ALTERED THIAMINE PROPYL DISULFIDE METABOLISM IN RATS WITH LIVER INJURY INDUCED BY CARBON TETRACHLORIDE AND D-GALACTOSAMINE

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(Received September 20, 1982)

The urinary excretion of 2-hydroxypropyl methyl sulfone (2HPMS) and methyl propyl sulfone (MPS) after oral administration of thiamine propyl disulfide (TPD) was examined in rats with liver injury induced by carbon tetrachloride (CCl_4) and D-galactosamine (GAL), in order to investigate the effect of liver damage on TPD metabolism. In the CCl_4 -treated rats, excretion of 2HPMS and MPS markedly decreased at an early stage, but MPS thereafter started to be excreted rapidly as compared with the control rats. On the other hand, 2HPMS continued to be excreted in lesser amounts in the CCl_4 -treated rats than in the control rats even after 72 h. In the GAL-treated rats, excretion of 2HPMS and MPS did not vary so greatly at a dose of 300 mg/kg, but alteration of excretion of 2HPMS and MPS was clearly observed at a dose of 600 mg/kg and slightly more at a dose of 1200 mg/kg. In comparison with the CCl_4 -treated rats, delay of MPS excretion was not observed but excretion of 2HPMS more delayed in the GAL-treated rats. Concerning the amount excreted in the 0–72 h urine, decrease in 2HPMS and increase in MPS was the same as the CCl_4 -treated rats. From light microscopic examination, the liver of rats treated at a dose of 1000 mg/kg of GAL was found to be injured over a long period. From these results, TPD metabolism was suggested to be impaired in proportion to the severity of liver damage, and decrease of 2HPMS excretion was confirmed in liver-injured rats, as well as the patients with liver disease. Our previous suggestion that measurement of 2HPMS excreted in the urine was a useful test for quantification of liver disease was supported.

Keywords—metabolism of thiamine propyl disulfide; methyl propyl sulfone; 2-hydroxypropyl methyl sulfone; liver injury; carbon tetrachloride treatment; D-galactosamine treatment

INTRODUCTION

Our previous paper¹⁾ showed that the amount of 2-hydroxypropyl methyl sulfone (2HPMS), a major metabolite of thiamine propyl disulfide (TPD), excreted in the 0–48 h urine in the patients with liver disease was significantly less than that in the normal subjects, and that the decrease was approximately proportional to the severity of liver disease. It was thus demonstrated that TPD metabolism was impaired in the patients with liver disease.

In this paper, we studied the urinary excretion of 2HPMS and methyl propyl sulfone (MPS) in rats with liver injury induced by carbon

tetrachloride (CCl_4) and D-galactosamine (GAL), in order to confirm the result in the patients with liver disease obtained in the previous report.¹⁾ MPS is a minor metabolite and also a precursor of 2HPMS.²⁾ CCl_4 is a well-known hepatotoxic agent which causes centrilobular necrosis³⁾ and decreases activities of liver microsomal drug metabolizing enzymes.^{4,5)} These changes recovered to the normal after several days. GAL produces liver injury which resembles human viral hepatitis,⁶⁾ but the effect on liver microsomal drug metabolizing enzymes is not apparent. Therefore, we also examined the effect of GAL on liver microsomal drug metabolizing enzymes

in this paper.

MATERIALS AND METHODS

Chemicals — TPD was kindly donated by Takeda Chemical Industries, Ltd., Osaka. CCl_4 and D-galactosamine-HCl (GAL) were purchased from Nakarai Chemicals, Ltd., Kyoto. Other chemicals were the same as before.⁷⁾

Animals and Pretreatment with Chemicals — Male Sprague-Dawley rats weighing 170–250 g were used throughout the experiments. CCl_4 was intraperitoneally injected at a dose of 1 ml/kg. GAL dissolved in 0.9% NaCl was intraperitoneally injected at a dose of 300, 600, 1000 or 1200 mg/kg.

TPD Administration and Collection of Urine — TPD (10 or 50 mg/body) dissolved in 0.9% NaCl containing an equivalent mole of HCl was orally administered 24 h after a single injection of CCl_4 or GAL. Urine samples were collected at 12 h intervals up to 48 h and then for 24 h after administration of TPD. Control urine was collected for 12 h before administration of TPD.

Quantitative Determination of 2HPMS and MPS in Urine — One ml of urine instead of 5 ml used in human experiments was used for the extraction of 2HPMS and MPS. Extraction procedures and methods of quantitative determination of 2HPMS and MPS were conducted in the same way as reported before,⁷⁾ using Shimadzu gas chromatograph GC-3BF_P with flame photometric detector.

