DETERMINATION OF URINARY METABOLITES OF THIAMINE PROPYL DISULFIDE IN HUMANS

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Gas chromatographic procedure for the quantitative determination of methyl propyl sulfone (MPS), 2-hydroxypropyl methyl sulfone (2HPMS) and 3-hydroxypropyl methyl sulfone (3HPMS) in urine was developed. By using this procedure, the urinary excretion of MPS, 2HPMS and 3HPMS after oral dose of thiamine propyl disulfide (TPD) was investigated in healthy adults, in order to assess their ability of drug disposition including metabolism. Among these metabolites, 2HPMS was predominant and the other two were minor. 2HPMS was excreted most in the 24–36 h urine and the time course of excretion of this metabolite was almost the same among these subjects. In 4th day urine, a significant amount of 2HPMS was still excreted. On the contrary, the time course of excretion of MPS varied among subjects and also at different occasions in the same subjects. 3HPMS was excreted most in the 0–12 or 12–24 h urine and more rapidly excreted than 2HPMS. Two-fold interindividual differences in the amount of 2HPMS in the 0–48 h urine occurred and intraindividual difference was also observed. Inter- and intraindividual differences in the amount of MPS in 0–48 h urine were much larger than that in 2HPMS. In 3HPMS, these differences were slightly less than in 2HPMS. Sex difference in the excretion of MPS and 2HPMS was not observed.

Keywords —metabolism of thiamine propyl disulfide; methyl propyl sulfone; 2-hydroxypropyl methyl sulfone; 3-hydroxypropyl methyl sulfone; gas chromatography; flame photometric detector; rate of drug metabolism; drug disposition; interindividual difference; intraindividual difference

INTRODUCTION

It has occasionally been seen that a dose of a drug which cause therapeutic effect in one person produce toxicity or no response in another. This difference in response to drug is attributable at least in part to the difference in the rate of drug metabolism. From this viewpoint, the present standardized dosage regimen, except for a few drugs possessing a narrow range between therapeutic and toxic effects, is considered to be unsatisfactory. For safe, effective and individualized drug therapy, a dose should be determined after the rate of drug metabolism in each patients has accurately been estimated. Up-to-date, this type of study has been carried out by various investigators but results obtained are still insufficient for practical application.

The rate of drug metabolism is altered by many factors including pathological state.^{1,2)} The liver is the most important organ for drug metabolism and therefore the rate of drug metabolism has especially been examined in patients with liver disease. Despite the occasional conflicting results, it seems to be unquestionable that the rate of drug metabolism is impaired in these patients. This suggests also that the disorder of hepatic function could be predicted from decreased rate of drug metabolism in patients with liver disease compared to healthy subjects.

In order to resolve these problems described above, inter- and intraindividual differences in the metabolism of a test drug and the range of the rate of drug metabolism in healthy subjects must first be investigated. For this purpose, a drug such

as antipyrine, which is well absorbed, least bound to plasma protein, evenly distributed in body water and extensively metabolized, has widely been utilized as a test drug. It is obvious, however, that this drug is not suitable to apply to patients with liver disease. We therefore planned to examine the urinary excretion of oxidative metabolites of propylthio moiety of thiamine propyl disulfide (TPD) after the oral administration as an index of the rate of drug metabolism. The reason for the choice of TPD as a test drug is that TPD is a propylthio derivative of vitamin B₁ and its administration under various pathological states is considered to be more safe compared to other drugs.

The metabolism of TPD was extensively studied in rats^{3,4)} and rabbits⁵⁾ with ³⁵S-labeled TPD, and the propylthio moiety was found to be excreted into urine as methyl propyl sulfone (MPS), 2-hydroxypropyl methyl sulfone (2HPMS) and 3-hydroxypropyl methyl sulfone (3HPMS), together with methylsulfonyl propionic acid. The former three metabolites were produced by mixed function oxidases from methyl propyl sulfide which was formed by successive reduction of disulfide linkage and methylation of the resultant propyl mercaptan. On the other hand, in man, only the amount of these metabolites excreted in 0–24 h urine was determined by isotope dilution method,⁵⁾ but no other studies were carried out in detail.

In this report, we describe a simple and highly sensitive method for the quantitative determination of above three TPD metabolites by using gas chromatograph equipped with flame photometric detector, and also provide the detail about the urinary excretion of these metabolites in healthy adults.

MATERIALS AND METHODS

Subjects — The study of urinary excretion of TPD metabolites in normal subjects was conducted in a total of 19 healthy subjects (12 men and 7 women) ranging in age from 21 to 52 years old in our laboratory, and 4 subjects among them further served as volunteers for assessment of the

intraindividual variation on 4 or 5 occasions in a year. No attempts was made to control the life styles and diets during the course of the study.

