are in accord with those of Holder *et al.*,[4] who found that BHT-alcohol was absorbed, metabolized, and excreted in the bile more rapidly than BHT after intraperitoneal administration to rats.

The present results suggest that the biotransformation of BHT to BHT-QM proceeds mainly through BHT-alcohol. A possible explanation of this pathway is that BHT-alcohol is dehydrated directly or after being converted to some conjugate (s). It is generally accepted that metabolic reduction of alcohols proceeds through dehydration followed by hydrogenation.[18] On the other hand, 4-hydroxy-BHT does not seem to be dehydrated *in vivo* to BHT-QM, although it was reported to be transformed *in vitro* to BHT and BHT-alcohol.[10]

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Chem. Pharm. Bull.
29(12)3741—3747(1981)

Fundamental Pharmacokinetic Behavior of Sulfadimethoxine, Sulfamethoxazole and Their Biotransformed Products in Dogs[1]

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(Received July 24, 1981)

Plasma concentration profiles of sulfadimethoxine, sulfamethoxazole and their biotransformed products (N^4-acetate and N^1-glucuronide) in dogs were determined and their pharmacokinetic parameters were calculated by using a two-compartment open model. The apparent partition coefficients between chloroform and phosphate buffer were also determined. Decline in plasma levels of sulfadimethoxine and sulfamethoxazole was considerably accelerated by N^4-acetylation and N^1-glucuronidation. The elimination of sulfadimethoxine-N^1-glucuronide from plasma was more rapid than that of sulfadimethoxine-N^4-acetate, and a similar tendency was observed for sulfamethoxazole and its biotransformed products. N^4-Acetylation or N^1-glucuronidation of sulfadimethoxine and

sulfamethoxazole decreased the lipid solubilities markedly in comparison with those of original sulfonamides.

Keywords——sulfadimethoxine; sulfamethoxazole; biotransformation; N^4-acetylation; N^1-glucuronidation; plasma concentration profile; elimination rate; pharmacokinetic parameters; partition coefficients; sulfonamides

Any biotransformation of a drug results in new compounds whose physicochemical properties differ from those of the original drug. Such a biotransformed product may display different pharmacokinetic behavior from the original drug. Therefore, elucidation of the correlation between biotransformation and pharmacokinetic behavior is important before the clinical use of any drug.

In this work, we carried out fundamental pharmacokinetic studies in dogs on sulfadimethoxine (SDM) and sulfamethoxazole (SMX), which are sulfonamides widely used in the treatment of infectious diseases, as well as four biotransformed products, SDM-N^4-acetate (SDM-Ac), SDM-N^1-glucuronide (SDM-Gl), SMX-N^4-acetate (SMX-Ac) and SMX-N^1-glucuronide (SMX-Gl), identified as the metabolites excreted in human urine.[2–10]

Materials and Methods

Materials——Commercially available SDM and SMX were recrystallized from ethanol (mp 201°C and 169°C, respectively). SDM-Ac and SMX-Ac (mp 222°C and 226°C, respectively) were synthesized by acetylation of SDM and SMX.[11,12] SDM-G1 and SMX-G1 were obtained from human urine after drug administration. They were separated by column chromatography, eluted, and purified. SDM-G1 was 97.2% pure (as the ammonium salt), mp 160—170°C (dec.). UV $\lambda_{max}^{H_2O}$ nm (ε): 267 (19300). IR ν_{max}^{KBr} cm^{-1}: 3700, 3000 (OH, NH_2, NH_4^+), 1620—1560 (COO^-), 1160 (SO_2). PMR (δ in D_2O): 3.88 (1H, s, OCH_3), 3.92 (1H, s, OCH_3), 6.55 (1H, s, pyrimidine proton), 6.75 (2H, d, $J_{AB}=9$ Hz, aromatic ring proton). SMX-G1 was 83% pure (as the ammonium salt), mp 165—173°C (dec.). UV $\lambda_{max}^{H_2O}$ nm (ε): 269 (13800). IR ν_{max}^{KBr} cm^{-1}: 3000—3500 (OH, NH_2, NH_4^+), 1560—1620 (COO^-), 1145 (SO_2). PMR (δ in DMSO-d_6): 2.37 (3H, s, CH_3), 6.12 (1H, s, isoxazole proton), 6.58 (2H, d, $J_{AB}=9$ Hz, aromatic proton). All other chemicals were of reagent grade.

