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THE FATE AND URINARY METABOLITES OF THIAMINE PROPYL DISULFIDE IN RABBIT AND MAN*

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Abstract—The metabolic fate of the propyl mercaptan moiety of thiamine propyl disulfide (TPD) has been studied in rabbit and man for comparison with the previous results obtained with rat. About 70–80 per cent of the radioactivity was excreted into the first 48 hr urine, when ^{35}S-labeled TPD was administered to rabbits by either s.c., i.v. or p.o. route. More than 90 per cent of the urinary metabolites was identified as methyl propyl sulfone (MPS), 2-hydroxypropyl methyl sulfone (2-HPMS), 3-hydroxypropyl methyl sulfone (3-HPMS), methylsulfonyl propionic acid (MSPA) and inorganic sulfate. Following oral administration, inorganic sulfate and MSPA were the predominant products, accounting for about 70 per cent of the urinary metabolites. On the other hand, MPS, 2-HPMS, MSPA and inorganic sulfate were excreted in approximately equal amounts (20–28 per cent) after parenteral administration. Time course changes on metabolites composition were also studied. In the urine of the healthy men who received TPD, was also confirmed the occurrence of MPS, 2-HPMS and 3-HPMS by the isotope dilution method. These results show that there are little species differences among rat, rabbit and man in the metabolism of the propyl mercaptan moiety of the drug.

THIAMINE propyl disulfide† (TPD) is a propyl analogue of thiamine allyl disulfide which is formed by the incubation of thiamine with minced garlic at pH 8[1] and it was prepared synthetically by Matsukawa *et al.*[2,3] TPD shows not only vitamin B_1 activity equivalent to thiamine in animals and birds, but also better intestinal absorption and higher affinity to erythrocytes than thiamine itself.[4,5] TPD has been widely used as a thiamine substitute or therapeutic agent in Japan and therefore it is interesting to study the metabolic fate of this compound in animals and man. The previous papers[6,7] demonstrated that the propyl mercaptan moiety of the drug was rapidly and almost quantitatively excreted into the urine when administered to rats. More than 90 per cent of the urinary metabolites was identified as methyl propyl sulfone (MPS), 2-hydroxypropyl methyl sulfone (2-HPMS), 3-hydroxypropyl methyl sulfone (3-HPMS), methylsulfonyl propionic acid (MSPA) and inorganic sulfate. Further studies showed that 2-HPMS, MSPA and inorganic sulfate were the major and terminal products and

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† Thiamine propyl disulfide (Alinamin) is 2-(2-methyl-4-aminopyrimidin-5-yl)methylformamido-5-hydroxy-2-penten-3-yl propyl disulfide, abbreviated TPD (Merck Index, 7th edition). The detailed descriptions concerning the chemical and biological properties of the compound can be found in the following literatures. B. C. JOHNSON, *Ann. Rev. Biochem.* **24**, 419 (1955); C. KAWASAKI, *Vitamin and Hormones* **21**, 69 (1963).

that inorganic sulfate could not be formed from the methylsulfonyl compounds such as MPS, 2-HPMS and 3-HPMS. These findings led us to propose a novel metabolic pathway responsible for the conversion of foreign alkyl mercaptans[8,9] to methylsulfonyl compounds. The present experiments were undertaken to clarify the metabolic fate of the drug in rabbit and man for comparative study concerning species difference.

EXPERIMENTAL

Compounds and methods used in the present studies are described below, otherwise the same as those reported previously.[6,7]

Administration of the drug and collection of the urine

Rabbit. Four male albino rabbits, weighing about 3 kg, were administered TPD-^{35}S (outer) p.o., s.c. or i.v., as shown in Fig. 3. The animals were then restrained on their backs and urine was collected by catheterization into flasks containing diluted hydrochloric acid for 6 hr. Thereafter the animals were housed in individual metabolism cages for subsequent collection of the urine. Water (50 ml per rabbit) was given p.o. by stomach tube immediately after the drug administration and once 6 hr later. Each urine sample was subjected to the subsequent chemical analyses.

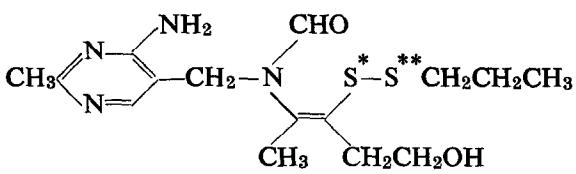

FIG. 1. Structural formula of thiamine propyl disulfide (TPD). TPD labeled with ^{35}S at the inner sulfur (*) or the outer sulfur (**) of S—S bond is designated as "inner or outer labeled," respectively.

