The Fate of Sulphadimethoxine in Primates Compared with other Species

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1. The metabolism of sulphadimethoxine (2,4-dimethoxy-6-sulphanilamido-pyrimidine) was examined in nine species of primates and nine species of non-primates. 2. The main metabolite of the drug in the urine in man, rhesus monkey, baboon, squirrel monkey, capuchin, bushbaby, slow loris and tree shrew was sulphadimethoxine N^1 -glucuronide. In the green monkey, although the main metabolite was N^4 -acetylsulphadimethoxine, the N^1 -glucuronide was also a major metabolite. 3. In the dog, rat, mouse, guinea pig, Indian fruit bat and hen the N^1 -glucuronide was a minor metabolite in the urine, whereas in the cat, ferret and rabbit this glucuronide was not found in the urine. 4. All the species examined except the dog excreted some N^4 -acetylsulphadimethoxine, which was the major metabolite in the green monkey, rabbit and guinea pig. 5. In the tree shrew, a doubtful primate, N^1 -glucuronide formation was similar to that in the other primates. 6. It is suggested that the slow excretion of the drug by the rat may be due partly to strong binding of the drug to tissue proteins and that the strength of binding may vary with species. 7. In the rat the amount of N^1 -glucuronide found in the urine is not a true indication of the extent of this conjugation since much more of the conjugate was found in the bile (7% of the dose) than in the urine (1%). In the rabbit, no N^1 -glucuronide was found in the bile or urine, but a small amount of sulphadimethoxine N^4 -glucuronide was found in the bile of the rat (0.5% of dose) and rabbit (0.8%).

Earlier work from this laboratory (Bridges, Kibby & Williams, 1965) indicated that in man the main metabolite of sulphadimethoxine (Madribon; 2,4-dimethoxy-6-sulphanilamidopyrimidine) in the urine was an N -glucuronide in which the glucuronyl group was attached to the sulphonamide nitrogen (N -1) of the drug. A study of the fate of the drug in several species suggested that the formation of such a glucuronide might be peculiar to man and the rhesus monkey (Bridges, Kibby, Walker & Williams, 1968). The metabolites of the drug in urine were therefore examined in 18 species including man, eight subhuman primates and nine other animals. N^1 -Glucuronide excretion was detected in 15 of these species, but the N^1 -glucuronide was a major metabolite of the drug in urine only in the

primates. Part of this work has been briefly reported (Adamson, Bridges & Williams, 1966).

EXPERIMENTAL

Materials. Sulphadimethoxine, m.p. 200–201°C, [^{35}S]-sulphadimethoxine, m.p. 200–201°C, specific radioactivity 35 $\mu\text{Ci}/\text{mg}$, and N^4 -acetylsulphadimethoxine, m.p. 210–211°C, were gifts from Roche Products Ltd., Welwyn Garden City, Herts., U.K. The ammonium salt of sulphadimethoxine N^1 -glucuronide, m.p. 150–160°C (decomp.) and $[\alpha]_D^{25} + 4.5^\circ$ (c 5 in water), the sodium salt of sulphadimethoxine N^4 -glucuronide, m.p. 149°C, and the potassium salt of sulphadimethoxine N^4 -sulphate were synthesized as described by Bridges *et al.* (1965, 1968).

Animals and dosing. The animals used are listed in Tables 1 and 2. They were obtained mainly from dealers in the London area and were maintained on an appropriate diet. Sulphadimethoxine was taken by humans in tablet form. The subhuman primates were given the powdered drug concealed in fruit or honey. It was given by stomach tube to the other species in water or propane-1,2-diol, except the dog, which received tablets concealed in food. Cannulation of the bile duct in certain species was carried out as described by Abou-El-Makarem, Millburn, Smith & Williams (1967). For intraperitoneal injection, the sodium salt of the drug was dissolved in water.

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Determination of metabolites. Metabolites in urine were separated by paper chromatography and determined as described by Bridges *et al.* (1968).

