Cetirizine or hydroxyzine solutions ($(1.3-2.7) \times 10^{-3}$ mol L^{-1} and 0.15 mol L^{-1} in KCl), initially acidified with HCl to pH 1.8, were titrated from pH 1.8 to 11.5 in the presence of different volumes of *n*-octanol (volume ratio octanol:water = 0.03−0.917). The alkalimetric titrations were conducted under argon at 25 ± 0.1 °C.

The log *P* values were estimated from difference Bjerrum plots[40] and refined with a nonlinear least-squares procedure[44] by including previously determined pK_a values as unrefined contributions. The detailed experimental procedures can be found elsewhere.[40,44]

Lipophilicity Measurement Using Centrifugal Partition Chromatograhy (CPC). Log *D* measurements in *n*-octanol/buffer were performed using an Ito multilayer coil separator−extractor (P.C. Inc., Baltimore, MD), and those in *n*-dodecane/water using a horizontal flow-through centrifugal partition chromatograph with a coil planet type centrifuge (Pharma-Tech Research Corp., Baltimore, MD). The apparatus was equipped with PTFE tubing (poly(tetrafluoroethylene), 3.00-mm i.d., 3.94-mm o.d.), a Kontron model 420 HPLC pump (Kontron, Zürich-Müllingen, Switzerland), and a Kontron model 432 UV−vis detector coupled with a Hewlett-Packard 3392A integrator (Avondale, PA). A flowmeter (Deeside Industrial Estate, Queensferry, U.K.) was used to measure the precise value of flow rates. The samples were dissolved in aqueous phosphate buffers (0.02 mol L^{-1}) or octanol depending on conditions. All measurements were performed at room temperature. The wavelength of detection was varied according to λ_{max}, which depended on pH. The detailed experimental procedures can be found elsewhere.[45]

CPC is based on a liquid−liquid partitioning system where centrifugal and Archimedan hydrodynamic forces allow maximal retention of the stationary phase. The distribution coefficient (log *D*) can be calculated by eq 1 or 2, respectively, when the aqueous or organic phase is the eluent:

$$\log D = \frac{(t_R - t_0) \cdot U}{V_t - U \cdot t_0} \quad (1)$$

$$\log D = \frac{V_t - U \cdot t_0}{(t_R - t_0) \cdot U} \quad (2)$$

where t_0 and t_R are the retention times of the solvent front and solute, respectively, U is the flow rate of the mobile phase, and V_t is the total capacity of the column. The value of t_0 was measured using a highly polar solute ($K_2Cr_2O_7$ for pH > 4.5 or $CoCl_2$ for acidic pH) or a highly lipophilic nonretained solute (biphenyl) depending on the conditions of measurement.

Determination of Rate Constants of Transfer. Transfer rate constant were determined in the triphasic system water/octanol/water using Koch's flask.[17] A 40-mL phosphate buffer solution (0.06 mol L^{-1}) of cetirizine was placed in compartment A of the flask, 40 mL of phosphate buffer solution (0.06 mol L^{-1}) at the same pH was placed in compartment B, and 80 mL of octanol was gently added on top of the two compartments. The flask was connected to a rotavapor (Büchi R-124, Flawil, Switzerland) ensuring a constant rate of stirring (35 rpm). The small neck of the flask (used to fill the system) was closed with a Teflon stopper. The concentrations of cetirizine were determined spectrophotometrically at regular time intervals using a Perkin-Elmer Lambda 11 UV−vis spectrometer (Perkin-Elmer Ltd., Beaconsfield, Bucks, U.K.). The measurements were automated by coupling the spectrometer to a personal computer (Epson AX-2, Düsseldorf, Germany). Circulation through the spectrometer was ensured by a peristaltic pump (Ismatec, Glattbrugg-Zürich, Switzerland) equipped with silicon tubes (Elkay, Shrewsbury, MA). The flow rate was 13 mL min^{-1}, and the volume of the tubing was 5 mL. The transport processes were performed at room temperature and monitored for 45−125 min, depending on conditions. The transfer rate constants were obtained by a nonlinear regression analysis of the data points performed with the GraphPad-Prism 1.03 software (GraphPad Software, San Diego, CA).

Iterative Calculation of log *P* Values from pK_a and log *D* Values. The relationship between log *D* and the log P_{oct} values of the anionic, cationic, and zwitterionic forms of cetirizine is described by eq 3:

$$\log D = \log[P^Z \cdot f^Z + P^C \cdot f^C + P^A \cdot f^A] \quad (3)$$

where *P* and *f* are the partition coefficients and molar fractions of the different species, respectively. The partitioning of the dicationic form is not taken into account. By extrapolating the molar fraction as a function of pK_a and pH, the partition model of eq 4 can be obtained as follows:

$$\log D = \log\left[P^Z \cdot \frac{10^{pK_{a3}-pH}}{X} + P^C \cdot \frac{10^{pK_{a2}+pK_{a3}-2\cdot pH}}{X} + P^A \cdot \frac{1}{X}\right] \quad (4)$$

where

$$X = 1 + 10^{pK_{a3}-pH} + 10^{pK_{a2}+pK_{a3}-2\cdot pH} + 10^{pK_{a1}+pK_{a2}+pK_{a3}-3\cdot pH}$$

