Pharmacokinetics, N1-glucuronidation and N4-acetylation of sulfadimethoxine in man

T.B. Vree, E.W.J. Beneken Kolmer, M. Martea, R. Bosch, Y.A. Hekster and M. Shimoda

Introduction

The pharmacokinetics of sulfadimethoxine (4-sulfanilamido-2,6-dimethoxypyrimidine) has been studied in man and many species, such as Beagle dogs [1], cats [2], hens [3-5], lions [6 7], lobsters [8], pigs [9], primates [10] and ruminants [11]. In man a small percentage of sulfadimethoxine undergoes acetylation at the N4-position; parent drug and N4-acetyl conjugate account for 10-15% of the mass balance, leaving 80% for other metabolic pathways [12 13]. Figure 1 shows the possible metabolic pathways.

Recently we have demonstrated that in addition to N4-acetylation (conjugation, phase II) of sulfonamides, phase I metabolic reactions also take place at the N1-substituent. For instance, oxidation reactions have been demonstrated for sulfamethoxazole, sulfatroxazole, sulfadimidine and sulfamerazine in man, ruminants, reptiles and molluscs [14-17].

In man, methoxysulfonamides, such as sulfadimethoxine, exhibit extremely long half-lives, a low percentage of N4-acetyl conjugation, a low percentage of renal excretion of parent drug and N4-acetyl conjugate, resulting in an incomplete mass balance (20%) [18 19]. This suggests that slowly performed oxidation reactions at the N1-substituent should be considered. Oxidative attack of sulfadimethoxine may occur at the methoxy groups, giving rise to *O*-dealkylation reactions. The resulting hydroxy metabolites of sulfadimethoxine and/or their *O*-glucuronides will be (very) water-soluble and would elute in the high pressure liquid chromatogram (HPLC) simultaneously with the water-soluble endogenous waste products. Therefore, it would be difficult to recognize the *O*-demethylated metabolites in human urine, if they should exist.

In addition, species-dependent glucuronide conjugates have been reported for sulfadimethoxine [10 20-23], which are assumed to be N1- or N4-glucuronides. However, no renal excretion rate–time profiles or plasma concentration–time curves of sulfadimethoxine glucuronides nor a full pharmacokinetic analysis of sulfadimethoxine have been published.

The aims of this investigation were to demonstrate in man that for sulfadimethoxine in addition to N4-acetylation, either *O*-dealkylation pathways exist, or some other conjugations take place, and to make the mass balance complete. Pharmacokinetic parameters of this drug based on HPLC methodology instead of the Bratton and Marshall analysis were assessed. Therefore, 10 volunteers were selected (fast and slow acetylators) and an oral dose of 10 mg/kg sulfadimethoxine was administered.

This investigation shows that *O*-demethylation of sulfadimethoxine is not present in man, but that N1-glucuronidation and N4-acetylation take place.

Keywords
Chromatography, high pressure liquid
Clearance
Metabolism
Pharmacokinetics
Protein binding
Sulfadimethoxine

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Abstract

Sulfadimethoxine is metabolized by *O*-dealkylation, N4-acetylation and N1-glucuronidation. In man, only N1-glucuronidation and N4-acetylation takes place, leading to the final double conjugate N4-acetylsulfadimethoxine-N1-glucuronide. The N1-glucuronides are directly measured by high pressure liquid chromatography. When N4-acetylsulfadimethoxine is administered as parent drug, 30% of the dose is N1-glucuronidated and excreted. Fast acetylators show a shorter half-life for sulfadimethoxine than slow acetylators (27.8 $\pm$ 4.2 h versus 36.3 $\pm$ 5.4 h; P = 0.013), similarly the half-life of the N4-acetyl conjugate is also shorter in fast acetylators (41.3 $\pm$ 5.2 h versus 53.5 $\pm$ 8.5 h, P = 0.036). No measurable plasma concentrations of the N1-glucuronides from sulfadimethoxine are found in plasma. N1-glucuronidation results in a 75% decrease in protein binding of sulfadimethoxine. N4-acetylsulfadimethoxine and its N1-glucuronide showed the same high protein binding of 99%. Approximately 50-60% of the oral dose of sulfadimethoxine is excreted in the urine, leaving 40-50% for excretion into bile and faeces.

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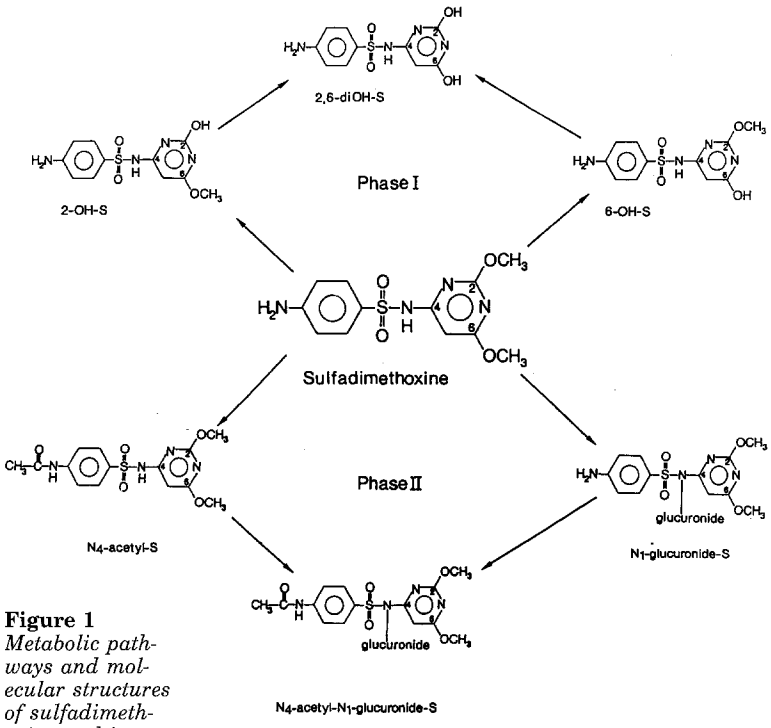

