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Research Article

Binding of Sulfonamides to Carbonic Anhydrase: Influence on Distribution Within Blood and on Pharmacokinetics

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Four new aromatic sulfonamides were synthesized and purified by standard techniques. Two were unsubstituted, primary sulfonamides and two possessed substituents on the sulfonamide nitrogen. The affinity of the inhibitors for the enzyme carbonic anhydrase was determined in terms of the inhibitory potency, which was found to be dependent on the presence of an unsubstituted sulfonamide group. Binding studies were performed in erythrocyte suspensions using a range of concentrations and the unbound, extracellular concentrations were determined by high-performance liquid chromatographic (HPLC) assay. The dissociation constant of binding and the total binding capacity of the erythrocytes were estimated by nonlinear regression using a two-site binding model. The affinity of the compounds for erythrocytes reflected their inhibitory potency against the enzyme. Binding to plasma proteins was more dependent on lipophilicity and pK_a and was stronger for the substituted sulfonamides. Pharmacokinetic studies in rats showed that the unsubstituted sulfonamides with a high affinity for carbonic anhydrase in erythrocytes have longer half-lives and lower clearance values than the substituted sulfonamides which were more strongly bound to plasma proteins. However, comparison of unbound clearance values showed that the variations in molecular structure, which produced differences in carbonic anhydrase binding and in distribution, also produced variations in susceptibility to elimination processes.

KEY WORDS: pharmacokinetics; carbonic anhydrase; sulfonamides; binding; erythrocytes.

INTRODUCTION

Drugs which are eliminated rapidly must be administered frequently or even continuously to maintain effective concentrations at the effector site. Among approaches that have been adopted to overcome this problem are the formulation of prolonged-release dosage forms, synthesis of metabolically more stable derivatives, and administration as a prodrug.

Within a homologous series of drug compounds, it has often been reported that those compounds which distribute outside the plasma or are bound within plasma are eliminated least rapidly (1,2). Therefore, increasing the binding of drugs, either outside plasma or to plasma proteins, may be expected to prolong the duration of action of compounds which would otherwise be eliminated rapidly. Bound drug would be sequestered away from the site of elimination and act as a reservoir, and dissociation of drug from the binding site would limit the rate of elimination (3). Unfortunately, an increase in lipophilicity, associated with an increase in binding to plasma proteins (4,5), is often accompanied by an increase in the susceptibility of the compound to metabolic attack (4).

Carbonic anhydrases (CA) I and II are the most abundant members of a family of isoenzymes. These isoenzymes occur predominantly within erythrocytes (6,7) and may provide an alternative site for sequestration of drugs. The total concentration of CA isoenzymes in erythrocytes is sufficient to create a large reservoir of drug (7,8). Structural requirements for binding to the enzyme have been extensively characterized (9–12) and binding does not depend directly on lipophilicity. Also, since CA is an intracellular protein, the erythrocyte cell membrane may provide a significant diffusional barrier between drug and organs of elimination. The pharmacokinetics of compounds which are bound strongly to CA are dependent on the extent of such binding (13) and their elimination is greatly accelerated by displacement from CA binding (14,15).

In order to investigate the possibility that CA could act as a suitable binding site for a drug, so as to reduce its rate of elimination, a homologous series of compounds was synthesized from phenylsalicylate and para-substituted benzene sulfonamides with a range of affinities for CA isoenzymes. The affinity of these compounds for CA, their distribution in blood, and their pharmacokinetics in rats were investigated.

METHODS

Materials and Equipment

Phenylsalicylate and sulfanilamide were purchased from BDH (U.K.), and homosulfanilamide from Hopkins and