Distribution of [^{35}S]sulphadimethoxine in rats. Two female rats (Wistar albino; 300g) were each injected intraperitoneally with [^{35}S]sulphadimethoxine (100mg/kg; 5 $\mu\text{Ci}/\text{rat}$) dissolved in dimethyl sulphoxide (0.5ml). The urine and faeces were collected daily for 3 days and their radioactivities counted. Then the rats were killed and ^{35}S was determined in certain organs and the residual carcass. Radioactivity was measured in a Packard Tri-Carb model 3214 scintillation spectrometer.

The radioactivity in urine (0.5ml) was counted after being added to a dioxan scintillator fluid (20ml), which consisted of naphthalene (60g), 2,5-diphenyloxazole (PPO) (4g), 1,4-bis-(5-phenyloxazol-2-yl)benzene (POPOP) (0.2g), methanol (AnalaR; 100ml) and ethylene glycol (20ml) made up to 1 litre of solution in dioxan. Faeces (5g) were homogenized in a dioxan-methanol (1:1, v/v) mixture (25ml). The radioactivity of the homogenate (0.5-1ml) was counted suspended in 20ml

of gel scintillator, which consisted of a 5% suspension of Cab-O-Sil (Packard Instrument Co.) in the dioxan scintillator fluid. Tissues (liver, kidney, intestine) were dissolved in 25% (w/v) NaOH, then neutralized with acetic acid, and the radioactivities of suitable samples counted in the gel scintillator. Rat carcasses (approx. 250g) were dissolved in 25% NaOH by heating at 50°C for 24 h, and suitable samples of the solution were neutralized with acetic acid and their radioactivities counted in the gel scintillator. The recovery of ^{35}S 10 min after intraperitoneal injection of 5 μCi of [^{35}S]sulphadimethoxine in dimethyl sulphoxide into rats was 97-98%.

Plasma concentrations of [^{35}S]sulphadimethoxine. Twentytwo female Wistar albino rats (about 300g each) were each injected intraperitoneally with [^{35}S]sulphadimethoxine (1.5 μCi ; 100mg/kg) in 0.5ml of dimethyl sulphoxide. Two animals at a time were killed by stunning at 0.5, 1, 2, 4, 8, 12, 18, 25, 30, 72 and 168 h after the dosing. The throats were cut and the blood (10ml from two rats) collected, mixed with heparin (0.1 ml) and then centrifuged at 2000g for 5 min. The radioactivity of the

Table 1. *Urinary excretion of sulphadimethoxine in different species*

Sulphadimethoxine (100mg/kg unless otherwise stated) was given orally in water. The numbers of animals used and their sex (M = male; F = female) are shown in parentheses in the first column.

Animal	Group or other description	% of dose excreted in urine	
		In 24 h	In 48 h
Man (4M)*	Caucasian	25 (19-30)	—
Old World monkeys (Cercopithecidae)			
Rhesus monkey (4F)	<i>Macaca</i>	39 (32-52)	55 (44-73)
Green monkey (2F)	<i>Cercopithecus aethiops</i>	12, 11	17, 15
Baboon (3M)	<i>Papio</i>	42 (28-50)	55 (39-68)
New World monkeys (Cebidae)			
Squirrel monkey (3F)	<i>Saimiri</i>	9 (7-11)	12 (11-13)
Capuchin monkey (2M)	<i>Cebus</i>	23, 28	—
Prosimii			
Bushbaby (2M)	<i>Galago</i>	16, 22	22, 27
Slow loris (2M)	<i>Loris</i>	7, 10	11, 13
Tree shrew (3F)	<i>Tupaia</i>	26 (20-29)	37 (33-38)
Carnivores			
Dog (3F)†	Mongrel	26 (13-32)	36 (26-42)
Cat (2F)	Domestic	33, 35	40, 38
Ferret (3F)	Albino	13 (7-16)	23 (19-25)
Lagomorph			
Rabbit (3F)	Chinchilla	46 (39-52)	73 (50-85)
Rodents			
Rat (6F)	Wistar albino	16 (6-21)	33 (17-48)
Mouse (15F)‡	Albino	55 (52-59)	64 (60-67)
Guinea pig (3M)	English	26 (20-37)	40 (34-52)
Bat (Chiroptera)			
Fruit bat (4F)	Indian (<i>Pteropus giganteus</i>)	13 (9-14)	17 (14-19)
Bird (Galliform)			
Hen (2F)	Light Sussex	24, 42	38, 58

* Dose 33 mg/kg.