Figure 1
Metabolic pathways and molecular structures of sulfadimethoxine and its metabolites and conjugates in man and animals

Methods

Subjects

10 Healthy volunteers [6 males, 4 females; 3 Asian (subjects A-C) and 7 Caucasian; 6 fast and 4 slow acetylators] with ages ranging from 20-46 years, participated in the study. 2 Volunteers (1 slow and 1 fast acetylator) also took N4-acetylsulfadimethoxine. The drugs were administered orally in gelatine capsules in a single 10 mg/kg dose. The study had the approval of the Hospital Ethics Committee.

Drugs

Sulfadimethoxine was obtained from Sigma (No. S-7007; St. Louis, USA) and N4-acetylsulfadimethoxine was synthesized [16]. 2-Hydroxysulfadimethoxine and 6-hydroxysulfadimethoxine were a gift from Dr. K. Hoji (Daiichi Seiyaku, Tokyo, Japan).

Table 1
Capacity factors of sulfadimethoxine and its conjugates

Compound*	Capacity factor (k')	Ratio k'†	
		N4/S	glucuronide/aglycon
2-OH-S	1.83		
6-OH-S	4.33		
S-glucuronide	13.28		0.53
N4-glucuronide	17.23	1.30	0.65
Sulfadimethoxine	25.15		
N4	26.53	1.05	

*S: sulfadimethoxine; N4: N4-acetylsulfadimethoxine; 2-OH-S: 2-hydroxysulfadimethoxine; 6-OH-S: 6-hydroxysulfadimethoxine.
†Glucuronide: N1-glucuronide; aglycon: S, N4.

Drug analysis

Sulfadimethoxine with its hydroxy metabolites, N4-acetyl conjugates and N1-glucuronide conjugates in plasma and urine were measured by HPLC analysis as previously published [18 24]. In short, the analysis is as follows. The HPLC system consisted of a Spectra Physics SP 8775 autosampler, a Spectra Physics SP 8800 ternary HPLC pump, a Kratos Spectroflow 757 UV detector, and a Spectra Physics SP 4290 integrator. The column was Cp Spher C8 5 μM, 250 mm × 4.6 mm i.d. (Chrompack, Middelburg, the Netherlands) with a guard column 75 mm × 2.1 mm, packed with pellicular reversed phase, 10 μM (Chrompack). The mobile phase was a mixture of acetonitrile and buffer (6.75 ml of 89% orthophosphoric acid and 2 ml diethylamine adjusted with distilled water to 1,000 ml). At $t = 0$, the mobile phase consisted of 10% acetonitrile and 90% buffer. During the following 35 min the mobile phase changed linearly into 25% acetonitrile and 75% buffer. At 36 min ($t = 36$) the mobile phase was changed within 1 min into the initial composition. The flow rate was 1.2 ml/min. UV detection was achieved at 271 nm. The capacity factors of the compounds are shown in Table 1.

Concentration

The concentrations of sulfadimethoxine-N1-glucuronide were measured using calibration curves where peakheights of sulfadimethoxine-N1-glucuronide were expressed versus sulfadimethoxine concentrations in urine released by deglucuronidation of urine samples containing different concentrations (peak heights) of the glucuronide. The calibration curve is constructed with the help of the following formula:

$$[S_{gluc}] = \triangle[S] \cdot M_{Sgluc}/M_S \qquad \text{(Eq. 1)}$$

where $\triangle[S]$ represents the difference in the concentration of sulfadimethoxine before and after deconjugation, and M is the molecular mass ($r = 0.995$). A similar procedure was followed for the measurement of N4-acetylsulfadimethoxine-N1-glucuronide [24].

Deconjugation

Deglucuronidation was carried out with 100 μl plasma or urine, 100 μl deglucuronidase (50,000 U/ml betaglucuronidase, type LII (lyophilized powder from limpets *Patella vulgata,* Sigma, St. Louis, USA; cat.no. G 8132) and 800 μl potassiumdihydrogen phosphate buffer of pH 3.8.

Sampling procedures

Blood. 2 ml Of blood samples were collected at regular time intervals by means of fingertip puncture with Monolet® lancets (Monoject, St. Louis, USA). After centrifugation plasma samples were stored at −20°C until analysis.

Urine. Urine was collected on spontaneous voiding. The total time of sample collection varied between 200 and 300 h (7 times the expected half-life of 30-40 h). Urinary pH was measured immediately after collection. Measurements were made with a Radiometer instrument

(Copenhagen, Denmark; PMH61). Urine samples of 5 ml were stored at −20°C until analysis.

Sample preparation

Plasma samples. Plasma samples (100 µl) were deproteinized with 0.4 ml 0.33 *M* perchloric acid, centrifuged and 20 µl of the supernatant was injected onto the column.

Urine samples. Urine samples were diluted 10 times with distilled water and 100 µl was injected onto the column.

Validation

The intra-day variability of a urine sample containing sulfadimethoxine and its conjugates is 3.4% for sulfadimethoxine, 1.7% for its N1-glucuronide, 2.1% for N4-acetylsulfadimethoxine and 1.6% for its N1-glucuronide (n = 5). The values for the inter-day variability are 2.5%, 2.7%, 6.0% and 1.6%, respectively [24].