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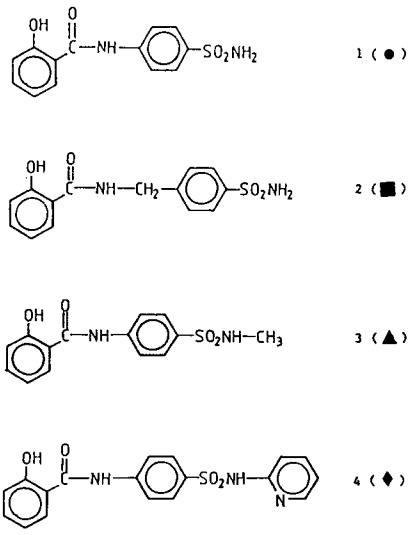
Williams (U.K.). N^1-Methyl and N^1-pyridyl sulfanilide were acquired from ICI (Alderley Park, U.K.) and purified by crystallization from ethyl acatate. Melting points were determined in open capillaries. Mass spectra, proton magnetic resonance spectra, and elemental analyses were obtained on instruments used in-house by ICI, Alderley Park, U.K. High-performance liquid chromatographic (HPLC) analyses were performed using a Kontron 410 LC pump, a Rheodyne 7125 injection valve, a Du Pont Zorbax ODS 250 × 4.5-mm column, and a Waters 481 variable wavelength detector. Internal standard solutions were 100 or 1 μ*M* solutions of 2 for assay of 1, 3, or 4 and of 1 for assay of 2 (see Scheme I for structures). The mobile phase was a mixture of methanol, acetonitrile, and an aqueous component (14.6 g Na_2SO_4, 2% CH_3COOH), 20:20:60 (1 and 2) or 20:25:55. Wavelengths of detection were 285 nm (all media except blood) or 300 nm (blood). The coefficients of variation of the assay were less than 5% over the concentration range studied and the lower limits of determination were 3.5 μ*M* (1), 4.8 μ*M* (2), 0.5 μ*M* (3), and 1.3 μ*M* (4) (16). Nonlinear regression was performed with the PHARMG program on a Sirius microcomputer. Choices between models were based on statistical criteria (*F* test) and on the ability to obtain precise parameter estimates.

Synthesis of Compounds

The four compounds (Scheme I) were synthesized by a simple condensation reaction of a para-aminobenzene sulfonamide with phenyl salicylate. The method for 1 was based on that of Islam *et al.* (17). Phenyl salicylate and sulfanilamide were heated at 200–300°C, the solidified reaction mixture was washed with ethyl acetate, and the product was purified by recrystallization from ethanol.

Compound 2 was synthesized by heating homosulfanilamide with phenyl salicylate in acetonitrile under reflux at 80°C for 3 hr. After cooling, the reaction mixture was mixed with equal volumes of water and ethyl acetate and the organic phase washed with 2 *N* HCl and with saturated $NaHCO_3$ and dried over Na_2SO_4. The solvent was evaporated, and the residue washed with toluene. Purification was achieved by repeated recrystallization from ethyl acetate.



Scheme I. Structures of four aromatic sulfonamides: compounds 1, 2, 3, and 4.

Compound 3 was synthesized by the same method as 1, starting with N^1-methyl sulfanilamide. The product was crystallized from ethyl acetate.

Compound 4 was synthesized by the same method as 1, starting with N^1-pyridyl sulfanilamide. The product was purified by the addition of methanol to a solution in dimethyl sulfoxide (DMSO) of the reaction products. The resulting precipitate was dried under vacuum at 120°C for 24 hr.

The compounds were characterized by melting point, PMR, mass spectroscopy, pK_a, and elemental analysis. The characteristics of the compounds are shown in Table I.

Enzyme Inhibition Studies

Inhibition constants were determined for the four compounds and for two reference compounds, acetazolamide (AZ) and sulfanilamide (SA), using the pH-stat method of Alsen and Ohnesorge (18). Fresh, heparinized human blood was obtained from the antecubital vein of a healthy, male volunteer, and rat blood from male Sprague–Dawley rats by cardiac puncture under an ether anesthetic. Hemolysates were prepared by adding 9 vol of distilled water to 1 vol of blood, centrifuging, and diluting the supernatant lysate in 30 m*M* barbital buffer. An inhibition constant was estimated by nonlinear regression of catalyzed reaction rate against inhibitor concentration using a noncompetitive model of inhibition (19,20).

$$V_{OBS} = V_{CAT}/(1 + I/K_I)$$

where V_{OBS} is the observed rate at inhibitor concentration I, V_{CAT} is the catalyzed rate in the absence of inhibitor, and K_I is the inhibition constant.