In Vitro Experiments — Rats were sacrificed at 24, 48, 72 and 96 h after treatment of GAL (1000 mg/kg), and livers were immediately removed. Small pieces of each liver were used for the histological examination, and the remainder was thoroughly perfused with 0.9% NaCl. Microsomes were isolated by usual manner and suspended in 0.25 M sucrose–0.1 mM EDTA–10 mM Tris-HCl buffer (pH 7.4). Protein content was determined by the method of Lowry *et al.*⁸⁾ Cytochrome P-450 was determined by the method of Omura and Sato⁹⁾ using the molar extinction coefficient of $91 \text{ mM}^{-1} \text{ cm}^{-1}$. *N*-Demethylation of benzphetamine was assayed by

measuring formaldehyde by the method of Nash.¹⁰⁾

Histological Examinations — Liver obtained above was fixed in 10% formalin and embedded in the usual manner in paraplast. Slice of 5 μ in thickness was stained with hematoxylin-eosin and examined by light microscopy.

RESULTS

TPD Metabolism in CCl_4 -Treated Rats

The urinary excretion of 2HPMS in the control and CCl_4 -treated rats was indicated in Fig. 1.

FIG. 1. Cumulative Excretion of 2HPMS in Control (○) and CCl_4 -Treated Rats (●)

TPD was administered at a dose of 10 mg/body. Open circle and bar represent mean and S.D. of three control rats.

FIG. 2. Cumulative Excretion of MPS in Control (○) and CCl_4 -Treated Rats (●)

For details see legend to Fig. 1.

CCl_4 -treated rats showed the similar excretion pattern from each other though there were some differences in the amount among rats. By comparison with the control rats, CCl_4 -treated rats excreted a very small amount of 2HPMS in the first 24 h urine and then excreted rather rapidly up to 48 and 72 h after administration of TPD in rats A and B, and rat C, respectively. The amount of 2HPMS excreted in the 0–72 h urine was undoubtedly less in all CCl_4 -treated rats compared to the control rats and delay of excretion of 2HPMS was observed.

As can be seen in Fig. 2, excretion of MPS in the CCl_4 -treated rats was also different from that in the control rats. In the control rats, MPS was excreted in approximately equal amount in the 0–12 and 12–24 h urines, and thereafter was not excreted any more. On the other hand, excretion of MPS in the CCl_4 -treated rats was depressed in the first 12 h urine, especially in rat B, and then rapid increase of excretion was observed. Like 2HPMS, the excretion of MPS was

distinctly delayed but the amount excreted in the 0–72 h urine increased by three-fold in rat A and two-fold in rats B and C comparing to the control rats.

TPD Metabolism in GAL-Treated Rats

In rats treated with GAL at a dose of 300 mg/kg, excretions of 2HPMS and MPS were not varied so large comparing to those in the control rats (Figs. 3-1 and 4-1). However, the amounts of 2HPMS excreted in the 0–12 and 12–24 h urines were considerably less in two of three rats than in the control rats.

Increasing the dose of GAL to 600 mg/kg, two or all of three rats showed quite different excretion of 2HPMS or MPS, respectively, comparing with those in the control rats (Figs. 3-2 and 4-2). For excretion of 2HPMS, rat G showed the similar excretion profile to the control rats but the amount excreted in the first 12 h urine was less than that in the control rats. Excretion in other two rats were very different from that in the control rats. Rat I continued to excrete very small

FIG. 3. Cumulative Excretion of 2HPMS in Control (○) and GAL-Treated Rats (●)

TPD was administered at a dose of 50 mg/body. GAL treatment was carried out one day prior to administration of TPD at a dose indicated in figures. Results of control rats are expressed as mean of three experiments.

amount over a long period, and rat H showed the similar excretion to rat I up to 48 h after administration of TPD, but thereafter showed rapid increase of excretion. The amount excreted in the 0–72 h urine was slightly larger in rat G but much less in rats H and I than in the control

FIG. 4. Cumulative Excretion of MPS in Control (○) and GAL-Treated Rats (●)
For details see legend to Fig. 3.

TABLE I. Time Course of Effect of D-Galactosamine on Contents of Microsomal Protein and Cytochrome P-450, and Activity of Benzphetamine N-Demethylase

Time after administration (h)	Control (N=8)	24 (N=4)	48 (N=4)	72 (N=4)	96 (N=5)
Protein (mg/g liver)	21.0±1.88	20.4±3.62	17.1±4.16 ^{b)}	14.7±2.86 ^{a)}	18.5±2.93
Cytochrome P-450 (nmol/mg protein)	0.87±0.10	0.76±0.08	0.71±0.32	0.31±0.10 ^{a)}	0.49±0.18 ^{a)}
Benzphetamine N-demethylase (units/mg protein)	8.61±1.29	10.23±0.94	5.21±3.39 ^{b)}	3.29±2.18 ^{a)}	4.87±1.58 ^{a)}

Rats were treated with D-galactosamine (i.p., 1000 mg/kg) and then sacrificed at the time indicated at the top of column. Results are expressed as mean ± S.D. N represents the number of rats used. A unit was defined as 1 nmol of HCHO formed per min. a, b) Values are significantly different from the control (a) $p < 0.01$, b) $p < 0.05$.

rats. Comparing with the amount excreted in the 0–48 h urine, even rat G excreted less than the control rats who already excreted almost all 2HPMS. For excretion of MPS, two types of excretion profiles were observed. One was observed in rats I and G. They excreted more amount over a longer period than the control rats did. The other was observed in rat H, excreting less amount in the first 36 h urine than in the control rats, and excreting rapidly during the period of 36–72 h. The amount excreted in the 0–72 h urine increased by about two and four-fold in rats I and G, and in rat H, respectively.