Protein binding

Protein binding (*in vivo*) of sulfadimethoxine and N4-acetylsulfadimethoxine was measured in volunteer's plasma samples by means of Ultrafree® drug filters (Worthington, Freehold, USA). The average protein binding (± SD) in each volunteer was calculated from 6 plasma samples obtained at different time intervals and over the concentration range of 10-100 µg/ml.

Protein binding of the N1-glucuronides of sulfadimethoxine and N4-acetylsulfadimethoxine are carried out *in vitro*. Plasma of a volunteer is spiked with urine of the same volunteer, containing the N1-glucuronides. For this, 50 µl urine is diluted 20 times with 0.95 ml volunteer plasma, equilibrated for 15 min at 37°C. This protein binding is compared with 50 µl urine diluted with 0.95 ml 0.067 *M* KH_2PO_4 buffer, pH 7.4, equilibrated at 37°C for 15 min (0% binding), and with 50 µl urine diluted with 0.95 ml blank pooled patient plasma.

For the validation of this method of spiking and to eliminate the effect of urine constituents, known concentrations of sulfadimethoxine and N4-acetylsulfadimethoxine in human urine, were spiked in blank pooled patient plasma and the plasma of the volunteer.

Renal clearance

The apparent and true renal clearance values of parent drug and conjugates were calculated from the average renal excretion rate in each urine sample, divided by the plasma concen-

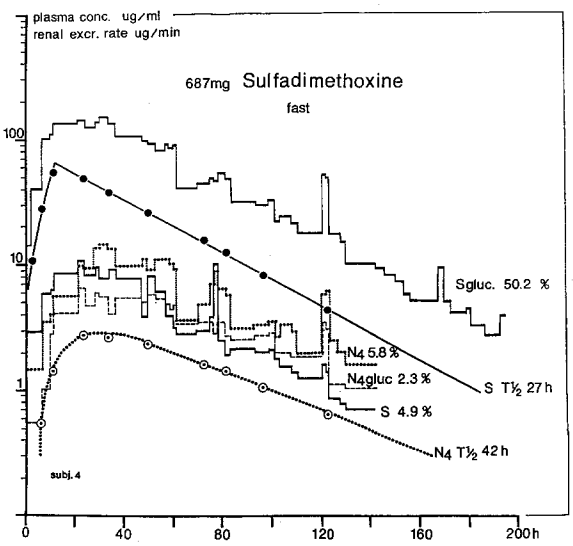

Figure 3
Plasma concentration–time curves and renal excretion rate–time profiles of sulfadimethoxine (S), and its conjugates N4-acetylsulfadimethoxine (N4), sulfadimethoxine-N1-glucuronide (S_{gluc}) and the double conjugate N4-acetylsulfadimethoxine-N1-glucuronide ($N4_{gluc}$) in a fast acetylator after an oral dose of 687 mg sulfadimethoxine

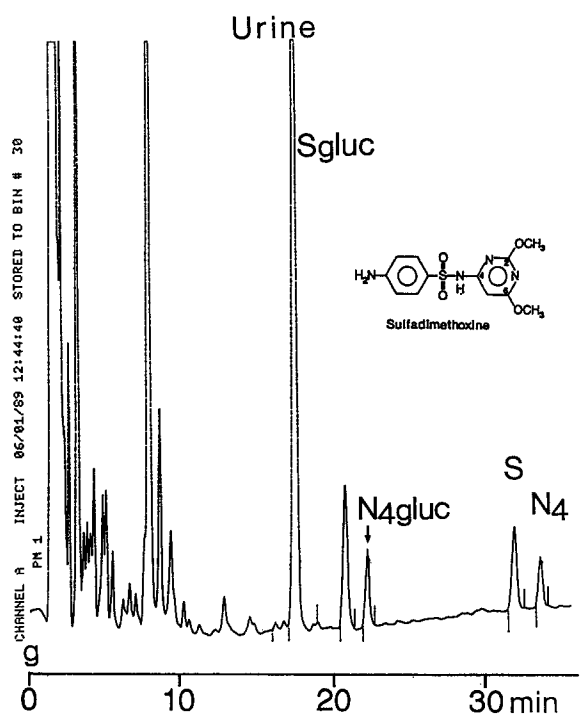

Figure 2
High pressure liquid chromatogram of a urine sample containing sulfadimethoxine (S), and its conjugates N4-acetylsulfadimethoxine (N4), sulfadimethoxine-N1-glucuronide (S_{gluc}) and the double conjugate N4-acetylsulfadimethoxine-N1-glucuronide ($N4_{gluc}$)

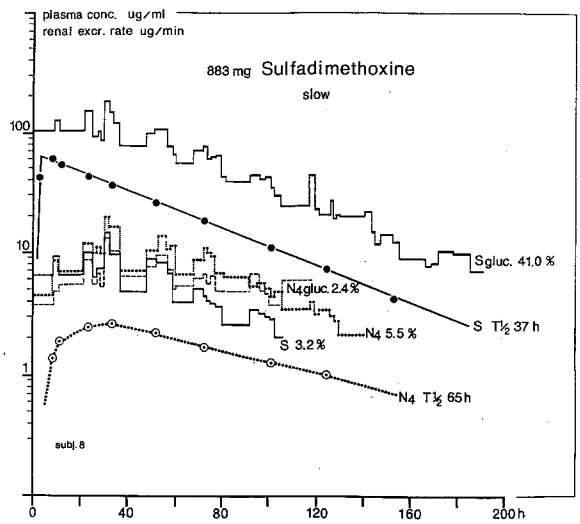

Figure 4
Plasma concentration–time curves and renal excretion rate–time profiles of sulfadimethoxine (S), and its conjugates N4-acetylsulfadimethoxine (N4), sulfadimethoxine-N1-glucuronide (S_{gluc}) and the double conjugate N4-acetylsulfadimethoxine-N1-glucuronide ($N4_{gluc}$) in a slow acetylator after an oral dose of 883 mg sulfadimethoxine