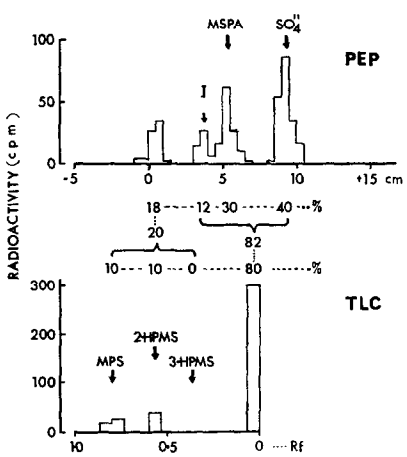

FIG. 2. Paper electrophoresis (PEP) and thin layer chromatography (TLC) of the urinary metabolites. Sample: 3–6 hr urine obtained from the rabbit injected TPD-^{35}S (outer) 3 mg i.v. PEP was carried out on filter paper, Toyo Roshi No. 51A in a 0·1 M sodium acetate solution, pH 7·1 (8·1 V/cm for 2·5 hr). TLC was developed on Silica Gel G thin layer plate with benzene: acetone (1:1, v/v).

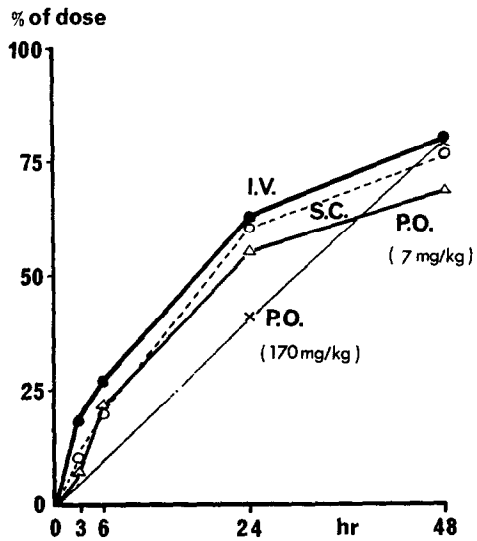

FIG. 3. Urinary excretion of the radioactivity in rabbits following the p.o., s.c. or i.v. administration of the drug. Four rabbits were administered TPD-^{35}S (outer) 7 or 170 mg/kg p.o., 3 mg/kg s.c. or 3 mg/kg i.v., respectively.

Man. Nine healthy adult men were orally given nonlabeled TPD (500 mg/man, about 9 mg/kg of body weight) and the first 24-hr urine was collected and subjected to determination of MPS, 2-HPMS and 3-HPMS by the isotope dilution.

Determination of urinary metabolites in rabbits

Quantitative determination of each urinary metabolite was performed by a combined use of paper electrophoresis and thin layer chromatography followed by determination of the radioactivity. The procedures were in details described in the previous paper.[7] A typical result on a urine sample from the rabbit is shown in Fig. 2. The accuracy of this combined method was already reported in our previous paper.[7]

Preparation of radioactive MPS, 2-HPMS and 3-HPMS

The radioactive samples of MPS, 2-HPMS and 3-HPMS which were used in the isotope dilution experiments were prepared from the urine of rats fed TPD-^{35}S (outer) by addition of the respective authentics as carriers. Two male Sprague–Dawley (JCL) rats, weighing 245 and 280 g, were orally given TPD-^{35}S (outer) ($7{\cdot}93 \times 10^6$ cpm) equivalent to 73 mg/rat) and the urine was collected for 48 hr. The combined urine contained 76 per cent of the administered radioactivity. About 60 per cent of the urinary radioactivity was extracted with chloroform:methanol (5:1, v/v) at pH 6. After evaporation of the solvent, the extract was subjected to preparative thin layer chromatography using silica gel G ($1 \times 150 \times 200$ mm plate; acetone:benzene = 1:1, v/v). Three radioactive bands on the chromatograms, corresponding to those of the authentic MPS, 2-HPMS and 3-HPMS, were scraped off and eluted with chloroform, respectively. After evaporation of the solvent, to each eluate were added the respective authentic samples of MPS (206 mg), 2-HPMS (196 mg) and 3-HPMS (112 mg). The

eluates containing MPS and 2-HPMS were subjected to repeated recrystallization until the specific radioactivity reached a constant value. The solvent systems for recrystallization were heptane:benzene (7:1·5, v/v) for MPS and benzene for 2-HPMS. Because of difficulty of recrystallization, 3-HPMS was checked for its purity by infrared spectrometry and the quantity was determined by measuring the optical density at 1312 cm^{-1} characteristic of 3-HPMS molecule. The yield and specific activity were as follows; MPS: 111·5 mg, 330 cpm/mg; 2-HPMS: 147·6 mg, 9190 cpm/mg and 3-HPMS: 111·9 mg, 1054 cpm/mg.

