Formation and elimination of sulphamethoxazole hydroxylamine after oral administration of sulphamethoxazole

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The formation and elimination of sulphamethoxazole hydroxylamine in relation to the pharmacokinetics of the parent compound and its N₄-acetyl metabolite were investigated in six healthy subjects after a single oral dose of 800 mg sulphamethoxazole. The apparent half-lives of sulphamethoxazole and its metabolites were approximately 10 h, indicative of formation rate-limited metabolism. The mean residence time of the hydroxylamine metabolite was 5.5 ± 1.5 h. The renal clearance of sulphamethoxazole hydroxylamine was 4.39 ± 0.91 l h⁻¹. The urinary recovery of sulphamethoxazole accounted for $16.5 \pm 5.5\%$ of the dose, N₄-acetyl-sulphamethoxazole for $46.2 \pm 6.6\%$ and the hydroxylamine metabolite for $2.4 \pm 0.8\%$. The remaining 35% of the dose was unaccounted for. Acetylator phenotype was determined using sulphadimidine. The renal excretion of sulphamethoxazole hydroxylamine was $1.9 \pm 0.9\%$ in slow acetylators ($n = 3$) and $2.8 \pm 0.3\%$ in fast acetylators ($n = 3$); for N₄-acetyl-sulphamethoxazole the values were $48 \pm 6\%$ and $44 \pm 8\%$, respectively. Sulphamethoxazole is metabolized, although to a limited extent, to a hydroxylamine metabolite. This metabolite may be important for the pathogenesis of adverse reactions.

Keywords sulphamethoxazole pharmacokinetics sulphamethoxazole hydroxylamine

Introduction

The high incidence of side-effects to sulphamethoxazole (SMX) in individuals infected with the human immunodeficiency virus (HIV) [1] has renewed interest in the metabolism of this drug. It is postulated that the hydroxylamine metabolite (NOH-SMX) is critical in the pathogenesis of adverse reactions to sulphonamides [2–7]. In HIV seropositive individuals, the detoxification capacity for reactive products of sulphonamide metabolism seems to be decreased owing to glutathione deficiency [8]. Although NOH-SMX may be an important metabolite, *in vivo* data on its information and elimination are limited [6, 9, 10].

Sulphamethoxazole hydroxylamine (NOH-SMX) results from the hydroxylation of the para-amino group of SMX, a cytochrome P450-dependent reac-

tion [6]. Besides hydroxylation, the para-amino group of SMX can also be acetylated resulting in the parallel formation of N₄-acetyl-sulphamethoxazole (N₄-AcSMX) [11]. *N*-acetyl transferase exhibits a marked genetic polymorphism and the rate of acetylation of some sulphonamides (sulphadimidine, sulphamerazine, sulphadiazine, and sulphapyridine) depends on the acetylator phenotype [11]. A prominence of the slow acetylator phenotype has been reported in patients with hypersensitivity reactions to sulphonamides [12]. However, for some sulphonamides (like SMX) slow and fast acetylators have not been distinguished [11].

In this study we document the formation and elimination of sulphamethoxazole hydroxylamine in re-

lation to the kinetics of the parent compound and the N₄-acetyl metabolite in healthy subjects (three slow, three fast acetylators).

Methods

Subjects

Six caucasians (four males, two females) ranging in age from 23 to 50 years (mean 34 years) participated after approval of the study by the Ethics Committee of our university hospital. Written informed consent was obtained from all participants. All subjects were drug-free and healthy by history, physical examination and routine biochemical assessment. An 800 mg dose of sulphamethoxazole was administered orally in two gelatine capsules on an empty stomach after an overnight fasting.

Sampling procedures

Blood samples were obtained by fingertip puncture at 1, 2, 4, 6, 8, 10, 12, 24, 32, 36, and 48 h after drug administration. Plasma was separated immediately by centrifugation and stored at -20°C until analysis. Urine was collected over 96 h and three 5 ml aliquots were stored immediately at -20°C until analysis.

Acetylator phenotype

Volunteers were phenotyped using sulphadimidine as described previously [13].

Chemicals

SMX and N₄-AcSMX were obtained from Hoffmann LaRoche, Mijdrecht, the Netherlands. NOH-SMX was obtained from Synthon BV, Nijmegen, the Netherlands. All other chemicals were of analytical purity and were obtained from commercial sources.

