Pharmacokinetics of Sulfanilic Acid and 1-Naphthylamine-4-sulfonic Acid in the Rabbit

KERRY ANN McMAHON and W. J. O'REILLY▲

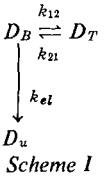
Abstract □ Blood level data were obtained in the rabbit after rapid intravenous administration of sulfanilic acid and 1-naphthylamine-4-sulfonic acid. The data were fitted to a two-compartment model, and the model parameters were obtained. Calculated clearance values from the central compartment were compared with clearance values for inulin obtained after rapid intravenous injection. Inulin and sulfanilic acid clearances from the central compartment were similar (11–16.3 ml./min. for sulfanilic acid and 4–13.5 ml./min. for inulin). 1-Naphthylamine-4-sulfonic acid had a greater clearance value, 52–63 ml./min. These values support the view that 1-naphthylamine-4-sulfonic acid is actively secreted into the renal tubule while sulfanilic acid is not.

Keyphrases □ Pharmacokinetics, 1-naphthylamine-4-sulfonic acid, sulfanilic acid—after intravenous administration, rabbits □ Sulfanilic acid—blood level–time relationships after intravenous administration, pharmacokinetic parameters, rate constants, rabbits □ 1-Naphthylamine-4-sulfonic acid—blood level–time relationships after intravenous administration, pharmacokinetic parameters, rate constants, rabbits

Numerous organic anions have been shown to undergo active transport in the kidney (1). Despopoulos (2) reviewed the structural requirements for the renal transport of organic anions, including various sulfonic acids. To demonstrate active transport, Despopoulos relied mainly on measurement of the ability of kidney slices to accumulate compounds from the medium. Direct evidence of active renal transport *in vivo* may be obtained by renal clearance studies, usually by a method involving perfusion with the compound under test (3). The effect of inhibitors of the renal transport system on the excretion of the compound is also a useful indicator of active transport (4).

During studies on the pharmacokinetics of sulfonamides and some sulfonic acids, blood level curves were obtained for sulfanilic acid and 1-naphthylamine-4-sulfonic acid. Despopoulos (2) demonstrated that sulfanilic acid was not concentrated by kidney slices while 1-naphthylamine-4-sulfonic acid was. The renal slice uptake of 1-naphthylamine-4-sulfonic acid was inhibited by probenecid and anoxia, indicating that this compound was transported actively by the kidney while sulfanilic acid was not. It is of interest to examine the pharmacokinetics of the two compounds in the rabbit to determine if the data for intact animals support the evidence based on slice uptake studies.

When the individual compounds are administered to rabbits, sulfanilic acid is excreted to some extent as an *N*-acetyl derivative (5) while 1-naphthylamine-4-sulfonic acid is recovered quantitatively from the urine as the unchanged compound[1].



Scheme I

EXPERIMENTAL

The compounds used were obtained commercially. 1-Naphthylamine-4-sulfonic acid was purified by recrystallization. Male New Zealand white rabbits (2–2.5 kg.) were fasted overnight, and the compounds were administered by rapid intravenous injection into a median ear vein. Sulfanilic acid and 1-naphthylamine-4-sulfonic acid were given as sodium salts (50 mg. in 1 ml.); inulin was given as an aqueous solution (200-mg. dose). Blood samples were taken at frequent intervals from a marginal ear vein into a heparinized syringe.

At the end of the blood collection period, the animals were transferred to metabolism cages and all urine was collected for 3–5 days. Experiments were conducted at intervals of 14 days. The blood sample was placed immediately into a centrifuge tube containing sodium oxalate solution (1 mg. in 7 ml.) and stored in a refrigerator until assay. Plasma proteins were precipitated by addition of 15% trichloroacetic acid (2 ml.). The samples were then centrifuged[2] at 2000 r.p.m. for 10 min., and the supernate was filtered. Free acid and metabolite were assayed by the Bratton–Marshall technique (6). It was found that 1-naphthylamine-4-sulfonic acid bound strongly to blood and was not fully released by the protein precipitant. To correct for this binding, a standard curve for 1-naphthylamine-4-sulfonic acid was prepared using known amounts of acid in blood. Inulin in blood and urine was determined by a modification of the method reported in Colowick and Kaplan (7).