Plasma Level of Sulfonamides in Dogs——Female dogs weighing 11.0—15.0 kg were used in this study. They were anesthetized with pentobarbital sodium (30 mg/kg). Each sulfonamides was administered in doses of 10 mg/kg through the cephalic vein. About 3 ml of blood was withdrawn from the cephalic vein into a heparinized syringe, and the plasma was obtained by centrifugation. Plasma samples were deproteinized with 10% trichloroacetic acid, and the sulfonamides and their biotransformed products were analyzed by diazotization.[13]

Estimation of pharmacokinetic parameters was made according to the two-compartment open model by least-squares fitting of the plasma levels using a digital computer, NEC-ACOS 500 (NEC Co., Ltd.).

Glucuronic acid was measured by the naphthoresorcinol picrate method as modified by Ishidate *et al.*[14]

Determination of Apparent Partition Coefficients——Apparent partition coefficients (P') of sulfonamides and their biotransformed products between 0.1 M phosphate buffer (pH 7.4) and chloroform were studied.[15,16] To determine P', a drug solution of 1.0—5.8 μg/ml was made using buffer solution which had previously been saturated with chloroform. Ten ml of the drug solution was added to 10 ml of chloroform which had previously been saturated with the buffer solution. After shaking for 2 h at 18±1°C to achieve equilibrium, the drug concentration in the buffer was analyzed by diazotization.

Results

Elimination Profiles of SDM and the Two Biotransformed Products from Plasma

The plasma concentration profiles of each compound following bolus intravenous administration to three dogs are shown in Fig. 1.

The plasma levels of SDM declined much more slowly than those of SDM-Ac and SDM-Gl. The plasma levels of SDM-Gl declined most rapidly among those of the three compounds.

Elimination Profiles of SMX and the Two Biotransformed Products from Plasma

Plasma concentration profiles of SMX and the two biotransformed products following bolus intravenous administration to dogs are shown in Fig. 2.

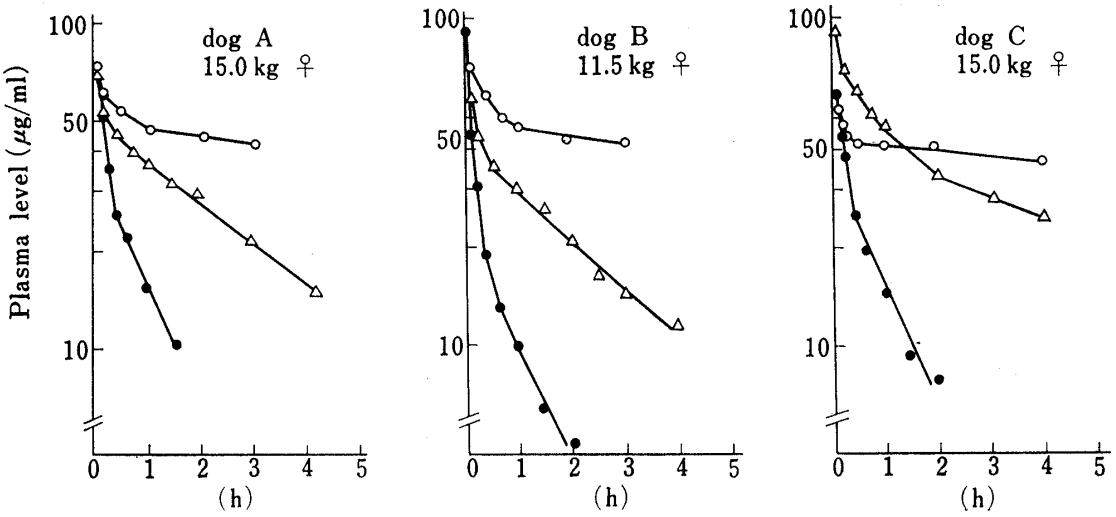

Fig. 1. Plasma Concentration Profiles of Sulfadimethoxine, Sulfadimethoxine-N^4-acetate and Sulfadimethoxine-N^1-glucuronide in Dogs

—○—: sulfadimethoxine
—△—: sulfadimethoxine-N^4-acetate
—●—: sulfadimethoxine-N^1-glucuronide

Each drug was intravenously administered into the cephalic vein at a dose of 10 mg/kg.