† Dose 200 mg/kg.

‡ Three groups of five animals.

plasma (0.2ml) was then counted in the gel scintillator (20ml).

RESULTS

Rate of excretion. The percentage of the dose of sulphadimethoxine excreted in the urine in 24 and 48h after the dosing is shown in Table 1. In the primates the drug is relatively slowly excreted. In the baboon and rhesus monkey some 40% of the dose is excreted in 24h, whereas in man the value is 25%. In the other primates values in the range 9–26% were obtained.

Among the non-primates the drug is most rapidly excreted by the rabbit (46% in 24h) and the mouse (55%) and most slowly by the rat (16%), ferret (13%) and fruit bat (13%), the other species showing values in between these extremes.

Metabolites in urine. The main excretory products of sulphadimethoxine are the unchanged drug, its *N*¹-glucuronide and its *N*⁴-acetyl derivative. The amounts of these metabolites excreted in 24h after the dosing in various species are shown in Table 2, and are expressed in two ways, namely as the percentage of the dose excreted in 24h and as a percentage of the amount of drug excreted in 24h. The latter indicates the relative amounts of each metabolite excreted. Table 2 shows that in

man and the other primates 30–65% of the metabolites excreted in 24h consists of *N*¹-glucuronide. Of the non-primates three, namely the cat, ferret and rabbit, excrete no *N*¹-glucuronide, whereas the other six eliminate less than 20% of the 24h excretion as *N*¹-glucuronide. However, in absolute amounts, the dog, mouse and hen are excreting as much *N*¹-glucuronide in 24h as are the green monkey, squirrel monkey and slow loris. The cat is known to have a defective glucuronic acid conjugation mechanism (see Williams, 1967) and the absence of a glucuronic acid conjugate was not unexpected.

In the carnivores, i.e. dog, cat and ferret, most of the drug is excreted unchanged (60–80% of the 24h excretion). Further, the dog, which is apparently unable to acetylate aromatic amines (see Williams, 1967), is the only animal listed in Table 2 that does not produce *N*⁴-acetylsulphadimethoxine. In the rabbit the drug is excreted almost entirely acetylated (91% of the 24h excretion), there being no *N*¹-glucuronide and very little (1%) unchanged drug. The drug is also highly acetylated (67%) in the guinea pig. In the primates acetylation varies widely from 2% in the capuchin monkey to 61% in the green monkey.

A perusal of Table 2 suggests that in primates,

Table 2. *Excretion of N¹-glucuronide by various species receiving sulphadimethoxine orally*

Doses and animals are as described in Table 1. The results given are averages, the numbers of animals used being indicated in Table 1.

Animal	Metabolites excreted in 24h						
	% of dose				% of 24h total		
	Total	Free drug	<i>N</i> ¹ -Glucuronide	<i>N</i> ⁴ -Acetyl	Free drug	<i>N</i> ¹ -Glucuronide	<i>N</i> ⁴ -Acetyl
Primates							
Man	25	1.8	16.3	5.3	7	65	21
Rhesus monkey	39	2.3	25.7	9.0	6	66	23
Green monkey	12	1.1	3.6	7.3	9	30	61
Baboon	42	7.6	26.5	3.4	18	63	8
Squirrel monkey	9	1.1	3.9	3.3	12	44	37
Capuchin	25.5	11.7	12.2	0.5	46	48	2
Bushbaby	19	0.6	11.8	6.7	3	62	35
Slow loris	8.5	2.8	4.2	1.3	33	49	15
Tree shrew	26	7.5	13.5	4.7	29	52	18
Non-primates							
Dog	26	16.6	4.9	0.0	64	19	0
Cat	34	27.9	0.0	6.1	82	0	18
Ferret	13	9.4	0.0	3.5	73	0	27
Rabbit	46	4.6	0.0	41.9	1	0	91
Rat	16	6.1	1.2	7.5	38	8	47
Mouse	55	26.4	6.6	10.5	48	12	19
Guinea pig	26	4.2	1.0	17.4	16	4	67
Fruit bat	13	7.3	1.3	3.0	56	10	23
Hen	33	21.1	5.3	3.0	64	16	9