Exploration of Conformational Space by Quenched Molecular Dynamics. All computations (QMD and MLP) were performed on Silicon Graphics workstations (Indigo R4000, Indy R4400) using the Sybyl software.[46] The conformational hypersurface of cetirizine was explored by quenched molecular dynamics (QMD).[25,47] This conformational search strategy is able to describe efficiently the main valleys of a conformational space.[48] The method comprises three steps:

(1) Three to five starting geometries, of different topology (neutral and zwitterionic) and conformation (extended and folded), were energy-optimized using the Tripos force field with Gasteiger−Marsili formal atomic charges[49] in order to remove initial high-energy interactions. High-temperature molecular dynamics (MD) calculations were then carried out at 2000 K. Each simulation was run for 100 ps with steps of 1.0 fs. The frame data were stored every 0.05 ps, giving 2000 frames. The starting velocities were calculated from a Boltzmann distribution. Finally, 10% of all conformers were randomly selected and saved in a database ultimately containing 200 conformers.

(2) All saved conformers were then subjected to energy minimization using the same force field as for the MD calculations. The Powell minimization method was applied with a gradient value of 0.001 to test for convergence. The maximum number of iterations was set at 3000. The energy-minimized conformers were then classified according to increasing energy content.

(3) The conformational similarity of the 200 energy-minimized conformers was investigated by comparing every possible pair of conformers. The two criteria of comparison were the force field energy and the rms (root mean square) distance difference calculated by the option MATCH of Sybyl over all heavy atoms and polar hydrogen atoms. Then an ad hoc FORTRAN program calculated the mean and standard deviations of the rms values. Two conformers were considered identical when their energy difference was ≤ 3 kcal mol^{-1} and their rms distance difference was less than or equal to the rms mean minus the standard deviation. When this was the case, one of the two conformers was eliminated from the database, and it was always the one of higher energy.

Using this conformational search strategy, 52 conformers were identified for zwitterionic and 28 for neutral cetirizine.

Molecular Electrostatic Potential and Molecular Lipophilicity Potential Calculations. The MEP (molecular electrostatic potential) was calculated with the classical Coulomb equation using Sybyl software.[46] The atomic charges were calculated using the Gasteiger−Marsili approach,[49] and the dielectric constant was set to 1 to simulate the gas phase.

The lipophilicity range covered by the "virtual" log *P* values of individual conformers is hypothesized to be a more dynamic descriptor of lipophilicity behavior than the experimental (average) log *P*, and it might even be of relevance in predicting the permeation of a drug across biological membranes. Because no direct experiment can give access to the virtual log *P* value of each conformer of a flexible compound, a theoretical approach has recently been developed in our laboratory.[50] The MLP (molecular lipophilicity potential) method allows to calculate log P_{oct} values as a function of the 3D structure, giving access to the virtual log *P* of all conformers identified in the conformational space of a molecule.[25] This was done using CLIP1.0 software.[23,24,51]

Plasma Protein and Serum Binding. The stock solution of [^{14}C]cetirizine was approximately 37 MBq/mL in distilled water. The solution was kept protected from light at −20 °C. The glucose buffer saline was of pH 7.4 and contained 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgSO_4$, and 5 mM glucose. The concentration of [^{14}C]cetirizine in buffer and protein solutions was determined in duplicate in a Packard liquid scintillation counter (Packard Tri-Carb 460 CD).

Stock solutions of proteins were made in Sörensen's phosphate buffer (66.6 mM, pH 7.4). The concentration of proteins in pure solutions was determined by UV spectrophotometry using $\epsilon_{278nm} = 35\,720$ M^{-1} cm^{-1} for AAG and $\epsilon_{279nm} = 35\,400$ M^{-1} cm^{-1} for HSA. The concentration of GG was determined by the method of Lowry.[52] In serum or plasma, AAG was measured with an immunodiffusion system (NOR-Partigen AAG, Behring) and HSA by a colorimetric method using purple bromocresol with the albumin Sigma kit (no. 625). A serum pool (five healthy subjects) was used for the serum binding experiments.

Equilibrium dialysis was used in the plasma protein or serum binding assays. Aliquots of protein solutions or serum were added to Teflon cells (0.25 mL/chamber), and dialysis was performed against buffer (pH 7.4) containing various concentrations of cetirizine (approximately 10−200 μmol L^{-1}). As previously observed the pH of frozen serum samples was approximately 8.[53] Concentrated lactic acid was then added to defrozen serum to adjust the pH to 7.35−7.40. Preliminary studies showed that dialyzing the serum according to the above procedure maintained a physiological pH in the system as checked at the end of the dialysis ($7.35 < pH < 7.40$). Dialysis was performed at 37 °C for 2 h, under constant stirring (20 rpm), without apparent accumulation of fluid on the protein or serum side of the dialysis chamber. The two chambers were separated by a semipermeable membrane (Spectra/Por, cutoff 12−14 kDa). Preliminary studies showed that equilibrium with respect to the free drug fraction was achieved within 2 h. At equilibrium the concentration in each compartment was measured by liquid scintillation counting.

Cetirizine binding to HSA was studied in the presence of the HSA site markers warfarin and diazepam. The curves, with and without inhibitor, were then analyzed according to classical inhibition models.[54] The binding data were calculated by an iterative nonlinear regression program using the least-squares criterion (MicroPharm[55]).

Brain Extraction Ratio. The brain distribution of cetirizine was determined using the rapid intracarotid bolus injection technique described by Oldendorf.[28] This technique measures the first-pass extraction of a compound by the brain. Full details have been published.[56]

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