Identification of MPS, 2-HPMS and 3-HPMS in the human urine

The 24-hr urine (10·7 l.) from nine healthy men receiving the drug was concentrated to 1500 ml by rotary evaporator *in vacuo* at 45–50° and divided into three equal portions. The known amounts of the radioactive MPS (26,300 cpm and 89·3 mg) were added to each portion. Each mixture was then extracted several times with 4–6 vol. of chloroform:methanol (5:1, v/v). The radioactivity was completely extracted into the solvent layer. After evaporation of the solvent, the residues were again extracted with chloroform to remove the major part of nonradioactive materials without any appreciable loss of the radioactivity. The extracts obtained from the three portions were then applied on individual silicic acid columns and eluted by the following solvents, respectively. MPS was eluted by benzene–acetone (1:1, v/v) and 2-HPMS and 3-HPMS in order by benzene–acetone (1:1, v/v). The latter two were further purified by TLC using ethyl acetate. The purity of these fractions was checked by infrared spectrometry using individual authentic sample as a reference standard. The quantity of each metabolite was then determined by infrared spectrometry at 1132–1140 cm^{-1} and 1310–1312 cm^{-1} characteristic to the sulfonyl group.

RESULTS AND DISCUSSION

Urinary excretion of TPD-^{35}S (outer) in rabbits

Figure 3 shows the excretion curves of radioactivity in the urine of rabbits receiving TPD-^{35}S (outer) orally, s.c. or i.v. Irrespective of difference in the administration routes, about 70–80 per cent of the dose was excreted into the 48-hr urine. Upon oral ingestion, the larger dosage (170 mg/kg) induced some delay in the urinary excretion at the earlier phase but the total excretion rate exceeded that with the smaller dosage (7 mg/kg) after 48 hr. These results show that the propyl mercaptan moiety of the drug is well absorbed from the digestive tracts and excreted mainly in the urine in rabbits. These results are quite consistent with the previous findings with rats.[6,7]

Urinary metabolites in rabbits

The composition of metabolites in the first 24-hr urine after the drug administration is shown in Table 1. When the drug was orally given in a small dose (7 mg/kg), MSPA and inorganic sulfate were the most predominant metabolites and 2-HPMS was the subsequent product. The larger dose (170 mg/kg) caused an increased excretion of inorganic sulfate and MPS with a decrease of MSPA and 2-HPMS. On the other hand, when the drug was administered either i.v. or s.c., MPS, 2-HPMS, MSPA and inorganic sulfate were excreted in approximately equal amounts (20–28 per cent). In other words, the parenteral administration, as compared with the oral one, caused a

decreased excretion of inorganic sulfate with an increased excretion of MPS, which is an intermediary product. This point is the most remarkable difference in the metabolite patterns which were observed between rabbit and rat, for in the latter i.v. injection induced an increased excretion of inorganic sulfate with a decrease of MPS. Another noticeable difference is the excretion of 3-HPMS. Namely, little or no 3-HPMS was

TABLE 1. THE COMPOSITION OF METABOLITES IN THE 24-hr URINE FOLLOWING THE ADMINISTRATION OF TPD-^{35}S (OUTER) TO RABBITS AND RATS

Animal	Rabbit				Rat*	
Application	p.o.	p.o.	i.v.	s.c.	p.o.	i.v.
Dose (mg/kg)	7	170	3	3	10	6
Neutral metabolites	%	%	%	%	%	%
MPS	3·0	12·5	20·0	20·0	3	2
2-HPMS	15·0	5·5	20·0	22·5	29	23
3-HPMS	2·0	0	0	0	14	1
Acidic metabolites						
MSPA	37·5	19·0	22·5	23·0	15	10
Inorg. sulfate	32·5	55·5	28·5	23·5	27	50
Unidentified I	10·0	8·5	7·0	9·5	6	7
Unidentified II	0	0	0	0	5	3

* For comparison, the data on rats are referred to from the previous paper.[7]

detected in the rabbit urine, whereas the significant amounts of 3-HPMS were consistently recognized in the rat urine.[3]

Time course changes of metabolites composition were then investigated. Figures 4 and 5 show the percentage distribution of each metabolite in the urine following the administration. When the drug was orally given (7 mg/kg), MSPA was the most