Table 2
Mean values of the pharmacokinetic parameters of sulfadimethoxine in fast and slow acetylators

Parameter*	Fast (n = 6)	Slow (n = 4)	P
Dose (mg/kg)	10	10	
Half-life S (h)	27.8 ± 4.20	36.3 ± 5.42	0.013
Half-life N4 (h)	41.3 ± 5.19	53.5 ± 8.54	0.036
MRT S (h)	46.7 ± 9.34	61.9 ± 3.51	0.014
MRT N4 (h)	73.7 ± 6.95	99.3 ± 7.24	0.014
MRT N4 intrinsic (h)	27.8 ± 5.88	30.1 ± 9.19	1.00
TRT S (h)	165.0 ± 22.6	217.3 ± 9.84	0.014
TRT N4 (h)	235.3 ± 26.0	319.2 ± 49.6	0.014
CL (ml/min)	5.11 ± 2.97	4.03 ± 0.32	0.59
V (l)	13.05 ± 4.69	14.89 ± 0.97	0.46
Dose excreted (%)			
S	3.85 ± 0.85	4.00 ± 1.80	0.92
S_{gluc}	45.15 ± 8.56	41.80 ± 7.75	0.33
N4	5.30 ± 3.27	4.90 ± 1.50	1.00
$N4_{gluc}$	4.15 ± 1.26	2.05 ± 0.55	0.043
Renal clearance (ml/min)			
S	0.25 ± 0.17	0.21 ± 0.06	1.00
N4	4.00 ± 0.85	4.06 ± 0.79	1.00
Creatinine	133 ± 18.4	138 ± 28.0	1.00
Protein binding (%)			
S	99.4 ± 0.72	99.0 ± 0.05	0.095
S_{gluc}	25.4 ± 7.7	21.1 ± 13.2	0.594
N4	nd	nd†	−‡
$N4_{gluc}$	99	99	−‡
Albumin (g/l)	50.8 ± 4.1	47.8 ± 1.3	0.333

*S: sulfadimethoxine; N4: N4-acetylsulfadimethoxine; MRT: mean residence time; TRT: total residence time.
†nd: not detected *in vivo*.
‡ −: no data with N4, and 2 data with N4-glucuronide; Wilcoxon signed rank test.

tration at midpoint of the measured time interval. For the calculation of the apparent renal clearance values the total plasma concentration was used; for the 'true' renal clearance values the free or unbound plasma concentration was used.

Acetylator phenotype
Volunteers were phenotyped according to their ability to acetylate sulfadimidine as described earlier [13 17].

Statistics
Regression lines, standard deviations and Wilcoxon tests were calculated according to standard statistical procedures [25]. Curve fitting was carried out by means of Elsmos. AUC was calculated using the trapezoidal rule. Total body clearance (CL) = dose/AUC_∞ (F = 1). Mean residence times (MRT) of the conjugates were calculated according to Veng Pedersen [26].

Results

The capacity factors found for parent drug and the metabolites and the constant ratio between them are given in Table 1. An HPLC of a urine sample containing sulfadimethoxine and its N4-acetyl conjugates and N1-glucuronide conjugates is seen in Figure 2. No hydroxy metabolites of sulfadimethoxine are present in plasma and urine. Figure 3 shows the plasma concentration–time curves and renal excretion rate–time profiles of sulfadimethoxine and its N4-acetyl conjugates and N1-glucuronide conjugates in a 'fast' acetylator. The glucuronide conjugates could not be detected in plasma. The main compound in the urine is sulfadimethoxine-N1-glucuronide, which accounts for 40% of the ad-

Table 3
Pharmacokinetic parameters of sulfadimethoxine and its conjugates

Parameter*	Subjects									
	fast acetylators						slow acetylators			
	A	B	C	D	E	F	G	H	I	J
Dose (mg; 10 mg/kg)	521	592	633	687	698	656	915	883	767	677
Half-life S (h)	30	32	30	27	27	20	35	37	38	35
Half-life N4 (h)	47	40	47	42	34	37	47	65	47	55
AUC_∞ S	2,652	2,979	3,107	1,626	1,068	1,626	4,103	3,302	3,021	2,734
AUC_∞ N4	269	377	150	241	148	52.2	307	307	118	283
AUCM S	128,695	196,256	169,603	142,544	74,384	31,373	281,046	201,540	192,105	167,172
AUCM N4	20,726	26,933	12,839	17,994	10,138	3,412	29,070	32,104	10,775	30,094
MRT S (h)	48.5	52.0	56.9	47.4	45.7	29.4	65.4	57.3	63.6	61.1
MRT N_4	77.1	71.4	85.5	74.8	68.7	65.3	94.6	104.5	91.6	106.3
MRT_{int} N4	31.0	19.3	28.7	28.9	23.0	35.9	26.2	43.5	28.0	23.0
TRT S (h)	177	184	181	160	164	123	217	216	230	207
TRT N4 (h)	259	223	271	240	200	221	282	377	274	345
CL (ml/min)	3.28	2.62	3.54	3.81	7.15	10.23	3.55	4.19	4.24	4.13
V (l)	9.54	8.16	12.1	10.8	19.6	18.0	13.9	14.4	16.2	15.1

*Based on parallel renal excretion rate–time profiles; S: sulfadimethoxine; N4: N4-acetylsulfadimethoxine; MRT_{int}: intrinsinc mean residence time of the conjugate N4; TRT: total residence time.