Drug analysis

SMX and its metabolites were assayed by h.p.l.c. using a Spectra Physics SP8810 pump (Eindhoven, The Netherlands), a 757 Separations u.v. detector (Hendrik Ido Ambacht, The Netherlands) and a C8 (5 μm) column (Synthon, Nijmegen, The Netherlands) (250 mm $\times$ 4 mm i.d.). The mobile phase consisted of 45 ml of a mixture of 20 g orthophosphoric acid and 5 g tetramethylammonium chloride in 1 l water, 45 ml dimethylformamide and 180 ml acetonitrile, adjusted to 1 l with water. The mobile phase was degassed with helium before use. The flow rate was 1.5 ml min^{-1} . Detection was at 271 nm. The retention times of NOH-SMX, SMX, and N₄-AcSMX were 8.6, 10.1 and 13.3 min, respectively, and the chromatographic peaks were identified using authentic standards. Intra- and interday variation in the assay of both samples and standards was $<5\%$. Quantitation limits, determined with a signal-to-noise ratio of 3, for SMX, N₄-AcSMX, and NOH-SM were 0.0055, 0.01, and 0.01

$\mu\text{g ml}^{-1}$, respectively, in plasma and 0.10 $\mu\text{g ml}^{-1}$ for all compounds in urine. The hydroxylamine metabolite was stable in anaerobic plasma and urine.

Sample preparation

After thawing, the sample (100 μl) was diluted with 900 μl 0.33 M trichloroacetic acid, centrifuged at 3000 g, and the supernatant injected onto the column.

Data analysis

Renal clearances were calculated from urinary recoveries of the compounds (corrected for molecular weight) divided by the corresponding AUC values. Curve fitting to a one compartment model with first order drug absorption was carried out using the Mediware® computer program 15. AUC values were calculated using the linear trapezoidal rule with extrapolation to infinity using $C(\text{last})/k$. Oral clearance (CL_o) of SMX and its mean residence time (MRT) were calculated by standard methods [14].

Statistics

The Mann-Whitney test was used to compare results. Pearson correlation coefficients were calculated. Differences were considered significant when $P < 0.05$.

Results

Plasma concentrations and renal excretion rates of SMX, NOH-SMX and N₄-AcSMX in a representative subject are shown in Figure 1. Mean pharmacokinetic parameters are summarised in Table 1.

The elimination half-life of SMX was 9.5 h while the half-lives of N₄-AcSMX and NOH-SMX were 11.1 and 11.2 h, respectively (NS). The mean residence time of SMX was 14.0 h and those of N₄-

Figure 1 Plasma concentrations and renal excretion rates of sulphamethoxazole (SMX) and its N₄-acetyl (N₄-AcSMX) and hydroxylamine metabolites (NOH-SMX) in one subject after an 800 mg oral dose of sulphamethoxazole.

Table 1 Mean ($\pm$ s.d. and range) of pharmacokinetic parameters describing the fate of sulphamethoxazole in six healthy subjects

Parameter	SMX		N ₄ -AcSMX		NOH-SMX	
	Mean $\pm$ s.d.	(Range)	Mean $\pm$ s.d.	(Range)	Mean $\pm$ s.d.	(Range)
C _{max} ($\mu\text{g ml}^{-1}$)	46.9 $\pm$ 8.7	(35.6–61.4)	8.1 $\pm$ 1.1	(6.5–9.1)	0.3 $\pm$ 0.1	(0.2–0.4)
t _{max} (h)	0.7*	(0.6–1.6)	5.7*	(5.1–9.0)	2.9*	(2.1–8.1)
t _{1/2,z} (h)	9.5 $\pm$ 0.7	(8.7–10.5)	11.1 $\pm$ 1.4	(9.3–13.4)	11.2 $\pm$ 1.2	(9.8–12.2)
MRT (h)	14.0 $\pm$ 0.9	(12.8–15.4)				
MRT (m) (h)			5.5 $\pm$ 1.5	(2.8–6.7)	5.2 $\pm$ 1.7	(3.2–6.4)
AUC (mg l ⁻¹ h)	675 $\pm$ 102	(501–813)	189 $\pm$ 29	(161–227)	6 $\pm$ 1	(4–7)
CL _o (l h ⁻¹)	1.2 $\pm$ 0.2	(1.0–1.6)				
CL _R (l h ⁻¹)	0.22 $\pm$ 0.05	(0.14–0.28)	2.45 $\pm$ 0.35	(1.91–2.97)	4.39 $\pm$ 0.91	(3.44–5.51)
Ae(∞) (%)	16.5 $\pm$ 5.5	(8.3–23.4)	46.2 $\pm$ 6.6	(36.9–54.4)	2.4 $\pm$ 0.8	(0.9–3.2)

*Median.