THEORY

The two-compartment open model (Scheme I) has been used to describe the pharmacokinetic behavior of many drugs (8), where D_B represents drug in a central compartment including the blood; D_u and D_T represent drug amounts in the urine and a peripheral compartment, respectively; and k_{el}, k_{12}, and k_{21} are apparent first-order rate constants describing the elimination of the compound and its distribution into tissue. The interpretation and mathematical solution of this model has been extensively discussed (8–10).

After intravenous injection, drug concentration in the central compartment may be calculated from Eq. 1:

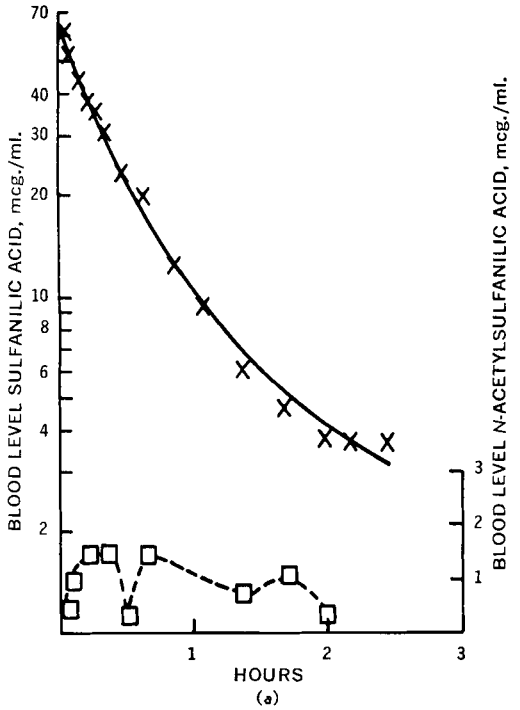
$$C_b = Ae^{-r_1 t} + Be^{-r_2 t} \qquad \text{(Eq. 1)}$$

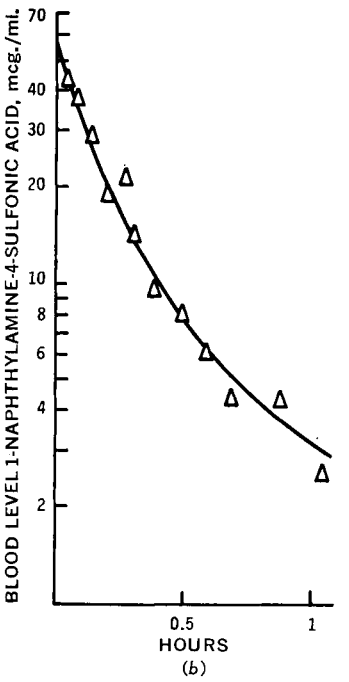
where C_b is the concentration of drug in plasma; and A, B, r_1, and r_2 are hybrid rate parameters containing the three rate constants of the model and the term C_b^0, the concentration of drug initially ($t = 0$) present in the central compartment, *i.e.*, immediately after injection. The methods of curve fitting and computer analysis to obtain the constants of the model were described previously (11).

RESULTS

Examples of the blood level curves obtained are shown in Fig. 1. The curves do not differ substantially except that 1-naphthylamine-4-sulfonic acid is excreted much more rapidly. The hybrid rate

[1] Unpublished data from this laboratory.
[2] Sorvall GLC-1 centrifuge.





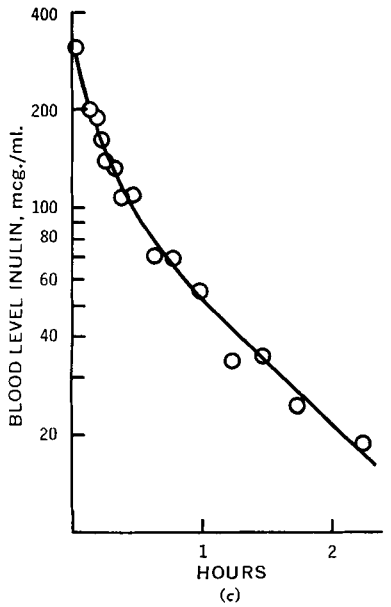


Figure 1—*Blood level curves for sulfanilic acid* (×), *1-naphthylamine-4-sulfonic acid* (Δ), *and inulin* (○) *in the rabbit. The solid line is the computer-generated least-squares fit to the respective data. In Fig.* 1a, *blood levels of* N-*acetylsulfanilic acid* (□) *are shown (right-hand linear scale). These levels are small and have considerable experimental error; no attempt was made to fit* N-*acetylsulfanilic acid data by computer.*