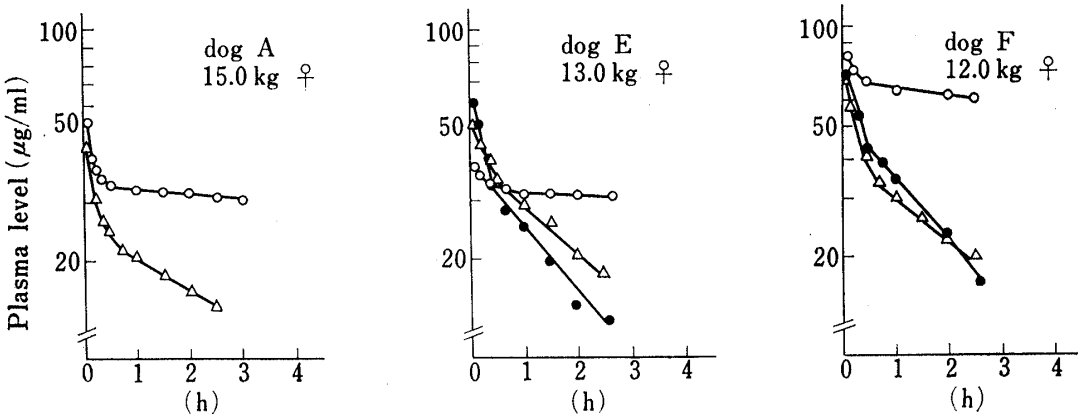

Fig. 2. Plasma Concentration Profiles of Sulfamethoxazole, Sulfamethoxazole-N^4-acetate and Sulfamethoxazole-N^1-glucuronide in Dogs

—○—: sulfamethoxazole
—△—: sulfamethoxazole-N^4-acetate
—●—: sulfamethoxazole-N^1-glucuronide

Each drug was intravenously administered into the cephalic vein at a dose of 10 mg/kg.

TABLE I. Pharmacokinetic Parameters for Sulfadimethoxine, Sulfadimethoxine-N^4-acetate and Sulfadimethoxine-N^1-glucuronide following Intravenous Administration to Dogs

Animal	Parameters	Drugs: Sulfadimethoxine	Sulfadimethoxine-N^4-acetate	Sulfadimethoidexine-N^1-glucuron
Dog A 15.0 kg ♀	k_{21}	6.23	4.90	2.28
	k_{el}	0.135	0.551	1.85
	k_{12}	3.62	4.31	2.84
	$t_{1/2}(\beta)$ (h)	8.14	2.44	1.04
	V_1	1.30	1.55	1.51
	V_2	0.753	1.36	1.88

Animal	Parameters	Drugs: Sulfadimethoxine	Sulfadimethoxine-N^4-acetate	Sulfadimethoxine-N^1-glucuronide
Dig B	k_{21}	2.62	5.47	2.32
11.5 kg	k_{el}	0.0843	0.860	3.78
♀	k_{12}	1.50	6.41	5.04
	$t_{1/2}(\beta)$ (h)	13.0	1.82	0.813
	V_1	1.39	1.04	0.738
	V_2	0.796	1.21	1.60
Dog C	k_{21}	6.17	3.81	2.58
15.0 kg	k_{el}	0.0802	0.515	1.80
♀	k_{12}	5.13	3.47	2.81
	$t_{1/2}(\beta)$ (h)	15.9	2.67	0.967
	V_1	1.90	1.17	1.66
	V_2	1.58	1.07	1.81
Mean	k_{21}	5.01±0.974	4.73±0.399	2.39±0.0759
±S.E.[a]	k_{el}	0.100±0.0145	0.642±0.0895	2.47±0.532
	k_{12}	3.42±0.859	4.73±0.712	3.56±0.604
	$t_{1/2}(\beta)$ (h)	12.3 ±1.84	2.31±0.206	0.939±0.0539
	V_1	1.53±0.153	1.25±0.124	1.30±0.234
	V_2	1.04±0.219	1.22±0.0679	1.77±0.0680

a) Standard error of estimate.