C_{max} = Maximum plasma drug concentration, t_{max} time at which C_{max} occurs, t_{1/2,z} = plasma duration half-life, MRT = mean residence time, MRT_(m) = mean residence time of metabolite after administration of drug, (MRT_(m) = MRT_m - MRT), MRT = MRT parent compound, AUC = area under plasma concentration-time curve, CL_o = oral clearance (Dose/AUC), CL_R = renal clearance (Ae(∞)/AUC), Ae (∞) = amount excreted in urine (molar).

AcSMX and NOH-SMX formed from SMX were 5.5 and 5.1 h, respectively.

The urinary recoveries of parent drug and metabolites were similar in all subjects. Sulphamethoxazole accounted for 16.5 $\pm$ 5.5% (percentage $\pm$ s.d.) of the dose, N₄-AcSMX, for 46.2 $\pm$ 6.6%, and NOH-SMX, for 2.4 $\pm$ 0.8%. Three subjects acetylated sulphadimidine rapidly and three slowly. Renal excretion of NOH-SMX accounted for 1.9 $\pm$ 0.9% of the dose of SMX in the slow acetylators and 2.8 $\pm$ 0.3% in the fast acetylators. For N₄-AcSMX, the values were 48 $\pm$ 6% and 44 $\pm$ 8%, respectively.

The clearance (metabolic- and renal clearance) of SMX was 1.21 $\pm$ 0.21 l h⁻¹. The mean renal clearance of SMX was 0.22 $\pm$ 0.05 l h⁻¹, which differed significantly from that of N₄-AcSMX (2.45 $\pm$ 0.35 l h⁻¹) and NOH-SMX (4.39 $\pm$ 0.91 l h⁻¹) (paired *t*-test, *P* < 0.05). The renal clearance of SMX but not of its metabolites correlated with the urine pH (SMX, *r* = 0.91, all samples).

Discussion

Metabolism

After a single 800 mg oral dose of sulphamethoxazole, the renal excretion of SMX, N₄-AcSMX, and NOH-SMX accounted for 65% of the dose. The oral clearance of SMX (1.2 l h⁻¹) was much greater than its renal clearance (0.2 l h⁻¹). The fate of 35% of the dose is unknown.

The hydroxylamine metabolite accounted for 2.4% of the recovered dose of SMX, a figure in agreement with those reports by others [6, 9]. Since NOH-SMX is unstable and can be oxidised to nitroso derivatives [15], it is possible that its urinary recovery is less than the amount formed in the liver. However, under physiological conditions glutathione prevents the auto-oxidation of NOH-SMX [15].

Mechanism of renal clearance

After glomerular filtration, SMX undergoes net passive reabsorption while data for N₄-AcSMX when corrected for plasma binding indicate that this compound undergoes net active excretion [16]. Urine flow and pH govern passive reabsorption and influence the renal excretion of SMX but not that of N₄-AcSMX. This difference in excretion is discernable from respective excretion rate-time profiles. Thus, the excretion rate-time curve of N₄-Ac-SMX was smooth and parallel to the corresponding plasma concentration-time curve, while that of SMX had an irregular profile. The shape of the excretion rate-time profile of NOH-SMX was similar to that of N₄-AcSMX and, in a separate experiment, it was shown that the excretion of NOH-SMX is not dependent on urine pH (data not shown). This suggests that NOH-SMX undergoes limited passive tubular reabsorption. The high renal clearance rates of N₄-AcSMX and NOH-SMX compared with that of SMX are associated with similar apparent half-lives.

Acetylator phenotype

A previous study indicated that SMX clearance is unrelated to acetylator phenotype [16], and we now show that the excretion of N₄-AcSMX is similar in both fast and slow acetylators of sulphadimidine. Overrepresentation of the slow acetylator phenotype has been reported in patients with sulphonamide hypersensitivity reactions [12]. Thus, it was suggested that in slow acetylators more of the parent drug may be available for metabolism by cytochrome P450 to the hydroxylamine metabolites [12]. However, our data do not indicate an increased hydroxylamine formation in slow sulphadimidine acetylators, although the number of subjects studied was limited.

This study has documented the formation and renal excretion of the N₄-acetyl and hydroxylamine metabolites of sulphamethoxazole in healthy subjects.

Sulphamethoxazole hydroxylamine may be critical in the pathogenesis of adverse reactions although the

contribution of *N*-hydroxylation to the metabolism of sulphamethoxazole was found to be small.

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