Binding Within Erythrocytes

Fresh, heparinized whole blood from either rat or human was centrifuged, and the plasma removed. The packed erythrocytes were washed three times with 2 vol of Krebs–Ringer bicarbonate (KRB) buffer and resuspended in KRB to a hematocrit of 0.4 (Micro Hematocrit, Hawksley & Son Ltd.). Solutions of the compounds in erythrocyte suspensions were prepared by the addition of stock solutions of KRB to the suspensions prepared above. After 30 min (established as sufficient for equilibration), the suspension was centrifuged and the supernatant buffer recovered. The buffer was refined by ultrafiltration (Centrifree MPS-1 micropartition system, Amicon, Mass.) to remove protein released by hemolysis. There was no loss due to adsorption during ultrafiltration. Ultrafiltrates were assayed by HPLC without further treatment. Binding parameters were estimated by nonlinear regression of unbound concentration C_U against total concentration (C_T) using a model incorporating one specific binding site with a dissociation constant K_d and a capacity M. In addition, a high-capacity, low-affinity binding site was included and a proportionality constant (f) relating the concentration bound at this site to C_U was estimated. This constant is equal to the ratio of M/K_d for this second

Table I. Characteristics of the Four Compounds

Characteristic	Compound 1	Compound 2	Compound 3	Compound 4
Reaction yield (%)	50	30	60	40
Melting point (°C)	265–266 (Lit., 257)[a]	183 (Lit., 184)[b]	194	258–260
PMR ([$^2H_6$] DMSO)	δ 6.9–8.1 (8 H aromatics)	δ 9.4 (1H, s, NH)	δ 10.0 (1H, s, CONHAr)	δ 10.6 (1H, s, OH)
	7.3 (2H, s, SO_2NH_2)	6.8–8.1 (8H aromat)	6.8–8.1 (8H aromat)	6.8–8.1 (12H aromat)
		7.3 (2H, s, SO_2NH_2)	6.3 (1H, s, SO_2NH)	
		4.7 (2H, d, NCH_2Ar)	2.6 (3H, d, SO_2NHCH_3)	
pK_a	7.72	7.92	7.27	9.82
Elemental analysis	$C_{13}H_{12}N_2O_4S$	$C_{14}H_{14}N_2O_4S$	$C_{14}H_{14}N_2O_4S$	$C_{18}H_{18}N_3O_4S$

[a] Source: Ref. 17.
[b] Source: Ref. 31.

binding site. By this model, the total concentration in the system is

$$C_T = F \cdot C_U + M \cdot C_U/(K_d + C_U)$$

where $F = 1 + f$. Solving for C_U,

$$C_U = [-(M + F \cdot K_d - C_T)^2 \pm [(M + F \cdot K_d - C_T)^2 + 4 \cdot F \cdot K_d \cdot C_T]^{1/2}]/(2 \cdot F)$$

F, M, and K_d were estimated using the positive root of the second term in the numerator. All concentrations are micromolar.

Distribution Within Whole Blood

Heparinized whole blood was obtained from male Sprague–Dawley rats by cardiac puncture using a wide-bore (19-G) needle, taking care to avoid hemolysis. Solutions in blood were prepared by the addition of whole blood to the residue of methanolic solutions of the compounds from which the solvent had been evaporated. After 30 min of equilibration the blood was centrifuged and the plasma removed. Plasma was assayed for the compounds by the addition of 50 μl of CH_3COOH, 50 μl of internal standard solution, and 200 μl of methanol to 200 μl of plasma followed by 30 min of shaking with 10 ml of ether. The organic phase was separated, the solvent evaporated, and the residue reconstituted in 200 μl of mobile phase, injecting 100 μl into the HPLC system. The blood-to-plasma (B/P) ratio was calculated.

Binding to Plasma Protein

Plasma was obtained from rat blood, taking care to avoid hemolysis. Solutions of the four compounds in plasma were prepared by the same method used to prepare solutions in blood. One-milliliter aliquots of spiked plasma were ultrafiltered as for the erythrocyte binding studies. Ultrafiltrates were prepared for assay by adding 200 μl CH_3COOH to 500 μl ultrafiltrate and extracted as for plasma.

Pharmacokinetic Studies

The jugular vein and carotid arteries of male Sprague–Dawley rats were cannulated following a previously published method (21). After allowing the animals to recover from surgery (minimum of 4 hr), the cannulae were tested for patency and the animals were placed in a cylindrical restraining cage (22) for administration of compounds and collection of blood and urine samples.