Materials — Alinamine-25[®] tablets, each of which contains 25 mg of TPD, were kindly gifted from Takeda Chemical Industries, Ltd., Osaka. MPS, 2HPMS and 3HPMS were synthesized by the methods reported previously.^{6–8)} All other chemicals used were of the analytical grade.

Administration of TPD and Collection of Urine — TPD (200 mg), 8 tablets of Alinamine-25[®], was administered orally at 11:00 a. m. Urine samples were collected at 12 h intervals up to 48 h after administration of TPD. Control urine sample was collected for 3 h before administration of TPD.

Quantitative Determination of Urinary TPD Metabolites — The TPD metabolites in urine were extracted with ethylacetate and determined by gas chromatography using a Shimadzu gas chromatograph GC-3BF_p with flame photometric detector (394 nm filter). A glass column (1.7 m × 3 mm) packed with 1% XE-60 on Chromosorb W (80–100 mesh) was used throughout the experiments. Extraction procedure and conditions of gas chromatography were described in RESULTS.

In analysis of gas chromatography-mass spectrometry (GC-MS), an instrument employed was a JEOL GC-MS model DMS-100. 1% XE-60 on Chromosorb W (1 m × 2 mm) and helium (1.0 kg/cm²) were used as column packing and carrier gas, respectively. Column temperature was raised at a rate of 10°C/min from 100°C. Ionizing voltage and current were 23 eV and 300 mA, respectively.

RESULTS

Quantitative Determination of TPD Metabolites

As shown in Fig.1, 10 ng each of MPS, 2HPMS and 3HPMS were separated with a symmetric peak except a peak of 3HPMS showing a little tailing under the conditions as follows: Oven temp.; 160°C, carrier gas: N₂ (1.0 kg/cm²), H₂ (0.45 kg/cm²), air (1.0 kg/cm²). Retention times for MPS, 2HPMS and 3HPMS were 0.8, 2.6 and

11.5 min, respectively. Under the same conditions, the ethylacetate extract of control urine did not afford any peaks interfering with those of MPS and 3HPMS, but showed a small peak at the

same retention time with that of 2HPMS.

For the actual quantitative determination of these metabolites, the column temperatures were set so that the peak of each metabolites revealed

FIG. 1. Gas Chromatogram of the extract from Control Human Urine and a Mixture of TPD Metabolites

—, the extract from control human urine; ---, a mixture of TPD metabolites. Ten ng of each metabolite was injected.

FIG. 2. Excretion Pattern of 2HPMS in Normal Subjects

Each point represents the mean $\pm$ S.D. (vertical bars) of 42 experiments.

FIG. 3. Excretion Patterns of MPS and 3HPMS in Normal Subjects

Each point represents the mean $\pm$ S.D. (vertical bars) of 42 (MPS) and 27 (3HPMS) experiments.

FIG. 4. Frequency Distribution of Amount of 2HPMS excreted in 0–48 h Urine in 19 Normal Subjects

from 2 to 3 min, holding other conditions the same as those described above. Under these conditions, a response of each metabolite increased in

proportion to a square of the amounts ranging from 5 to 100 ng (2HPMS and 3HPMS) or from 1 to 20 ng (MPS). Procedures of quantitative

FIG. 5. Frequency Distribution of Amount of 3HPMS excreted in 0-48 h Urine in 12 Normal Subjects

FIG. 6. Frequency Distribution of Amount of MPS excreted in 0-48 h Urine in 19 Normal Subjects

FIG. 7. Intraindividual Variation of Amount of 2HPMS excreted in 0-48 h Urine in 4 Normal Subjects
TPD was administered orally on the date indicated at the top of figures. The solid and broken lines represent mean and S.D., respectively. Coefficient of variation (C.V.) was calculated as follows; (S.D./mean $\times$ 100).

determination of TPD metabolites in urine were described below.

MPS: To 5 ml of sample urine, 10 μg of sulfolane dissolved in 0.1 ml of methanol was added as an internal standard and extracted once with 10 ml of ethylacetate by shaking for 20 min. After centrifugation at 2500 r.p.m. for 20 min, 8 μl of organic phase was injected into the gas chromatograph setting column temperature at 130°C. The amount of MPS was calculated from the ratio of peak height of MPS to that of internal standard, using the calibration curve. By this procedure, MPS in urine was determined with an accuracy of 99 ± 1.5 (S.D.)%.