parameters are shown in Table I. The values for the two compounds differ significantly, indicating marked differences in pharmacokinetic behavior between them. These values were used to obtain computer estimates of the constants of the model (Table II). The elimination rate constant of 1-naphthylamine-4-sulfonic acid is markedly higher than k_{el} for sulfanilic acid and shows that 1-naphthylamine-4-sulfonic acid is very rapidly eliminated from the blood. It is assumed that this elimination is mainly renal, since there is no evidence for metabolism or biliary excretion of the compound and urinary recovery is quantitative.

Sulfanilic acid is eliminated both as free acid and as an acetyl derivative. If it is assumed that the metabolism occurs in the central compartment, the elimination rate constant (k_{el}) may be split into a rate constant for urinary excretion of the free compound (k_u) and an acetylation rate constant (k_m) for the formation of metabolite. Both k_m and k_u may be calculated from the relationship:

$$\frac{D_u^{\infty}}{M_u^{\infty} + D_u^{\infty}} = f = \frac{k_u}{k_{el}} \qquad \text{(Eq. 2)}$$

where D_u^{∞} and M_u^{∞} represent the total amount of drug and metabolite, respectively, ultimately excreted in the urine (11). The values of f, k_m, and k_u are shown in Table II.

The apparent volume of the central compartment (V_b) may be calculated from the relationship:

$$V_b = \frac{\text{dose}}{C_b^0} \qquad \text{(Eq. 3)}$$

where $C_b^0 = A + B$ (8). The apparent renal clearance from the central compartment (C_f) was calculated from $C_f = k_{el}V_b$ (10). The term "apparent" renal clearance is used to distinguish the model compartment clearance used here from the renal clearance determined by classical methods. The apparent renal clearance of sulfanilic acid was calculated using k_u, the urinary excretion rate constant, instead of k_{el}. The inulin clearance was corrected for the total inulin recovered in the urine (f). It was assumed that the rest of the inulin had been eliminated *via* extrarenal processes such as tissue binding (12). The clearance values are shown in Table II.

Table I—Parameters of the Equation $C_b = Ae^{-r_1t} + Be^{-r_2t}$ for Blood Levels of Sulfanilic Acid, 1-Naphthylamine-4-sulfonic Acid, and Inulin following Intravenous Injection in the Rabbit[a]

Animal	A, mcg./ml.	r_1, hr.$^{-1}$	B, mcg./ml.	r_2, hr.$^{-1}$
		Sulfanilic Acid		
L	69.89 ± 7.18	4.04 ± 0.51	25.39 ± 5.06	0.869 ± 0.170
S	54.66 ± 2.16	2.78 ± 0.20	11.82 ± 1.46	0.549 ± 0.073
A	56.63 ± 2.00	3.25 ± 0.14	18.03 ± 1.35	0.844 ± 0.057
		1-Naphthylamine-4-sulfonic Acid		
L	47.12 ± 3.62	6.25 ± 0.79	9.88 ± 1.38	1.18 ± 0.25
S	51.78 ± 9.00	9.57 ± 1.91	7.14 ± 1.30	0.88 ± 0.34
A	44.94 ± 3.13	7.44 ± 0.51	9.95 ± 1.18	0.99 ± 0.24
		Inulin		
S	243.27 ± 24.94	6.39 ± 0.42	116.82 ± 11.83	0.760 ± 0.110
S	181.58 ± 58.04	11.95 ± 1.99	188.01 ± 19.15	0.834 ± 0.146
A	262.74 ± 27.21	16.31 ± 1.64	241.38 ± 10.47	0.372 ± 0.047

[a] Values are quoted ± SD of the parameter.