Doses of 8 μmol · kg^{-1} were administered via the jugular vein cannula in approximately 0.5 ml of polyethylene glycol 400 over 5 min at a constant rate (MS 16A infusion pump, Graseby-Medical, Watford, England). Sufficient blood to yield two 50-μl samples for analysis was collected passively from the carotid artery cannula into microfuge tubes containing 10 μl of heparinized saline. Samples were collected during the infusion period and for 30 min thereafter, rinsing the cannula with heparinized saline between samples. Two aliquots of 50 μl from each sample were transferred to silanized screw-topped culture tubes, to which was added 50 μl of internal standard solution, 200 μl of distilled water, 200 μl of 10% H_2SO_4, and 10 ml of ether. Extraction, evaporation, reconstitution, and analysis were as for plasma. In calculating the concentration in whole blood a correction was applied for the dilution of samples by the heparinized saline solution.

Blood concentration vs time data were analyzed by nonlinear regression. Pharmacokinetic models incorporating one or two compartments following intravenous infusion were fitted to the data. The terminal elimination rate constant (λ_z) was estimated as a parameter of these models. Areas under the curves were calculated using a combination of trapezoidal (rising concentrations) and log-trapezoidal (falling concentrations) methods; the area beyond the final concentration value (C_z) was estimated as C_z/λ_z.

Urine samples were collected by passive drainage for up to 24 hr after administration, transferring the rats from the restraining cages to metabowls after 2 hr. Urine was assayed for the compounds by the same method used for plasma. In

addition, 200 μl of each sample was incubated with 1000 U of β-glucuronidase (G-0751, Sigma, St. Louis, Mo.) at 37°C for 3 hr. The resulting solution was assayed for compound by the same method as for plasma, correcting for dilution.

RESULTS AND DISCUSSION

Inhibition Studies

Two isoenzymes with differing enzymatic activities and affinities for inhibitors have been reported to be present in erythrocytes of rat and human (7,8). However, over the concentration range used in the present study, only one inhibitable enzyme was needed to account for the variation of enzyme-catalyzed rate with inhibitor concentration. Estimated inhibition constants for the reference compounds AZ and SA were of the same order as those reported in the literature (7,19,21) (Table II). For the new inhibitors, the rank order of inhibitory potency was the same for both enzyme preparations. Figure 1 is a graph of relative enzyme activity against log of inhibitor concentration for 1 to 4. The unsubstituted sulfonamides 1 and 2 were 100- to 1000-fold more potent than the substituted sulfonamides 3 and 4. This may be predicted from the known structural requirement of a free aromatic sulfonamide group for significant enzyme inhibition (9,10). The inhibitory potencies of 1 and 2 are of the same order as that of AZ. The potency of 4 is of the same order as that of SA, and that of 3 is similar to those of monovalent anions (24).

Binding to Erythrocytes Suspended in Buffer

The binding of the compounds to erythrocytes was determined, in the absence of plasma, by allowing a known quantity of the compound to equilibrate in a volume of freshly prepared erythrocyte suspension. The concentration in the supernatant after centrifugation is assumed to be equal to the concentration unbound in the erythrocytes C_U. The binding experiments were performed at room temperature, but preliminary experiments showed no difference in binding to erythrocytes between this temperature and 37°C. The same is true of blood distribution and plasma binding studies.

Estimates of the binding parameters are given in Table III for both human and rat erythrocyte suspensions. In most cases F was small, but significantly greater than one, i.e., a component of the binding was nonspecific. In those cases where F was not significantly greater than one, the precision of the estimate of this parameter was poor. Fitting a model with two binding sites did not significantly improve the fit to the data. Thus, the presence of two binding sites (25) was not apparent over the concentration range studied. The estimated binding capacities (M) for human erythrocytes are within the range reported for the total cell-water concentration of CA (8). The binding capacities in rat erythrocytes are

Table II. Inhibition Constants for Reference and Experimental Compounds with CA from Both Human and Rat Erythrocytes[a]

Compound	K_I (μM)	
	Human CA	Rat CA
AZ	0.0322 ± 0.0037	0.0056 ± 0.0008
SA	12.1 ± 0.6	4.05 ± 0.25
1	0.0530 ± 0.0019	0.0298 ± 0.0019
2	0.0983 ± 0.0068	0.0943 ± 0.0080
3	136 ± 12	237 ± 14
4	21.3 ± 0.9	13.1 ± 1.5

[a] Estimates of K_I are given ± SE. The data are based on five replicate determinations of reaction rate at each concentration of inhibitor. The hemolysate was from a single subject for human CA, but from a pooled hemolysate from four animals for rat CA.