2HPMS: To 5 ml of control urine were added the known amount (10 or 20 μg) of 2HPMS and 20 μg of dibenzylsulfide dissolved in 0.1 ml of methanol as an internal standard. It was then extracted with 20 ml of ethylacetate by shaking for 20 min. After centrifugation at 2500 r.p.m. for 20 min, 6 μl of organic phase was injected into the gas chromatograph setting column temperature at 160°C. The amount of 2HPMS were calculated

from the ratio of peak height of 2HPMS to that of internal standard, using the calibration curve. By this procedure, however, the recovery of 2HPMS from urine exceeded 100%. This error was due to the small peak of the urinary constituent revealing the same retention time (2.6 min) with that of 2HPMS as described above. From GC-MS analysis of this peak, the retention time in gas chromatography and the fragment pattern in mass spectrometry were the same as those of 2HPMS (m/e :123(M^+), 94, 79, 59). This finding suggested that 2HPMS, though in trace amount, was also excreted in control urine and this affected the quantitative value, especially in the case that 2HPMS as a metabolite of TPD was excreted in a small amount. Therefore, the amount of 2HPMS derived from TPD was corrected by subtracting the amount in control urine from that in test urine. The amount of 2HPMS in control urine was estimated by subtracting the amount of added 2HPMS from the amount in 2HPMS-added control urine. The accuracy of this method was 100 ± 1.0 (S.D.)%.

FIG. 8. Intraindividual Variation of Amount of 3HPMS excreted in 0–48 h Urine in 4 Normal Subjects For other details see legend to Fig. 7.

3HPMS: Urine (5 ml) was extracted 5 times with 20 ml of ethylacetate with mechanical shaking. This extract was partially purified by silica gel column chromatography using 150 ml of ethylacetate-acetone (3:2) as an eluent, and the eluate was evaporated *in vacuo*. This residue was acetylated with acetic anhydride and pyridine in the usual manner. To the product remained after evaporation *in vacuo* was added the definite amount of diphenyl sulfone as an internal standard. This sample solution was injected into the gas chromatograph setting column temperature at 190°C. The amount of 3HPMS was calculated by the ratio of peak height of acetyl derivative of 3HPMS to that of internal standard, using the calibration curve. This procedure was rather tedious because of poor extractability and tailing of 3HPMS in gas chromatography, but the amount in urine was exactly determined with an accuracy of 93 ± 4.5 (S.D.)%.

Urinary Excretion of TPD Metabolites

The time course of excretion of TPD metabolites, 2HPMS, MPS and 3HPMS, was shown in Figs. 2 and 3, respectively. In all periods, 2HPMS was predominant, and MPS and 3HPMS were minor. The mean amounts of 2HPMS in 0–12, 12–24, 24–36 and 36–48 h urines were 3.2, 6.6, 8.6 and 6.3% of dose, respectively (Fig. 2). The maximum amount was excreted in the 24–36 h urine but the amounts in three periods after 12 h did not differ too much. Only the amount in 0–12 h urine was distinctly small. Such an excretion pattern was commonly seen in all subjects, but the amounts excreted in each period varied among individuals. As it was found that 2HPMS was slowly excreted, we examined the excretion of 2HPMS for 4 d following administration of TPD. In the 4th day urine, the amount of 2HPMS was found to be half of that in the second day urine, in which the maximum

FIG. 9. Intraindividual Variation of Amount of MPS excreted in 0–48 h Urine in 4 Normal Subjects
For other details see legend to Fig. 7.

amount was excreted. This finding indicated that 2HPMS continued to be excreted over a long period of time with a significant amount.

The excretion of MPS was maximum in the first 12-h urine, decreasing thereafter, although the amounts in 12–24 and 24–36 h urine were approximately equal (Fig. 3). Very large individual differences were seen in the amount of MPS excretion. Their excretion patterns could be divided into three types. The first one exhibited the maximum excretion in 0–12 h urine, the second in 12–24 h urine, and the third revealed two excretion peaks in 0–12 and 24–36 h urines. These types of excretion patterns were also seen at different occasions in one subject. The maximum excretion of 3HPMS was observed in either 0–12 or 12–24 h urine approximately in

FIG. 10. Comparison of Excretion of 2HPMS with That of 4-Hydroxyantipyrine (4-OH-AN) in 4 Normal Subjects

Antipyrine (300 mg) was administered orally and the urine was collected for 48 h following administration of antipyrine. 4-OH-AN in urine was determined by gas chromatographic method of Huffman et al.¹⁹⁾ with some modifications. The result was the mean of two experiments.

The result of 2HPMS excretion was the mean of 4 or 5 experiments.

the equal amount and then gradually decreased with time (Fig. 3). When counted individually, the number of subjects who excreted the maximum amount in 0–12 or 12–24 h urine was approximately equal.

