Effects of Dietary Pantethine Levels on Drug-Metabolizing System in the Liver of Rats Orally Administered Varying Amounts of Autoxidized Linoleate

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Summary The effects of dietary pantethine levels on the drug-metabolizing system were investigated under administration of varying amounts of autoxidized linoleate (AL) with rat liver microsomes and S-9 fractions. AL having 800 meq/kg of peroxide value and 1,700 meq/kg of carbonyl value was dosed to the rats of each group given drinking water containing 0 mg% (deficient), 6.25 mg% (normal), and 125 mg% pantethine (sufficient). The contents and activities of the enzymes in the drug-metabolizing system in the rat liver of each pantethine-level group changed essentially in a similar manner, that is, they were induced at an AL daily dose of 0.2 ml/100 g body weight (i.e., small dose) for 5 successive days and lowered at a daily dose of 0.4 ml/100 g body weight (i.e., large dose) by the same administration period, compared with respective non-AL groups in each of the three pantethine levels. In both non-AL and the small-dose AL, enzyme activities of the electron transfer system in rat liver microsomes, aminopyrin-N-demethylase activity, and metabolic activation of 2-acetylaminofluorene in S-9 fractions were significantly higher in the pantethine-deficient group than in the pantethine-normal and -sufficient groups. In the large-dose AL, the enzyme activities in the drug-metabolizing system decreased significantly in any pantethine levels, though the survival rate of the rats was higher in the pantethine-sufficient group than in the pantethine-normal groups. The results suggest that the pantethine relieves the effect of dosed AL on the drug-metabolizing system in rat liver.

Key Words autoxidized linoleic acid, microsome, cytochrome P-450, cytochrome b₅, electron transfer system, drug-metabolizing activity, aminopyrin-N-demethylase, S-9 activity, pantethine
Pantethine, d-bis-(pantothenyl-β-aminoethyl) disulfide, is a dimer of pantetheine, which is a derivative of pantothenic acid and cysteamine, and forms a portion of the structure of the coenzyme A. Yoshikawa et al. found that the pantethine decreased the injury in rat liver caused by carbon tetrachloride (1). It has also been reported that pantethine administered to rats improved a dermatopathy developed by an in vivo lipid peroxide (2, 3), and decreased a cardiotoxicity produced by an administration of adriamycin (4). These facts suggest that the metabolite of pantethine may function as one of the scavengers against in vivo lipid peroxidation which occurs mainly at the biomembrane.

Since the enzymes of the drug-metabolizing system are also present in the biomembrane, especially in the electron transfer system of liver microsomes, it seems likely that the system is affected by both lipid peroxides yielded in vivo via metabolization of ingested xenobiotics and the endogenous scavengers against the lipid peroxides. From the standpoint of nutritional science, there are several reports (5–10) on the effects of xenobiotics (mainly polychlorinated biphenyl) and scavengers on the changes in the drug-metabolizing system and lipid peroxide formation in vivo. There have been few studies on the effect of autoxidized fatty acid, including the peroxides, on drug-metabolizing activity in rat liver microsomes. Since lipid peroxides occur in foods, and accumulated (11, 12) especially in the liver after ingestion of them, it is important to clarify the effect of autoxidized fatty acids on the drug-metabolizing system. Previously, we have investigated in detail the effects of dose levels and dose periods of AL on the drug-metabolizing system in rat liver (13, 14). Both the content of cytochrome P-450 and the activity of the drug-metabolizing system were increased by a small dose of AL, and were decreased by a large dose of AL (13), although the content and the activity were decreased by the elongation of the dose period even in the case of a small dose of AL (14).

This time, we plan to investigate the effects of dietary pantethine levels on the drug-metabolizing system in rat livers with both a small and a large dose of AL. Thus, in the present study, changes in cytochrome P-450 and b5 contents, the enzyme activities of the electron transfer system, and drug-metabolizing activities are determined with microsomes or postmitochondrial 9,000 × g supernatant (S-9 fraction) prepared from liver of male Wistar rats administered a small or a large amount of AL for 5 successive days.

MATERIALS AND METHODS

Chemicals. Linoleic acid was of extra-pure reagent grade from Nakarai Chemical Co. (Kyoto), and its purity as determined by gas chromatography was about 95%. NADPH and NADH were purchased from the Oriental Yeast Co. (Tokyo). Bovine serum albumin, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and cytochrome c from horse heart were obtained from Sigma Chemical Co. (USA). Nutrient broth and agar powder were purchased from Difco (USA). The other chemicals were of guaranteed reagent grade from Nakarai Chemical Co.
Animals and diet. Male Wistar rats, 3 weeks old and each weighing about 40 g (JCL, Tokyo, Japan), were divided into 9 groups (5 rats/group), and were housed at approximately 23°C with a light and dark cycle of 12 h each. They were fed for one month on the same diet (Clea Japan Inc., Tokyo) as described in the previous reports (13, 14). The components of the diet consisted of 24.5% vitamin free casein, 45.5% cornstarch, 10.0% granulated sugar, 6.0% corn oil, 3.0% avicel, 2.0% KC flock, 1.0% α-starch, 1.0% vitamin mixture without pantothenic acid, and 7.0% mineral mixture. The drinking water containing 0 mg%, 6.25 mg%, or 125 mg% pantethine was given to the rats, which were accordingly designated the pantethine-deficient, -normal, and -sufficient groups, respectively. The diet and drinking water were provided ad libitum.