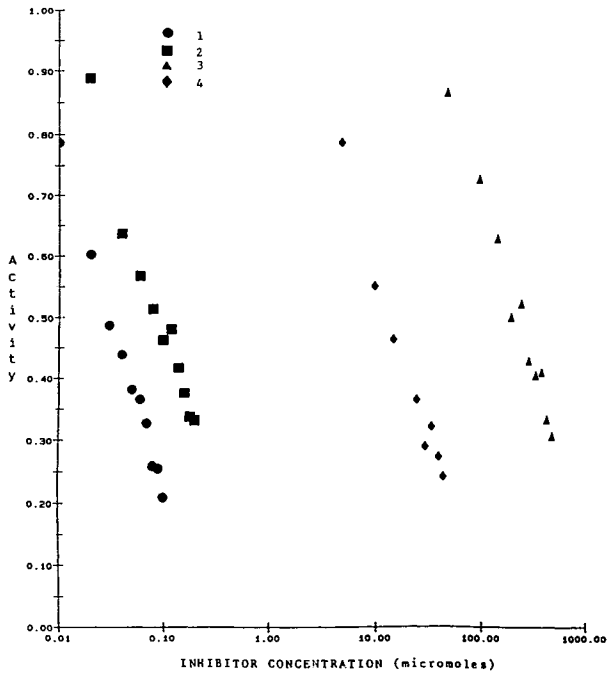


Fig. 1. Plot of relative enzyme activity against log concentration (μM) for four inhibitors, 1 (●), 2 (■), 3 (▲), and 4 (◆), with rat erythrocyte CA.

Table III. Binding Parameters for Compounds Within Erythrocytes from Both Humans and Rats

Compound	K_d (μM)	Capacity (μM)	F
Human erythrocytes			
1	0.197 ± 0.095	208 ± 9	2.04 ± 0.10
2	0.457 ± 0.090	268 ± 54	1.01 ± 4.90
3	12.0 ± 1.8	289 ± 31	1.00 ± 4.16
4	10.1 ± 3.6	159 ± 38	1.47 ± 7.04
Rat erythrocytes			
1	0.183 ± 0.130	225 ± 12	3.18 ± 0.12
2	0.680 ± 0.201	294 ± 11	1.65 ± 0.06
3	—	—	3.00 ± 0.15
4	—	—	4.24 ± 0.25

[a] All estimates of binding parameters given ± SE. The imprecision of the estimates of F arise due to constraining the value to be greater than or equal to one. The erythrocyte suspensions were prepared from the blood of a single male subject for humans. The blood of four animals was used to prepare the rat erythrocyte suspensions. The binding models were fitted to four replicate measurements at each concentration of compound in the erythrocyte suspension.

eightfold higher than a previous estimate of CA concentration in rat erythrocytes (7) but are consistent for the two compounds where this parameter could be estimated. Recently the concentration of one of the isoenzymes in rat erythrocytes has been reported to be higher than the previous estimate of the total concentration (26). This value is more consistent with the concentration of binding sites determined in the present study.

Binding to erythrocytes reflected the CA inhibitory potency of the compounds. The potent inhibitors 1 and 2 were bound strongly, whereas the less potent 3 and 4 were bound weakly in human erythrocytes and showed no specific binding in rat erythrocytes. Equivalence of inhibition (K_I) and dissociation (K_d) constants was not observed, presumably due to differences in the conditions between the two types of investigation.

Binding Within Whole Blood

The fraction unbound in plasma, f_U, was calculated from the ratio of unbound, C_U, and plasma, C_P, concentrations, assuming that C_U is equal to the concentration in the plasma ultrafiltrate. The fraction unbound in plasma did not vary for any of the compounds between 1 and 50 μM concentrations in plasma (Table IV). The unsubstituted sulfonamides, which bind more strongly to CA, had the highest values of f_U. In contrast, the substituted sulfonamides, with less affinity for CA, were more strongly bound to plasma proteins and had lower f_U values. Within this series of compounds the degree of binding to plasma proteins was related to the relative polarity of the compounds judged by the order of elution from the reversed-phase HPLC system: **2, 1, 4, 3.** The less polar, longer-retention time compounds were more highly bound to plasma proteins than the more polar analogues. Also, the degree of ionization of the compounds affects their affinity for plasma protein. Compound 4 has the lowest pK_a and the lowest f_U.