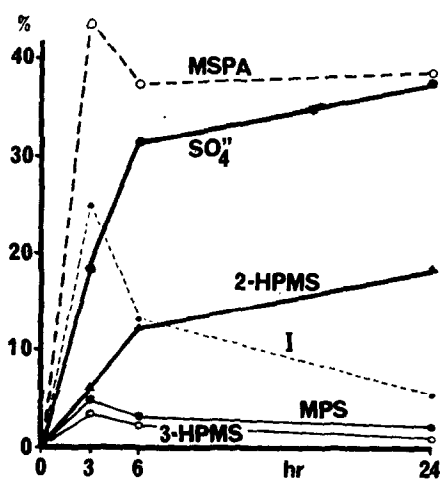

FIG. 4. Time course changes in the distribution of the urinary metabolites following the p.o. administration of the drug. A rabbit was administered p.o. TPD-^{35}S (outer) 7 mg/kg and urine was collected at 3, 6 and 24 hr. The values on vertical axis are expressed as percent of the total radioactivity in each urine sample.

predominant constituent throughout the period, while inorganic sulfate increased with time, amounting to about the same level as MSPA 24 hr after the administration. 2-HPMS gradually increased with time, while small amounts of MPS and 3-HPMS were excreted transiently at the initial stage. On the other hand, when the drug was administered parenterally, inorganic sulfate was the most predominant at the earlier phase, comprising 49 and 64 per cent of the radioactivity in the first 3-hr urine after i.v. and s.c. injection, respectively (Fig. 5). But, inorganic sulfate rapidly decreased and MPS, 2-HPMS and MSPA conversely increased. These patterns were very similar to those obtained with rats,[7] except that in rabbits the p.o. administration caused an increased excretion of MSPA, especially at the earlier phase.

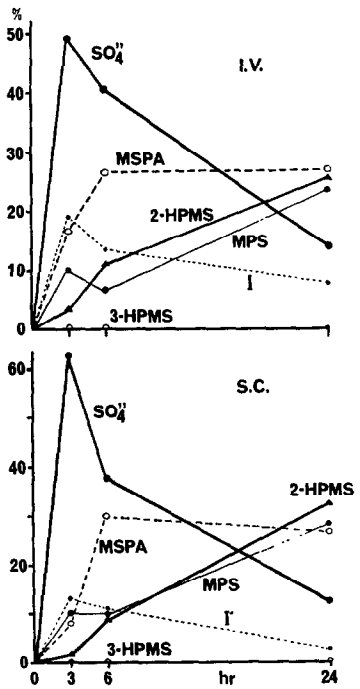

FIG. 5. Time course changes in the distribution of the urinary metabolites following the i.v. or s.c. administration of the drug. Two rabbits were injected TPD-^{35}S (outer) 3 mg/kg i.v. or s.c., respectively, and urine was collected at 3, 6 and 24 hr. The values on vertical axis are expressed as per cent of the total radioactivity in each urine sample.

Urinary metabolites in man

Table 2 shows the results of the isotope dilution experiments. Thus, MPS, 2-HPMS and 3-HPMS accounted for 4·5, 13 and 4 per cent of the dose, respectively. Although the occurrence of MSPA and inorganic sulfate as urinary metabolites has not been studied in man, the formation of three neutral compounds possessing the methylsulfonyl group has been thus established in all the tested species. Comparison of the excretion data obtained under comparable condition with rat (10 mg/kg, p.o.),[7] rabbit (7 mg/kg, p.o.) and man (9 mg/kg, p.o.) demonstrates that neutral metabolites which were excreted in the first day amounted to 13–36 per cent of the dose and that

TABLE 2. QUANTIFICATION OF MPS, 2-HPMS AND 3-HPMS IN HUMAN URINE BY THE ISOTOPE DILUTION

Metabolites	Added as carrier		Isolated	Urinary contents	
	mg	specific activity cpm/mg	specific activity cpm/mg	mg/man/day	% of dose*
MPS	80·0	330	263	6·7	4·5
2-HPMS	73·8	9190	4980	20·8	13·0
3-HPMS	89·3	1054	867	6·3	4·0

* Calculated as the propyl mercaptan moiety of the drug.

2-HPMS was the major component comprising about 50–70 per cent of the neutral metabolites in these species. From the present data, it was concluded that there were little species differences, at least from the qualitative viewpoint, in the biotransformation of the propyl mercaptan moiety of TPD among rat, rabbit and man.

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