DISCUSSION

Despopoulos (2) pointed out that renal clearances suggest active tubular secretion when they exceed the estimated glomerular filtration rates (GFR). The GFR is usually taken to equal the clearance of inulin or some other material not actively secreted such as creatinine or xylose (13). Clearance values based on single bolus dose pharmacokinetics may be taken only as tentative evidence for true renal clearance values. The two-compartment model clearance of polyfructosans was obtained in man after a single intravenous dose and compares favorably with the clearance of the same compound obtained by classical methods (14).

In the present experiments, the fast distributive phase (r_1) of inulin was very rapid; consequently, the fitting of the two-compartment model to the data was less accurate than with sulfanilic acid and 1-naphthylamine-4-sulfonic acid. Nevertheless, the range of clearance values obtained (4–13.5 ml./min.) agrees reasonably well with the range of literature values for the rabbit [1.7–12 ml./min. calculated on a 2.5-kg. weight (15–19)]. The wide variety of the values reported is probably related to the anomalous behavior of the rabbit since inulin clearance is urine flow dependent and urine flow varies with stress (20, 21). The apparent renal clearance of sulfanilic acid (Table II) is slightly higher than the apparent renal clearance of inulin and it may be concluded that sulfanilic acid is probably eliminated in a similar manner to inulin, with perhaps a small contribution by active tubular secretion. This supports the observation of Despopoulos (2) that sulfanilic acid is not actively transported into kidney slices or actively secreted into the tubules of chicken kidney *in vivo*.

1-Naphthylamine-4-sulfonic acid is not excreted as a metabolite, while sulfanilic acid is excreted to some extent as *N*-acetylsulfanilic acid, which is known to be transported into kidney slice preparations (2). It is possible that *N*-acetylsulfanilic acid in the circulation could compete with sulfanilic acid for a renal secretory mechanism. Nelson and O'Reilly (22) suggested possible competition between high blood levels of sulfisoxazole and acetyl sulfisoxazole for tubular secretory or reabsorption processes. Such competition cannot be ruled out in the present study but is rendered unlikely in view of the low levels of *N*-acetyl metabolite present, particularly during the early stages of sulfanilic acid excretion when the blood levels of sulfanilic acid are relatively high (Fig. 1). Later, the blood levels of both compounds are low and saturation of renal transport mechanisms would seem unlikely.

In most studies of renal clearance, quite high blood levels of drug and inhibitor are used to demonstrate inhibition of tubular secretion. For example, Arita *et al.* (18) used blood levels of 100 mcg./ml. of sulfonamide and 200–600 mcg./ml. of iodopyracet to demonstrate inhibition of tubular secretion of sulfisoxazole in rabbits. Jusko *et al.* (23) studied the effect of probenecid on the clearance of riboflavin in man. To reduce flavin clearance, serum levels of probenecid of 20–50 mcg./ml. compared to flavin levels of 0.3–0.5 mcg./ml. were required. However, *N*-acetylsulfanilic acid may influence the renal transport of sulfanilic acid, and this possibility requires further study.

Active secretion in the kidney tubule is generally considered to occur with substances such as diodone. Clearance values for this compound in the rabbit range from 25 to 40 ml./min. (17–20). The apparent renal clearance of 1-naphthylamine-4-sulfonic acid reported here (Table II) is slightly higher than the diodone value but well within the renal blood flow of the rabbit (80 ml./min.) (24). The 1-naphthylamine-4-sulfonic acid clearance is considerably higher than inulin or sulfanilic acid clearance and supports the view of Despopoulos (2) that the compound is actively secreted in the rabbit kidney.