TABLE II. Pharmacokinetic Parameters for Sulfamethoxazole, Sulfamethoxazole-N^4-acetate and Sulfamethoxazole-N^1-glucuronide following Intravenous Administration to Dogs

Animal	Parameters	Drugs: Sulfamethoxazole	Sulfamethoxazole-N^4-acetate	Sulfamethoxazole-N^1-glucuronide
Dog A	k_{21}	5.18	3.72	—
15.0 kg	k_{el}	0.0802	0.536	—
♀	k_{12}	4.64	3.70	—
	$t_{1/2}(\beta)$ (h)	16.5	2.68	—
	V_1	2.56	2.95	—
	V_2	2.30	2.94	—
Dog E	k_{21}	3.11	3.08	2.17
13.0 kg	k_{el}	0.0225	0.439	0.776
♀	k_{12}	0.710	1.20	1.67
	$t_{1/2}(\beta)$ (h)	37.9	2.26	1.74
	V_1	3.28	2.26	1.76
	V_2	0.748	0.882	1.36
Dog F	k_{21}	4.92	2.76	4.87
12.0 kg	k_{el}	0.0652	0.608	0.860
♀	k_{12}	2.32	2.79	4.55
	$t_{1/2}(\beta)$ (h)	15.7	2.43	1.63
	V_1	3.17	1.33	1.07
	V_2	1.49	1.34	1.01
Mean	k_{21}	4.41±0.531	3.18±0.230	3.52[b]
±S.E.[a]	k_{el}	0.0560±0.0141	0.528±0.0398	0.818[b]
	k_{12}	2.56±0.932	2.56±0.597	3.11[b]
	$t_{1/2}(\beta)$ (h)	23.3±5.93	2.46±0.0991	1.69[b]
	V_1	3.00±0.181	2.18±0.385	1.42[b]
	V_2	1.51±0.366	1.72±0.509	1.19[b]

a) Standard error of estimate. b) Arithmetic mean.

The plasma levels of SMX declined very slowly as compared to those of SMX-Ac and SMX-Gl.

Calculation of Pharmacokinetic Parameters

Pharmacokinetic parameters were estimated by least-squares fitting of the plasma concentration to the following equation,

$$C = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t}$$

where C is plasma concentration of a drug at time t after intravenous administration, β is the slope of the elimination phase and α is the slope of the residual concentration curve. The values of A, B, α and β were calculated by computer analysis of the plasma concentration data for each compound. Employing these values, the values of k_{12}, k_{21}, k_{el}, V_1, V_2 and $t_{1/2}$ (β) in the two-compartment open model were calculated by the method of Riegelman *et al.*[17,18] The pharmacokinetic parameters are listed in Tables I and II.

As shown in Table I, marked increases in the values of k_{el} of SDM-Ac and SDM-Gl were observed in all cases as compared with SDM, in spite of some fluctuation in the absolute values due to individual physiological differences. SDM-Gl was observed to have the highest k_{el} among the three compounds.

Similar results were observed for SMX and its biotransformed products, as shown in Table II.

Determination of Apparent Partition Coefficients for SDM, SMX and Their Biotransformed Products

Apparent partition coefficients (P') for SDM, SMX and their biotransformed products were determined, and the data are shown in Table III.

Tabel III. Apparent Partition Coefficients for Sulfadimethoxine, Sulfamethoxazole and Their Biotransformed Products in Chloroform/Phosphate Buffer (pH 7.4) System

Drug	Initial conc. in aqueous phase (mg/l)	P'[a] ± S.D.[b] (chloroform)	Number of experiments
SDM[c]	1.0	4.07 ±0.381	3
	5.0	4.16 ±0.137	3
	Mean	4.12 ±0.261	6
SDM-Ac[d]	1.0	0.391±0.038	3
	5.0	0.362±0.010	3
	Mean	0.391±0.047	6
SDM-Gl[e]	1.2	0.015±0.002	5
	5.2	0.015±0.005	5
	5.8	0.008±0.003	5
	Mean	0.013±0.005	15
SMX[f]	1.0	0.240±0.015	3
	5.0	0.140±0.015	3
	Mean	0.190±0.055	6
SMX-Ac[g]	1.0	0.035±0.015	3
	5.0	0.027±0.017	3
	Mean	0.030±0.016	6
SMX-Gl[h]	1.0	0.017±0.007	3
	5.0	0.003±0.001	3
	Mean	0.010±0.009	6