except possibly the green monkey, the main metabolite of sulphadimethoxine is the N^1 -glucuronide, and that in non-primates, except the rabbit and the guinea pig, the main excretory product is the unchanged drug. In the rabbit and guinea pig the main excretory product is the N^4 -acetyl derivative, as is also the case in the green monkey, although in this monkey 30% of the 24h excretion is N^1 -glucuronide.

Biliary excretion of sulphadimethoxine. The biliary excretion of the drug after intraperitoneal injection was studied in bile-duct-cannulated rats and rabbits. Two doe rabbits injected intraperitoneally with 100mg of drug/kg excreted 2.4 and 2.6% of the dose respectively in the bile in 6h. This material consisted roughly of equal parts of free drug (0.7% of the dose), N^4 -acetylsulphadimethoxine (0.9%) and sulphadimethoxine N^4 -

glucuronide (0.8%), there being no N^1 -glucuronide or N^4 -sulphate of the drug in the bile. Biliary excretion was also examined in nine rats. In three of these an average of 6% of the drug (100mg/kg) was excreted in the bile in 6h. In another six 10% (range 9–12%) of the dose was excreted in 24h and 13% (range 12–16%) in 48h. The 24h bile was analysed and found to contain five metabolites, namely unchanged drug (2.3%), N^4 -acetylsulphadimethoxine (0.3%), N^1 -glucuronide (6.8%), N^4 -glucuronide (0.5%) and N^4 -sulphate (0.1% of the dose). The main metabolite of sulphadimethoxine in bile in the rat is thus the N^1 -glucuronide, and it appears that the rat is producing more of the N^1 -glucuronide than is indicated by the urinary excretion, which is about 1% (Table 2).

Distribution of ^{35}S in the rat. The distribution of ^{35}S 3 days after the intraperitoneal injection of [^{35}S]sulphadimethoxine (100mg/kg) is shown in Table 3. Most (95%) of the ^{35}S has been accounted for. About 62% of the dose has been eliminated, 60% in the urine and 2% in the faeces. The rest, some 30%, is still in the tissues of the animal. The plasma concentrations of ^{35}S after intraperitoneal injection of the drug fell slowly (see Table 4), for the plasma concentration of the drug at 3 days after injection was still 72% of that at 0.5h whereas at 7 days it was 24% of the 0.5h value.

Table 3. *Distribution of ^{35}S in the excreta and tissues of rats given [^{35}S]sulphadimethoxine*

[^{35}S]Sulphadimethoxine (100mg/kg; 5 μCi) was injected intraperitoneally in dimethyl sulphoxide (0.5ml).

Location of ^{35}S 3 days after dosing	% of dose of ^{35}S	
	Rat 1	Rat 2
Urine	58.2	62.0
Faeces	2.7	1.3
Liver	1.9	1.7
Kidney	1.8	1.9
Intestine and contents	4.2	3.8
Blood (4ml)	3.0	2.9
Rest of carcass	23.7	20.2
Total	95.5	93.8

Table 4. *Plasma concentrations of [^{35}S]sulphadimethoxine in the rat*

Rats were injected with [^{35}S]sulphadimethoxine and killed in pairs as described in the text. The plasma concentration of the drug was calculated from the amount of ^{35}S present, assuming that the ^{35}S found was present as [^{35}S]sulphadimethoxine.