Table 4
*Percentage of the dose of sulfadimethoxine and its conjugates excreted in the urine**

Subject†	S	S_{gluc}	N4	$N4_{gluc}$	Total
A FmA	3.4	38.5	6.6	5.5	54.0
B FfA	3.8	42.6	10.7	5.4	62.5
C FmA	3.7	38.2	4.6	3.2	49.7
D FfC	4.9	50.2	5.8	2.3	63.2
E FmC	2.6	41.2	2.7	4.6	51.1
F FfC	4.7	60.2	1.4	3.9	70.2
Mean ±SD	3.85 ± 0.85	45.15 ± 8.56	5.30 ± 3.27	4.15 ± 1.26	58.45 ± 8.09
G SmC	6.4	53.0	6.6	2.6	68.6
H SmC	3.2	41.0	5.5	2.4	52.1
I SmC	4.2	36.7	3.1	1.4	45.4
J SfC	2.2	36.5	4.4	1.8	44.9
Mean ±SD	4.00 ± 1.80	41.80 ± 7.75	4.90 ± 1.50	2.05 ± 0.55	52.75 ± 11.6
Total	3.91 ± 1.22	43.81 ± 7.98	5.14 ± 2.60	3.31 ± 1.47	56.17 ± 9.26

*S: sulfadimethoxine; S_{gluc}: sulfadimethoxine-N1-glucuronide; N4: N4-acetylsulfadimethoxine; $N4_{gluc}$: N4-acetylsulfadimethoxine-N1-glucuronide.
†F: fast acetylator; S: slow acetylator; f: female; m: male, A: Asian; C: Caucasian.

ministered dose and for 70% of the total percentage in the mass balance based upon parent drug and known conjugates excreted in the urine.

Figure 4 shows the same data of sulfadimethoxine in a 'slow' acetylator. The half-life of elimination of sulfadimethoxine is slightly shorter in 'fast' acetylators than in 'slow' acetylators, (27.8 ± 4.20 h versus 36.3 ± 5.42 h; P = 0.013) as can be seen in Table 2. Also, the half-life of the conjugate N4-acetylsulfadimethoxine differs in fast (41.3 ± 5.19 h) and slow (53.5 ± 8.54 h) acetylators (P = 0.036). A similar statistical difference is observed for the mean residence times of both compounds (P = 0.014, Table 2).

Table 3 summarizes the individual pharmacokinetic parameters of sulfadimethoxine and its conjugates. The percentage of the dose of sulfadimethoxine and its conjugates excreted in the urine for all subjects is shown in Table 4. No difference in excretion can be found, except for the double conjugate N4-acetylsulfadimethoxine-N1-glucuronide (P = 0.043).

Renal clearance

The apparent renal clearance of N4-acetylsulfadimethoxine (4.02 ± 0.78 ml/min) is approximately 20 times as high as that of sulfadimethoxine (0.23 ± 0.13 ml/min). The renal clearance of sulfadimethoxine and its N4-acetyl conjugate is similar in fast and slow acetylators (Table 5) (P = 1.00).

Table 5
Renal clearance of sulfadimethoxine and its conjugate in man

Subject	Urine flow (ml/min)	Urine pH	Renal clearance (ml/min)*		
			S	N4	creatinine
A	0.98 ± 0.77	7.02 ± 0.77	0.16 ± 0.07	3.67 ± 1.07	151 ± 60.8
B	1.40 ± 1.17	6.45 ± 0.67	0.14 ± 0.04	3.50 ± 0.92	103 ± 28.9
C	1.29 ± 0.73	6.13 ± 0.62	0.15 ± 0.05	5.00 ± 1.23	139 ± 21.7
D	1.81 ± 1.28	6.75 ± 0.80	0.24 ± 0.12	3.71 ± 0.91	141 ± 20.9
E	1.23 ± 0.63	6.60 ± 0.45	0.23 ± 0.09	3.01 ± 0.97	145 ± 15.6
F	1.91 ± 1.80	5.69 ± 0.73	0.58 ± 0.18	5.09 ± 1.52	118 ± 52.3
Mean ±SD			0.25 ± 0.17	4.00 ± 0.85	133 ± 18.4
G	1.61 ± 1.36	5.97 ± 0.65	0.28 ± 0.11	4.25 ± 1.17	135 ± 33.4
H	3.28 ± 2.31	6.50 ± 0.61	0.23 ± 0.07	4.59 ± 1.25	171 ± 27.6
I	1.33 ± 1.30	6.49 ± 0.64	0.19 ± 0.04	4.51 ± 1.21	143 ± 25.9
J	1.60 ± 0.89	6.22 ± 0.78	0.13 ± 0.04	2.90 ± 1.07	103 ± 22.7
Mean ±SD			0.21 ± 0.06	4.06 ± 0.79	138 ± 28.0
Total			0.23 ± 0.13	4.02 ± 0.78	135 ± 21.4

*S: sulfadimethoxine; N4: N4-acetylsulfadimethoxine.