Next, the amounts of TPD metabolites excreted in 0–48 h urine were individually determined. As shown in Fig. 4, the amount of 2HPMS in 0–48 h urine varied considerably among subjects ranging from 17.3 to 36.1% with a mean of 24.5% of the administered dose. In spite of such a large interindividual difference as above, the polymorphism of frequency distribution which was well known, for example, in the hydroxylation of debrisoquine⁹⁾ was not observed for excretion of 2HPMS. The frequency distribution rather exhibited the continuous unimode. Similarly about two-fold variation among individuals was found in the amount of 3HPMS in 0–48 h urine ranging from 1.4 to 2.5% of the dose and the frequency distribution was approximately continuous (Fig. 5). On the other hand, the frequency distribution was discontinuous, and an enormous interindividual difference was seen in the amount of MPS in 0–48 h urine (Fig. 6).

Further, sex difference in the excretion of MPS and 2HPMS was investigated in 7 men and 8 women. A slightly more 2HPMS was excreted in women than men but this difference was not statistically significant. Similarly, the amount of MPS excreted in 0–48 h urine was not found to be significantly different between both sexes.

Finally, the excretion of MPS, 2HPMS and 3HPMS was examined on 4 or 5 occasions in a year. As shown in Fig. 7, the amount of 2HPMS in 0–48 h urine varied at different occasions in all subjects. Coefficient of variation in the subject A, B, C and D was 11.9, 24.2, 18.3 and 21.0%, respectively. The magnitude of intraindividual difference was roughly equal except for the subject A. Similar intraindividual difference was observed in the amount of 3HPMS in 0–48 h urine (Fig. 8). In the amount of MPS in 0–48 h urine, coefficient of variation in the subject A, B, C and D was 60.1, 39.4, 58.2 and 44.5%, respectively (Fig. 9). Intraindividual difference in the

excretion of MPS was about 2 to 3 times larger than that in the excretion of 2HPMS.

DISCUSSION

The amounts of MPS and 2HPMS excreted in urine after oral dose of TPD were determined by simple and sensitive method using gas chromatograph equipped with a flame photometric detector. In the case of determination of 3HPMS, the procedure was rather tedious but the value obtained was accurate. By using these procedures, urinary excretion of TPD metabolites in healthy adults was examined. Among the metabolites excreted in urine, 2HPMS was major, and MPS and 3HPMS were minor metabolites. This finding was similar with that reported by Suzuoki *et al.*, who examine only the amount in the first 24-h urine.⁵⁾ Our result clearly showed that a significant amount continued to be excreted over a period of several days. Such a prolonged excretion of the TPD metabolites in human was not the case in rats and rabbits³⁻⁵⁾ who excreted most of the metabolites in their first 24-h urines.

Large difference among individuals was observed in the amounts of three metabolites excreted in 0–48 h urine. It is not clear as to what extent this difference reflects the rate of drug metabolism, but it is evident that this difference reflects the ability of various process of drug disposition including metabolism. Of TPD metabolites examined in this study, MPS and 3HPMS were intermediate metabolites so that these amounts in urine were affected by further metabolism. In addition, the excretion of these metabolites in urine was in very small amounts. In these regards, the amounts of MPS and 3HPMS excreted in urine were unsuitable as a parameter to assess the ability of drug disposition including metabolism. Therefore, we used preferentially the amount of 2HPMS excreted in 0–48 h urine as a parameter. This amount varied about two-fold among healthy adults. Although such a large interindividual difference was observed, subjects were not divided into subgroups such as extensive-metabolizer and poor-metabolizer. Conney *et al.*¹⁰⁾ reported that

the half-lives of such drugs as phenylbutazone, antipyrine and phenacetin varied on different occasions in the same subject. Similar intraindividual difference was occasionally observed in the amount of 2HPMS excreted in 0–48 h urine. This result suggests that the excretion of 2HPMS is mainly regulated by environmental factors.

Recently, multiplicity of cytochrome P-450, a key enzyme of mixed function oxidases, has been reported by many investigators.¹¹⁻¹³⁾ Actually, it is known that a subject who rapidly metabolizes one drug does not always metabolize the other with the same rate.¹⁴⁻¹⁸⁾ For the examination of TPD metabolism which is utilized as a practical test to improve the standardized dosage regimen, it must be investigated what type of drug metabolism correlates to TPD metabolism. We first attempted to correlate the excretion of 2HPMS with that of 4-hydroxyantipyrine after oral dose of antipyrine, but unsuccessful. The amounts of both metabolites excreted in 0–48 h urine were not correlated as shown in Fig. 10. This problem therefore remains to be resolved.

In the following paper of this series, the excretions of MPS and 2HPMS in patients with liver disease are compared with those in healthy adults indicated in this paper. The decrease of 2HPMS excretion in 0–48 h urine in patients with various stages of liver disease and the correlation of this decrease to the abnormalities of several liver function tests are presented.

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