The distribution of compounds between plasma and erythrocytes within whole blood is reflected in the blood-to-plasma concentration ratio, *B/P*. The *B/P* value is the result of competition between binding within plasma and binding within erythrocytes. Since a high affinity for CA is associated with a low affinity for plasma proteins, both 1 and 2 have high *B/P* values, whereas 3 and 4 have values close to unity (Table IV). A graph of plasma concentration vs whole-blood concentration is shown in Fig. 2.

The overall fraction of the total concentration in blood that is unbound, f_{UB}, was calculated from the ratio of f_U to *B/P*, assuming the unbound concentrations in plasma and erythrocytes to be equal. The compound which binds most strongly to CA, compound 1, and that which binds most strongly to plasma proteins, compound 4, have similar fractions unbound in blood.

Table IV. Distribution of Compounds Within Blood: Blood-to-Plasma Concentration Ratios (*B/P*), Fractions Unbound in Plasma (f_U), and Fractions Unbound in Blood, All in Rat Blood[a]

Compound	*B/P* ratio	f_U (plasma)	f_{UB} (blood)
1	21.8 ± 1.7	0.139 ± 0.025	0.0064 ± 0.0016
2	5.32 ± 0.29	0.127 ± 0.012	0.0239 ± 0.0036
3	1.32 ± 0.17	0.065 ± 0.004	0.0490 ± 0.0094
4	1.99 ± 0.28	0.013 ± 0.003	0.0065 ± 0.0024

[a] Values are means ± SD from five determinations at each of four concentrations from 1 to 50 μM. The solutions were prepared in blood pooled from four rats.

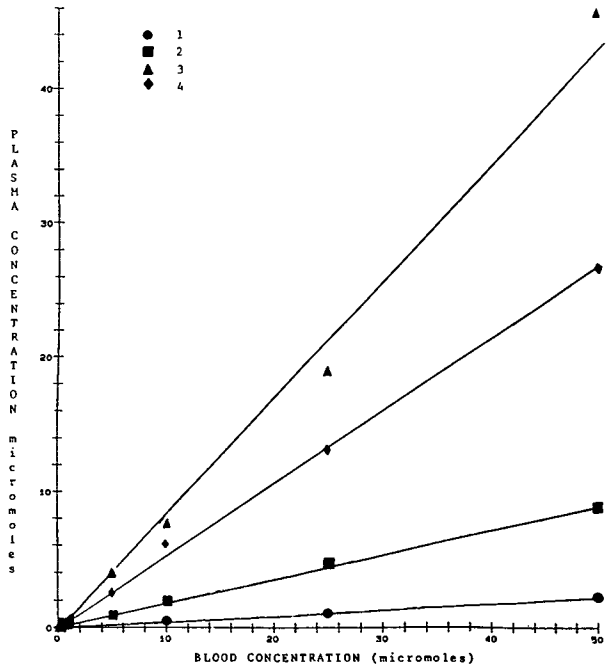


Fig. 2. Distribution of CA inhibitors, 1 (●), 2 (■), 3 (▲), and 4 (♦), between whole rat blood and plasma. Based on mean *B/P* ratio.

Pharmacokinetic Studies

Monoexponential or biexponential equations were used to describe blood concentration–time relationships after administering each compound to four rats. The terminal, elimination rate constant, λ_z, for each of the four compounds is given in Table V. In addition, the noncompartmental parameters, volume of distribution at steady state with respect to total drug (V_{BSS}) and to unbound drug in blood (V_{USS}), blood clearance (CL_B), and mean residence time in blood (MRT_B), were calculated from the blood concentration–time curve using standard methods (27). Values for these parameters are also given in Table V. Figure 3 shows log plasma concentration–time curves for the four compounds with the fitted lines.