Protein binding

In Table 6 the protein binding of sulfadimethoxine and its conjugates is shown. N4-acetylation does not change the protein binding of sulfadimethoxine (99-100%), while N1-glucuronidation results in a decrease in protein binding from 98% to 25%. Protein binding of the double conjugate N4-acetylsulfadimethoxine-N-glucuronide remains high, *e.g.* 99%. All subjects had a normal albumin plasma concentration (± 50 g/l).

N4-acetylsulfadimethoxine

Figure 5 shows the plasma concentration–time curves and renal excretion rate–time profiles of N4-acetylsulfadimethoxine (N4) and its metabolite sulfadimethoxine (S) in a fast acetylator (subject D) after the oral administration of 565 mg N4-acetylsulfadimethoxine. The elimination tends to be biphasic with half-lives of 7.5 and 22.5 h. Of the administered dose 50.4% are excreted unchanged, while 28.3% undergo N1-glucuronidation, and 4.7% undergo N4-deacetylation to sulfadimethoxine, which in turn might undergo N1-glucuronidation. In total 83.7% of the dose can be recovered from the urine. In a slow acetylator (subject G) a biphasic elimination is also observed, but the half-lives differ from that of the fast acetylator, *e.g.* 17.5 + 27.5 h (Table 7). 52.9% Of the dose could be recovered from the urine of this slow acetylator as parent drug and metabolites.

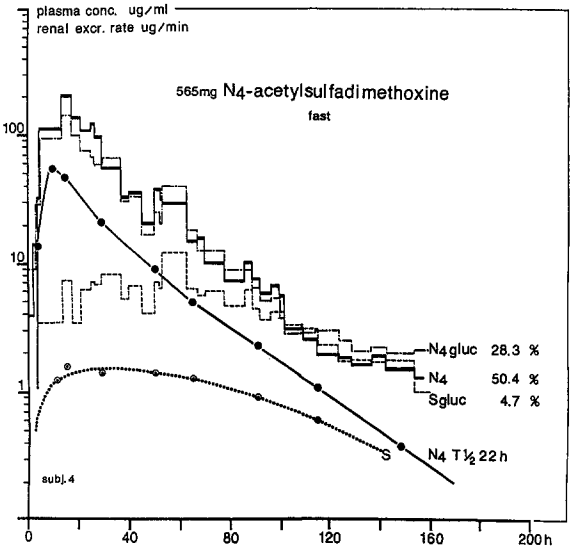

Figure 5
Plasma concentration–time curves and renal excretion rate–time profiles of N4-acetylsulfadimethoxine (N4), and its conjugates sulfadimethoxine-N1-glucuronide (S_{gluc}), and the double conjugate N4-acetylsulfadimethoxine-N1-glucuronide ($N4_{gluc}$) in a fast acetylator after an oral dose of 565 mg N4-acetylsulfadimethoxine

Discussion

Metabolism

Sulfadimethoxine can be metabolized by the following pathways: phase I: *O*-demethylation [18 19 27 28], and phase II: N4-acetylation and N1-glucuronidation. N1-glucuronidation of sulfadimethoxine has already been demonstrated by Adamson *et al.* [10], Bridges *et al.* [29], Uno *et al.* [21 22], and Walker and Williams [23] in the urine of man and primates. Phase I *O*-demethylation occurs in hens [27 28], snails [19], turtles

Table 6
Protein binding of sulfadimethoxine and its conjugates in man

Subject	Percentage protein binding*						Albumin concentration (g/l)
	in vivo†		*in vitro*‡		blank plasma§		
	S	N4	S_{gluc}	$N4_{gluc}$	S_{gluc}	$N4_{gluc}$	
A	99.8 ± 0.0	n.d.	30.6	n.d.	30	n.d.	54 ± 1
B	98.0 ± 2.0	n.d.	32.5	n.d.	28	n.d.	53 ± 2
C	99.4 ± 0.0	n.d.	29.7	n.d.	17	n.d.	56 ± 2
D	99.8 ± 0.0	n.d.	27.6	99	26	99	49 ± 2
E	99.8 ± 0.0	n.d.	19.3	n.d.	30	n.d.	47 ± 3
F	99.8 ± 0.1	n.d.	12.7	n.d.	11	n.d.	46 ± 1
Mean ±SD	99.4 ± 0.7		25.4 ± 7.7		23.6 ± 7.9		50.8 ± 4.1
G	99.0 ± 0.2	n.d.	15.2	99	21	99	48 ± 3
H	98.9 ± 0.1	n.d.	6.9	n.d.	26	n.d.	46 ± 3
I	99.0 ± 0.2	n.d.	24.9	n.d.	21	n.d.	49 ± 3
J	99.0 ± 0.0	n.d.	37.5	n.d.	31	n.d.	48 ± 1
Mean ±SD	99.0 ± 0.05		21.1 ± 13.2		24.8 ± 4.8		47.8 ± 1.3
Total	99.3 ± 0.59		23.7 ± 9.8		21 ± 10		50 ± 3

*S: sulfadimethoxine; N4: N4-acetylsulfadimethoxine; gluc: N1-glucuronide.
†*In vivo*: plasma subject + ultrafiltration; n.d.: concentration not detectable after filtration.
‡ *In vitro:* urine containing S-gluc or N4-gluc of a subject added to the subject's own plasma.
§blank plasma: urine of each subject containing approximately 100 μg/ml S_{gluc} or $N4_{gluc}$, added to a blank serum sample (mixed patient serum); subjects D and G took N4-acetylsulfadimethoxine.