These results indicate that bolus dose pharmacokinetic studies using a two-compartment model will give apparent renal clearance

Table II—Pharmacokinetic Constants for the Two-Compartment Model for Blood Levels of Sulfanilic Acid and 1-Naphthylamine-4-sulfonic Acid in the Rabbit

Animal	k_{el}, hr.$^{-1}$	f	k_m, hr.$^{-1}$	k_u, hr.$^{-1}$	k_{12}, hr.$^{-1}$	k_{21}, hr.$^{-1}$	V_b, ml.	C_f[a], ml./min.
				Sulfanilic Acid				
L	1.97 ± 0.27	0.637	0.72	1.25	1.10 ± 0.43	1.34 ± 0.48	525	11.0
S	1.65 ± 0.14	0.785	0.35	1.30	0.75 ± 0.20	1.04 ± 0.26	752	16.3
A	1.94 ± 0.07	0.720	0.54	1.40	0.74 ± 0.10	1.47 ± 0.21	670	15.6
				1-Naphthylamine-4-sulfonic Acid				
L[b]	3.59 ± 0.53	—	—	—	1.79 ± 0.84	2.06 ± 0.75	877	52
S	4.45 ± 0.27	—	—	—	4.09 ± 1.28	2.04 ± 0.90	849	63
A	3.50 ± 0.27	—	—	—	2.76 ± 0.50	2.28 ± 0.32	911	53
				Inulin				
S	2.007 ± 0.124	0.723	0.556	1.451	2.54 ± 0.29	3.01 ± 0.34	559	13.5
S	1.538 ± 0.141	0.738	0.403	1.135	4.70 ± 0.65	6.40 ± 1.72	540	10.2
A	0.767 ± 0.073	0.793	0.159	0.608	7.95 ± 0.77	8.04 ± 0.78	395	4.0

[a] For sulfanilic acid and inulin, $C_f = V_b k_u$. For 1-naphthylamine-4-sulfonic acid, $C_f = V_b k_{el}$; k_{el}, k_{12}, and k_{21} are quoted ± SD of the parameter.
[b] This animal died before inulin clearance was carried out.

values comparable to those obtained by more complex physiological methods without the assumption of the model.

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Oxime Acetates: Substrates for Acetylcholinesterase

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Abstract □ Oxime acetates function as typical substrates for acetylcholinesterase. Both acetate derivatives of *syn*-3- and *syn*-4-formyl-1-methylpyridinium iodide oximes are rapidly hydrolyzed. Both are highly water soluble and give large changes in absorbance upon hydrolysis. Hence, they have potential utility for spectrophotometric studies with the enzyme. The prior configurational assignment of the acetate derivative of *syn*-4-formyl-1-methylpyridinium iodide oxime has been confirmed by NMR spectroscopy. Vicinal aliphatic dioxime diacetates are hydrolyzed, but quite slowly, by acetylcholinesterase.

Keyphrases □ Acetylcholinesterase—oxime acetates as substrates □ *syn*-3- and *syn*-4-Formyl-1-methylpyridinium iodide oximes, acetate derivatives—configuration confirmation by NMR, acetylcholinesterase substrates □ Enzyme kinetics—oxime acetates as substrates for acetylcholinesterase □ 3-PAM acetate—reaction with isonitrosoacetone, substrate for acetylcholinesterase □ 4-PAM acetate—configuration, substrate for acetylcholinesterase □ NMR spectroscopy—configuration identification

It is becoming increasingly clear that the high selectivity and specificity of enzymes is a comparative rather than an absolute phenomenon. The substrate activity spectrum must ultimately be correlated with the enzyme's structure, conformation, and chemical mechanism. Hence, a knowledge of the activity spectrum provides information concerning these properties. It also provides flexibility in assay. We have observed that eel acetylcholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.7) catalyzes the hydrolysis of several *O*-acetyl oximes, including acetate derivatives of benzaldehyde oxime, *syn*-3- and *syn*-4-formyl-1-methylpyridinium iodide oximes[1] (*syn*-3- and *syn*-4-PAM acetates) (Structure I), and glyoxime and dimethylglyoxime (Structures II*a* and II*b*, respectively). In this article the kinetic constants for the enzymatic hydrolysis of the pyridinium compounds and semiquantitative results for the glyoximes are reported.

Acetylcholinesterase hydrolyses are quite sensitive to environmental conditions. Substrate turnover rates vary considerably with ionic strength, the presence or

[1] These compounds, which are closely related to the preferred oxime treatment compounds for organophosphonate poisoning (for example, pralidoxime chloride), have been generally referred to in the biochemical literature as 3-PAM and 4-PAM, respectively.