a) Apparent partition coefficient in chloroform/phosphate buffer (pH 7.4) system.
b) Standard deviation. *c*) Sulfadimethoxine. *d*) Sulfadimethoxine-N^4-acetate.
e) Sulfadimethoxine-N^1-glucuronide. *f*) Sulfamethoxazole.
g) Sulfamethoxazole-N^4-acetate. *h*) Sulfamethoxazole-N^1-glucuronide.

SDM has the largest value and SMX has a moderate value of P' between chloroform and phosphate buffer. A decreased value of P' was observed for SDM-Ac as compared to that of SDM. A similar relation was observed between SMX and SMX-Ac. Both SDM-Gl and SMX-Gl possess extremely small P' values when compared to the other compounds.

Discussion

In general, the main biotransformation reaction of sulfonamides in humans is N^4-acetylation, which is classified as a conjugation reaction *in vivo*. N^4-Acetylated products of sulfonamides usually show decreased solubility in body fluids compared to the original sulfonamides. Some sulfonamides are largely biotransformed to N^1-glucuronides, which have markedly increased solubility in body fluids.

We investigated the pharmacokinetic behavior of SDM and SMX, which are long-acting sulfonamides and are biotransformed to a large extent. Elimination patterns of the compounds in dogs were evaluated mainly in terms of variation in k_{el}, $t_{1/2}(\beta)$ and other pharmacokinetic parameters calculated from plasma levels. The results are shown in Figs. 1 and 2 and Tables I and II.

Several conclusions were reached concerning the relationship between pharmacokinetic behavior and biotransformation of SDM and SMX. Firstly, SDM and SMX are eliminated from plasma very slowly; they maintained high plasma levels for a long time ($t_{1/2}$ (β): SDM, 12.3±1.84 h; SMX, 23.3±5.93 h). Secondly, SDM-Ac and SMX-Ac are eliminated rapidly from plasma compared to SDM and SMX ($t_{1/2}$ (β): SDM-Ac, 2.31±0.206 h; SMX-Ac, 2.46± 0.0991 h). It seems clear that N^4-acetylation of long-acting sulfonamides such as SDM and SMX results in a large increase in elimination rate. Thirdly, SDM-Gl and SMX-Gl are most rapidly eliminated from plasma among the compounds tested in the study ($t_{1/2}$ (β): SDM-Gl, 0.939±0.0539 h; SMX-Gl, 1.69 h). It is also clear that N^1-glucuronidation of long-acting sulfonamides such as SDM and SMX results in the large increase in elimination rate.

Renal excretion patterns of SDM, SMX and their biotransformed products in dogs have been reported previously.[19,20] N^4-Acetylation of SDM and SMX results in a considerable increase in renal clearance ratio compared to the original sulfonamides. It is also demonstrated that N^1-glucuronidation of SDM and SMX results in an increase in renal clearance ratio compared to SDM-Ac and SMX-Ac.

The pharmacokinetic data given here support previous observations[19,20] on renal clearance of SDM, SMX and their biotransformed products.

A rough guide to the lipid solubility of a drug is provided by partition coefficient between an organic solvent, such as chloroform, and an aqueous phosphate buffer approximating the physiological pH. As shown in Table III, SDM possesses very high lipid solubility and N^4-acetylation decreases the lipid solubility. Further, N^1-glucuronidation of SDM results in negligible lipid solubility. A similar tendency is observed in SMX and its two biotransformed products, although the lipid solubility of SMX is originally less than that of SDM. The present data on the lipid solubility of SDM, SMX and their biotransformed products are mostly in accord with previous observations.[20–22]

Drug protein binding is another important factor controlling the pharmacokinetic behavior of a drug. It has been reported that N^4-acetylation and N^1-glucuronidation of SDM and SMX decreased the affinities for plasma protein compared to the those of original sulfonamides.[20–22] Judging from the pharmacokinetic behavior of SDM, SMX and their biotransformed products, such reduced protein binding of the biotransformed products seems to be an important factor in controlling the elimination rate of the compounds.

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