Comparison of λ_z values assumes that they all represent the true terminal-phase rate constant. Detection of a true terminal phase was difficult for compounds 3 and 4 because the concentrations in blood fell rapidly below the lower limit of determination. A terminal, elimination phase was detectable in blood for only two of four rats which received compound 4. Analysis of urine showed that elimination was complete within 120 min after administration for 3 and 4 (Table V), the majority of the material recovered in the urine being β-glucuronidase-cleavable conjugates. Compound 2, which is strongly bound within erythrocytes, has the lowest value of λ_z, but compound 1, which is more strongly bound than compound 2, has a λ_z value similar to the range seen with

Table V. Pharmacokinetic Parameters After Intravenous Administration to Four Rats

	Compound			
	1	2	3	4
λ_z (min^{-1})[a]	0.0634 ± 0.0058	0.0206 ± 0.0070	0.125 ± 0.040	0.0335–0.0724[b]
V_{BSS} (liters/kg)[c]	0.246 ± 0.030	0.249 ± 0.055	2.61 ± 2.43	0.254 ± 0.123
V_{USS} (liters/kg)[d]	38.4	10.4	53.1	38.8
MRT_B (min)[c]	15.9 ± 2.8	50.5 ± 14.3	7.90 ± 2.35	7.73 ± 2.15
CL_B (ml/min/kg)[c]	16.0 ± 4.5	5.48 ± 2.74	308 ± 193	34.0 ± 17.2
CL_U (ml/min/kg)[d]	2510	230	6260	5210
F_E (120 min)[e]	11.1	12.5	21.6	7.5
F_E (1440 min)[e]	15.4	24.3	18.6	7.2

[a] Parameters estimated by nonlinear regression from the data for each rat and given as mean ± SD for four rats for each compound.
[b] This parameter could be precisely estimated in only two rats for this compound.
[c] Parameters estimated by statistical moment theory from the data for each rat and given as mean ± SD for four rats for each compound.
[d] Calculated from V_{BSS} or CL_B and f_{UB}.
[e] The percentage of the dose recovered in the urine as unchanged compound plus glucuronidase-labile conjugates at the time in parentheses. Average of two to four rats for each compound.

compound 4. The differences between λ_z values for compounds 1, 2, and 3 are significant at $P < 0.05$.

Comparison of CL_B values is complicated by the difficulty in calculating an AUC value for compound 3 due to its rapid elimination. Nevertheless, it is apparent that clearance values of the compounds which bind to CA within erythrocytes are less than those of compounds which are not bound within erythrocytes, even if the latter compounds are strongly bound to plasma proteins. The high apparent CL_B value for 3 may be due to the extraction of a high proportion of the intravenous dose during its first passage through the lungs. Such a phenomenon has been reported for the conjugation of phenols by rat lung (28). The blood clearance of compound 1 is greater than the total cardiac output of plasma, indicating that the fraction of this compound that is bound to CA within erythrocytes is available for elimination.

The values of the volume of distribution at steady state, calculated from blood concentrations, are greater than the blood volume for all four compounds, indicating that they all distribute outside the blood. When administered intrave-