When a dose of GAL was further increased to 1200 mg/kg, all rats showed apparent decrease in excretion of 2HPMS compared to the control rats (Fig. 3-3). Only one of three rats began rapid

excretion after 48 h but the others showed no sign of recovery of excretion. Alteration of excretion of 2HPMS was slightly more than that in 600 mg/kg treated rats, though the similarity was observed among 600 and 1200 mg/kg treated rats. In excretion of MPS, increase was more clear in two rats than that in 600 mg/kg treated rats, while one rat showed the similar excretion to the control rats (Fig. 4-3).

Effect of GAL on Microsomal Drug Metabolizing Enzymes

Enlargement of liver due to accumulation of fat was observed at 24 and 48 h after injection of GAL at a dose of 1000 mg/kg. Effect on protein content, cytochrome P-450 content and activity of benzphetamine *N*-demethylase was indicated in Table I. Protein was not reduced at 24 h, but

FIG. 5. *Histological Change of Liver induced by GAL ($\times 100$)*
a), after 24 h; b), after 48 h; c), after 72 h; d), after 96 h.

reduced to 81 and 70% of the control level at 48 and 72 h, respectively. These decreases were significant. At 96 h, protein content was recovered to 90% of the control level. Cytochrome P-450 content did not significantly differ at 24 and 48 h from the control level. However, at 72 and 96 h, cytochrome P-450 content was reduced to 36 and 56% of the control level, respectively. Activity of benzphetamine *N*-demethylase was altered in a similar manner as the change of cytochrome P-450 content.

Histological Change of Liver induced by GAL

The histological appearances of liver at 24, 48, 72 and 96 h after administration of GAL at a dose of 1000 mg/kg exhibited features not found in the control rats (Fig. 5). After 24 h, foci of necrosis were disseminated over the lobles and mild inflammatory cell infiltration was observed in both foci of necrosis and Glisson's sheath. Councilman bodies, eosinophilic cells and vacuolar degeneration in cells were also found in lobles. After 48 h, inflammatory cell infiltration further increased in foci of necrosis and much more in Glisson's sheath compared to those observed after 24 h. After 72 h, foci of necrosis were substituted by fibrotic tissue with inflammatory cell infiltration. Kupper cells were enlarged and increased in number, and Glisson's sheath was enlarged and elongated, and exhibited moderate inflammatory cell infiltration. After 96 h, alterations in Glisson's sheath further increased compared to those observed after 72 h. This feature resembled that in human chronic hepatitis.

DISCUSSION

As well as the patients with liver disease, the rats with liver injury induced by CCl₄ and GAL showed altered urinary excretion of TPD metabolites. Especially, decrease and delay of excretion of 2HPMS were commonly seen.

In CCl₄-treated rats, excretion of 2HPMS was markedly depressed throughout the experimental period, whereas that of MPS was increased after 12 or 24 h. This means that CCl₄ suppressed (ω -1)-hydroxylation of propyl moiety of MPS to form 2HPMS through liver injury. It is known

that liver injury caused by CCl₄ is most severe at 24 to 48 h after injection of CCl₄ and then starts to recover. Accordingly, the amount of 2HPMS in the first 24 h urine was markedly reduced in all CCl₄-treated rats. The considerable difference in the excretion of 2HPMS after 24 h among rats A and B, and rat C was probably due to the difference in the degree of recovery from injury state.

In GAL-treated rats, excretion of 2HPMS and MPS showed a similar tendency as in CCl₄-treated rats, and the degree of the alteration of excretion depended on the dose of GAL and the degree of liver damage. In comparison with CCl₄-treated rats, recovery of excretion of 2HPMS was more delayed in GAL-treated rats. Concerning this phenomenon, it was confirmed that liver damage induced by GAL (1000 mg/kg) continued for rather long period with histological changes.

From these findings, differences in excretion of TPD metabolites among CCl₄- and GAL-treated rats, and rats given different doses of GAL were considered to be due to differences in the severity of liver damage and the degree of recovery from liver injury. In rats with liver injury, TPD metabolism was impaired in proportion to the severity of liver damage so that the appearance of metabolites in urine was altered in the amount and metabolite composition.

Cytochrome P-450 content in GAL (1000 mg/kg)-treated rats was not significantly reduced at 24 and 48 h. However, excretion of 2HPMS was depressed even for this period. This result suggested that decrease of excretion of 2HPMS did not directly correlate to that of cytochrome P-450 content, but some changes occurred under liver injury might influence TPD metabolism.

In our previous paper,¹⁾ it was suggested that the measurement of 2HPMS in the 0–48 h urine after oral dose of TPD was a useful test for quantification of liver disease in humans. Results obtained in this paper supported this suggestion.

Acknowledgement We thank Miss Seiko Inoue for her excellent technical assistance. This work was supported in part by Grant-in-Aid for

Scientific Research from the Ministry of Education, Science and Culture, which are gratefully acknowledged.

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