Table 7
Pharmacokinetic parameters of N4-acetylsulfadimethoxine

Parameter*	Fast acetylator	Slow acetylator
Subject	D	G
Dose mg	565	722
(mg/kg)	8.3	8.0
Parent drug		
Half-life N4 (h)	7.5 ± 22.5	17.5 ± 27.5
MRT N4 (h)	33.0	44.6
TRT N4 (h)	115	169
AUC_∞ N4 ($mg \cdot h \cdot l^{-1}$)	1,433	1,260
AUCM N4 ($mg \cdot h^2 \cdot l^{-1}$)	47,219	56,206
CL (ml/min)	6.57	9.55
V (l)	13.00	25.57
Metabolite		
Half-life S (h)	30.0	27.5
MRT S (h)	69.3	77.8
MRT S (h)	196	220
AUC_∞ S ($mg \cdot h \cdot l^{-1}$)	150.0	129.2
AUCM S ($mg \cdot h^2 \cdot l^{-1}$)	10,393	10,045
MRT_{int} S (h)	36.3	33.1
Percentage dose excreted		
– N4 parent	50.7 (60.6)	32.4 (61.3)
– N4-glucuronide	28.3 (33.8)	17.5 (33.1)
– S-glucuronide	4.7 (5.6)	3.0 (5.7)
– Total	83.7 (100%)	52.9 (100%)
– Renal clearance (ml/min)		
S metabolite	–†	–†
– N4 parent	3.30 ± 0.88	3.08 ± 0.72
– Creatinine	137 ± 19	143 ± 11
Albumin (g/l)	47 ± 2	49 ± 2

*S: sulfadimethoxine; N4: N4-acetylsulfadimethoxine; MRT: mean residence time; MRT_{int}: intrinsic mean residence time; Half-life: N4: biphasic plasma concentration–time curve.
†–: no plasma concentration.

[18] *etc.*, while N4-acetylation is the major pathway in pigs [9]. These three metabolic pathways compete for the sulfadimethoxine and the results depend on the enzyme composition of the species.

In our study it turned out that the percentage of N1-glucuronidation is high and similar in fast and slow human acetylators (P = 0.335). However, the percentage of N4-acetylsulfadimethoxine-N1-glucuronide is twice as high in fast as in slow acetylators (P = 0.043). Although this difference is statistically significant, it has no clinical implication, due to the low overall percentage (4 versus 2%).

Half-life

The half-life of elimination of sulfadimethoxine is smaller in fast acetylators than in slow acetylators (P = 0.013), while no differences in half-life can be observed between fast acetylators of Asian and of Caucasian origin (phenotype) (P = 0.338), as was observed for sulfadimidine [17]. The apparent half-life of the conjugate N4-acetylsulfadimethoxine is also smaller in fast than in slow acetylators (P = 0.036), but the calculated intrinsic mean residence time of the acetyl conjugate is similar in fast and slow acetylators (P = 0.556). These small but statistically significant differences in half-lives between fast and slow acetylators may be attributed totally to the differences in the formation of N4-acetylsulfadimethoxine-N1-glucuronide. The yield of N1-glucuronidation of N4-acetylsulfadimethoxine is comparable in fast and slow acetylators, as shown in Table 7, since 33% of the amount of N4-acetylsulfadimethoxine formed and present is N1-glucuronidated. The plasma concentration–time curves of the parent drug and N4-acetyl conjugate are not parallel, which is typical for sulfadimethoxine [13 14]. This is caused by the intrinsic long half-life values of 22-27 h of the N4-acetyl conjugate, which is nearly similar to that of the parent drug, and which is much longer than the 'normal' observed half-life values of 3-5 h for N4-acetylsulfonamides in general [13 16].

This long beta-phase half-life in the elimination must be the result of the acetylation–deacetylation equilibrium, which favours deacetylation. The alpha phase in the elimination of N4-acetylsulfadimethoxine approximates the elimination by renal excretion and deacetylation of the parent molecules (the administered molecules); this half-life is 7.5 h in the fast acetylator and 17.5 h in the slow acetylator. This slower intrinsic elimination of N4-acetylsulfadimethoxine in the slow acetylator contributes to the plasma concentration of sulfadimethoxine via the deacetylation, producing longer half-life values. The acetylation–deacetylation reactions are not an effective elimination pathway for sulfadimethoxine in man. The elimination is performed to a large extent by the N1-glucuronidation process, resulting in a decrease in protein binding and the subsequent renal excretion by glomerular filtration.

Species differences

In man, the half-life of sulfadimethoxine is about 30-40 h [12 13], in the lobster it is 80 h [8], in the snail 70 h [19], but in the cat [2], the dog [1] and cattle [11], the half-life is approximately 10 h. Metabolism of sulfadimethoxine in the latter species must therefore be fast. In the dog N1-glucuronidation is the only metabolic pathway responsible for the fast elimination [Russel FG, personal communication, 1989], as, due to the high rate of deacetylation, no N4-acetyl conjugate is observed, and no hydroxy metabolites of sulfadimethoxine were present [1 16]. In pigs, N4-acetylation followed by rapid renal excretion of the conjugate is the main elimination route, resulting in the short half-life of the parent compound, while no N1-glucuronidation takes place [9].

Cocks *O*-dealkylate sulfadimethoxine, which occurs mainly at the 6-position and is rate-limiting [27 28]. Turtles [19] and snails [20] *O*-dealkylate sulfadimethoxine predominantly at the 2-position. *O*-Demethylated products were also reported to be present as impurities in the synthesis of sulfadimethoxine [31].