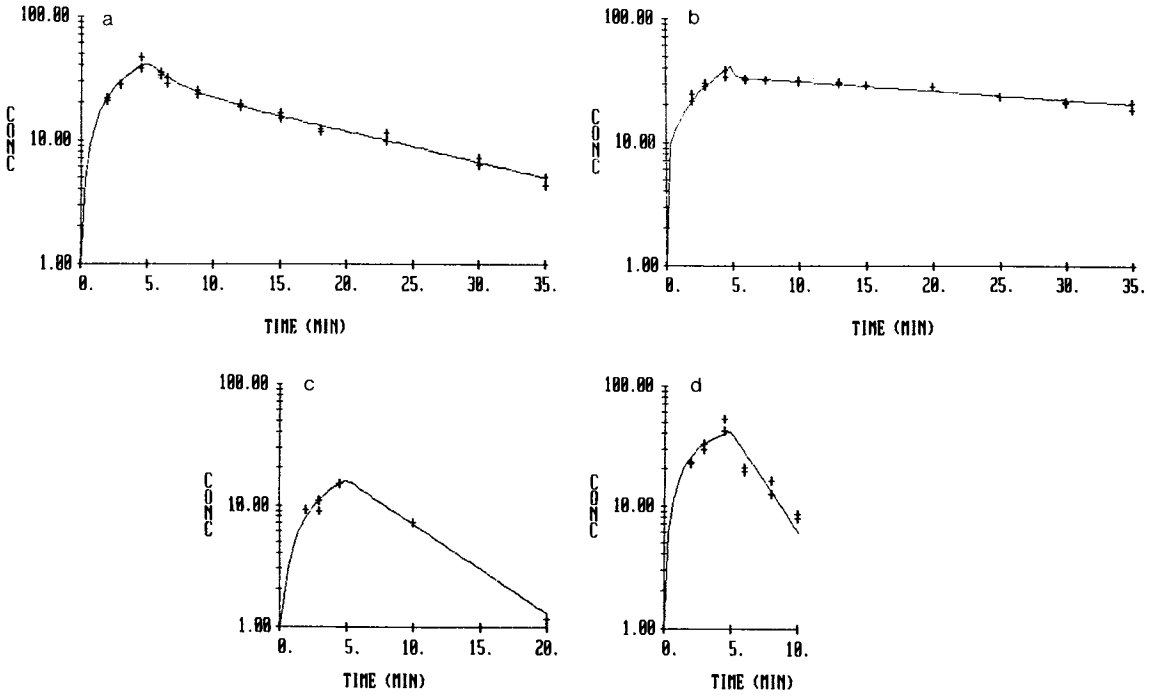


Fig. 3. Representative plots of log blood concentration (μM) against time (min) for each of four compounds with least-squares regression lines fitted: (a) compound 1, biexponential model; (b) compound 2, biexponential model; (c) compound 3, monoexponential model (d) compound 4, monoexponential model. Points represent duplicate determinations from each blood sample.

nously to rats, chlorthalidone, a compound that has a high affinity for CA, has also been found to distribute outside of blood, possibly to other sites which contain significant quantities of CA (28,29). The V_{BSS} value of compound 4 is not significantly different from those of 1 and 2, which are not different from each other. Therefore, binding either to plasma proteins or to CA can have a similar effect on the volume of distribution as viewed from blood. The values of V_{USS} are all very high, indicating that a large proportion of the amount of these compounds in the body is bound either to plasma proteins or to CA. Thus, only a small fraction of an administered dose would be able to produce a pharmacological response at any time.

The mean residence times viewed from whole blood (MRT_B) values are also given in Table V. There is no significant difference between the value for 3 and that for 4, but a significant difference in MRT_B exists between these compounds and compounds 1 and 2. The compounds which bind to CA have longer residence times than those which do not, even though the latter compounds bind more strongly to plasma proteins.

Using the values of fraction unbound in blood determined *in vitro*, approximate unbound clearance values (CL_U) were calculated from values of CL_B values determined *in vivo* ($CL_U = CL_B/f_{UB}$) (Table V). The unbound clearance of compound 2 is much less than those of the other three compounds. The unbound clearances of 3 and 4 are similar and are greater than that of compound 1 by a factor of two.

SUMMARY AND CONCLUSIONS

A series of structurally related sulfonamides has been synthesized and their potency as inhibitors of CA activity was used as a measure of affinity for CA isoenzymes. The order of potency observed was as expected from the structural differences. The unsubstituted sulfonamides were very potent inhibitors, while the substituted sulfonamides were almost without inhibitory activity. Affinity for CA was also reflected in the binding of the compounds within erythrocytes.

In contrast to binding within erythrocytes, the substituted sulfonamides bind more strongly to plasma proteins than the less polar compounds. A comparison of f_{UB} values shows that binding either to plasma protein or to CA within erythrocytes may result in a similar fraction unbound in blood.

Pharmacokinetic studies in rats demonstrated that the compounds that bind more strongly to CA within erythrocytes were eliminated less rapidly from blood. However, in some cases, differences in blood clearances were due to a difference in the unbound clearances of the compounds and were not dependent solely upon binding differences. Also, the relatively high blood clearance of compound 1 appeared to indicate that drug bound within erythrocytes may become available for elimination during the passage of blood through the eliminating organ.

Thus, binding to CA reduces the fraction of the drug in the body that is present unbound in the plasma and, so, may reduce the rate of elimination from the body. However, the structural changes required to generate and modulate affinity for CA may also produce variations in plasma binding and the vulnerability of the compounds to elimination processes. The overall effect on binding and clearance of increased affinity for CA depends on the balance between these influences.

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