Time after injection (h)	Concn. of drug ($\mu\text{g/ml}$)
0.5	420
1	422
2	375
4	372
8	366
12	362
18	360
25	369
72	303
168	100

DISCUSSION

Certain sulphonamide drugs have been shown to be excreted by man as stable N -glucuronides in which the glucuronyl residue is attached to the sulphonamide nitrogen ($N-1$) of the drug. These drugs are sulphathiazole (Uno & Ueda, 1963a), sulphamethoxazole (Ueda & Kuribashi, 1964), sulphadimethoxine (Bridges, Kibby & Williams, 1964, 1965), sulphamethomidine (Bridges, Walker & Williams, 1967, 1969) and sulphadimethoxy-pyrimidine (Walker & Williams, 1969). Uno & Ueda (1963b) noted that the N^1 -glucuronide of sulphathiazole, which occurred as a minor metabolite in human urine, did not occur in rabbit urine.

The present paper shows that 15 out of the 18 species given sulphadimethoxine orally excreted the N^1 -glucuronide of the drug in the urine. No N^1 -glucuronide was detected in the urine of the cat, ferret and rabbit. However, in the nine primates examined the N^1 -glucuronide occurred as a major metabolite in the urine, and these nine primates include members of almost every class of primate from the tree shrew, which is regarded by some authors as a doubtful primate (see Napier & Napier, 1967), to man (see Tables 1 and 2). In the non-primates the N^1 -glucuronide was either a minor metabolite or did not occur at all, the main excretory products being the unchanged drug and/

or its N^4 -acetyl derivative. These findings suggest that N^1 -glucuronide formation is a major reaction of sulphadimethoxine only in primates and that the tree shrew behaves as a primate in this respect.

Sulphadimethoxine is a drug that is rather slowly excreted in most of the species listed in Table 1. The most rapid urinary excretion during 2 days after the dosing is found in the rabbit (75%), mouse (64%), rhesus monkey (55%) and baboon (55% of the dose). In the rat the urinary excretion of the orally administered drug is relatively slow, being only 16% in 24h and 33% in 2 days. The distribution of the ^{35}S -labelled drug in the rat 3 days after intraperitoneal injection in dimethyl sulphoxide is shown in Table 3. About 62% of the dose is eliminated from the body in this time, mainly in the urine (about 60%), little (2%) being found in the faeces. Nearly one-third of the dose is still in the animal. The concentration of the drug in blood, measured as ^{35}S , also falls slowly. Other work in this laboratory has shown that sulphadimethoxine and its N^4 -acetyl derivative bind strongly to plasma proteins, and this suggests that the drug may bind firmly to tissue proteins, possibly more firmly in some species than others. Sulphadimethoxine N^1 -glucuronide, however, is poorly bound to proteins (Bridges *et al.* 1969), but the formation of this conjugate in primates does not appear to affect the rate of excretion of the drug, which is more rapid in some primates than others. In fact, urinary excretion occurs most rapidly in a species, the rabbit, in which this conjugate is not found.

There is an appreciable biliary excretion of sulphadimethoxine in the rat. This amounted to about 10% of the dose in 24h and, of this, 68% (6.8% of the dose) was present as the N^1 -glucuronide. The amount of N^1 -glucuronide found in the urine of the rat was only 1% of the dose in 24h (see Table 2). The rat was therefore synthesizing much more N^1 -glucuronide (possibly up to about 10% of the dose) than was indicated by the amount in the urine. The faecal excretion of the drug in the rat was small (about 2%; see Table 3), and therefore it follows that most of the drug and its metabolites

excreted in the bile must have been reabsorbed from the intestine, so that an enterohepatic circulation of at least a small part of the drug could be occurring in the rat. It seems likely that the sulphadimethoxine N^1 -glucuronide is hydrolysed in the gut, for glucuronides are not readily absorbed as such from the intestine (see Smith & Williams, 1966). Sulphadimethoxine N^1 -glucuronide (5mg/ml), when incubated at 37°C for 18h with a rat or human faecal homogenate (1ml) consisting of 1g of faeces suspended in 9ml of Todd-Hewitt broth, was found to be hydrolysed to the free drug. The autoclaved homogenate had no action on the glucuronide.

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