Species-dependent conjugation

The various conjugation reactions of sulfadimethoxine in man and the pig may indicate that man lacks an *N*-acetyl transferase isoenzyme that is present in pigs. Instead, sulfadimethoxine

is now slowly glucuronidated at the N1-position. Is the N1-glucuronidation pathway always present in every species, and/or is it only visible when the *N*-acetyl transferase activity is not present or not possible? For sulfadimethoxine the isoenzymes of glucuronidation and of N4-acetylation are present in man.

In the pig, the N1-glucuronidation of sulfadimethoxine or its conjugate N4-acetylsulfadimethoxine was not observed [9], which may indicate that this specific UDP-glucuronyl transferase isoenzyme is not present in the pig. Cats are known for the absence of glucuronidation reactions, and therefore sulfadimethoxine must undergo N4-acetylation [31]. In man, sulfadimidine, sulfamerazine and sulfamethoxazole show 1-2% N1-glucuronidation, while they are mainly subject to N4-acetylation [32]. Therefore, it is likely that the competition between N4-acetylation and N1-glucuronidation depends on two factors: the presence of the isoenzyme, and the rate constants for each reaction.

Mass balance

The oral dose of sulfadimethoxine is recovered for approximately 50-60% in urine, the main conjugate being sulfadimethoxine-N1-glucuronide. This conjugate has a relatively bulky structure (MW 487), which implies that it may also be excreted in the bile [33]. After administration of N4-acetylsulfadimethoxine in two volunteers, the conjugate N4-acetylsulfadimethoxine-N1-glucuronide shows a mass balance in urine of 83% and 53%, respectively. Also, this N1-glucuronide may be excreted in part in the bile and faeces.

Place of the glucuronidation

No plasma concentration of sulfadimethoxine-N1-glucuronide could be measured after an oral dose of 10 mg/kg in man. The renal excretion rate of the N1-glucuronide must therefore be high, and assuming a renal clearance equal to the glomerular filtration rate of 125 ml/min, the maximum plasma concentration would be approximately 0.1-0.3 μg/ml. This maximum concentration is just below the detection limit of 0.3 μg/ml. If the N1-glucuronide were excreted by active tubular secretion, the renal clearance would be higher and the plasma concentration even lower. It is also possible that the N1-glucuronide is formed in the kidney and immediately excreted. Then no detectable plasma concentration will be present either.

N4-acetylsulfadimethoxine is excreted by tubular secretion in the pig and in man. In man, 30% of the compound undergoes N1-glucuronidation and is excreted. This percentage is comparable to 40% of a sulfadimethoxine dose that is excreted as N1-glucuronide, which may indicate that both sulfadimethoxine and N4-acetylsulfadimethoxine are glucuronidated by the same (iso)enzyme. For N4-acetylsulfadimethoxine-N1-glucuronide no detectable plasma concentration is found, which would also occur if the kidney is the place of glucuronidation.

In the dog sulfadimethoxine-N1-glucuronide shows a half-life of 2.4 h, while that of sulfadimethoxine is 5 h, and that of the N4-acetyl conjugate is 4.7 h [34]. Therefore, it seems probable that the sulfadimethoxine-N1-glucuronide is excreted rapidly rather than that it is formed in the kidney.

Renal clearance

The apparent renal clearance of sulfadimethoxine is extremely low (0.25 $\pm$ 0.17 ml/min) and is independent of urine flow and urine pH. The compound is probably subject to active tubular reabsorption, as has been demonstrated in the pig [9] and the dog [Boom S, Russel FG, personal communication, 1989]. The apparent renal clearance of N4-acetylsulfadimethoxine is approximately 20 times as high as that of the parent compound. This conjugate is excreted by active tubular secretion [9 13 14]. The renal clearance of the N1-glucuronide conjugates must be high, since no measurable plasma concentration is found. In general, drug glucuronides are excreted by either glomerular filtration or filtration plus active tubular secretion [35].

Protein binding

The best measure of a drug's protein binding is *in-vivo* measurement in the plasma of subjects/patients. Then the problem of spiking plasma with the drug, which includes mixing and equilibration time are avoided. As the N1-glucuronides were not present in the blood, the plasma of each subject was spiked with his urine sample containing the N1-glucuronides. This way, by using the subjects' plasma albumin concentrations, a close approximation of the *in-vivo* situation was achieved. The protein binding of sulfadimethoxine *in vivo* or *in vitro* (spiked) was similar in each subject ($P = 0.855$), and is in agreement with the values reported earlier [36 37]. This experiment was used as a control experiment for the binding of N1-glucuronides. In dog the protein binding of sulfadimethoxine-N1-glucuronide is approximately 20% [38], and in man it was reported to be 30% [37]. This observation could lead to the assumption that the N1 region is the binding moiety in protein binding [37].

The water solubility and high free fraction in plasma enable a rapid renal excretion. The protein binding of N4-acetylsulfadimethoxine-N1-glucuronide remained high, 99%, and does not differ from that of N4-acetylsulfadimethoxine ($P = 0.951$). Thus the contribution of the N4-acetyl group to the protein binding is greater than any inhibiting effect resulting from the bulk of a glucuronide moiety at the N1 position.

Conclusion

The direct measurement of the N1-glucuronide conjugates of sulfadimethoxine and N4-acetylsulfadimethoxine enable the assessment of the pharmacokinetics, protein binding, renal excretion and mass balance of both compounds. Why sulfadimethoxine in man undergoes predominantly N1-glucuronidation instead of N4-acetylation must be explained by the lack of the suitable N-acetyltransferase isoenzyme in man and the presence of a suitable UDP-glucuronyltransferase isoenzyme. In animals, such as the pig, this situation is reversed.

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