Administration of AL. After the initial feeding for one month, AL prepared by the same way as previously described (13, 14) was orally administered to the rats of respective subgroups in each pantethine level at a daily dose of 0, 0.2, and 0.4 ml/100 g body weight for 5 successive days.

Preparation of S-9 and microsomes. The S-9 and microsomes were prepared from rat liver as noted previously (13, 14).

Measurements of the enzyme activities in electron transfer system. NADPH-cytochrome c reductase, NADH-ferricyanide reductase, and NADH-cytochrome c reductase activities were determined by the method of Omura and Takesue (15-17) as previously described (14).

Other measurement methods. Cytochrome P-450 and cytochrome b_5 contents were determined by the method of Omura and Sato (18) as previously described (13, 14). The metabolic activation of 2-AAF by S-9 fraction, which we termed S-9 activity, and aminopyrin-N-demethylase activity were determined according to the methods of Yahagi (19) and Kato et al. (8), respectively. The activities of GOT and GPT in serum were measured according to the method of Reitman and Frankel (20), using S. TA-Test Wako kit. Protein was determined by the procedure of Lowry et al. (21) with bovine serum albumin as the standard.

Statistical analysis. Student's t-test was used to determine statistical significance. The variability of the data is presented as mean ± SD.

RESULTS

Growth of rats and changes in body weight after AL administration

No difference between the pantethine-sufficient and -normal groups was observed in the growth of rats after the initial feeding for one month, but the growth of rats in these two groups was significantly different (p<0.001) from that of the pantethine-deficient group, as shown in Fig. 1. The pantethine-deficient rats showed not only bad growth but also roughness and loss of hair (Fig. 2), aging of skin, and significant rises of GOT and GPT (Table 1).
Then the rats of each group fed on the diets having three levels of pantethine were administered 0, 0.2, or 0.4 ml AL/100 g body weight/day for 5 successive days, respectively. With the small-dose administration of AL for 5 successive days, the body weight of rats in each group of all the pantethine levels kept increasing similarly to the respective non-AL groups. With the large-dose administration of AL for 5 successive days, the body weight in each group of the pantethine levels kept decreasing in contrast with the respective non-AL and the small-dose AL groups. In the pantethine-normal group, three rats died by large-dose AL adminis-
Table 1. The activities of GOT and GPT in serum after the initial feeding for one month.

<table>
<thead>
<tr>
<th>Pantethine level</th>
<th>GOT</th>
<th>GPT</th>
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<tbody>
<tr>
<td>Deficient</td>
<td>148.5 ± 19.0&lt;sup&gt;a,b&lt;/sup&gt; (n = 7)</td>
<td>29.4 ± 5.6&lt;sup&gt;a,b&lt;/sup&gt; (n = 7)</td>
</tr>
<tr>
<td>Normal</td>
<td>108.0 ± 7.2   (n = 4)</td>
<td>20.1 ± 2.2     (n = 7)</td>
</tr>
<tr>
<td>Sufficient</td>
<td>102.6 ± 5.6   (n = 6)</td>
<td>17.1 ± 2.6     (n = 7)</td>
</tr>
</tbody>
</table>

Values are mean ± SD. <sup>a</sup>Significant difference from the pantethine-normal diet at 0.001 < p < 0.005. <sup>b</sup>Significant difference from pantethine-sufficient diet at p < 0.001.

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Fig. 3. Changes in cytochrome P-450 content after AL administration. □, Non-AL; □, 0.2 ml AL/100 g body weight for 5 successive days; □, 0.4 ml AL/100 g body weight for 5 successive days.

Fig. 4. Changes in cytochrome b<sub>5</sub> content after AL administration. □, Non-AL; □, 0.2 ml AL/100 g body weight for 5 successive days; □, 0.4 ml AL/100 g body weight for 5 successive days.

tered for 4 days, but all the rats in the pantethine-sufficient group were active even under the same condition.

Changes in cytochrome P-450 and cytochrome b<sub>5</sub> contents

The change in cytochrome P-450 content is shown in Fig. 3. The contents of cytochrome P-450 in all the pantethine groups were increased by the small-dose AL, and were decreased by the large-dose AL. In the large-dose AL, the cytochrome P-450 content in the pantethine-sufficient group showed a higher level than that of the pantethine-normal group, though it was not significantly different.

The change in cytochrome b<sub>5</sub> content is shown in Fig. 4. The contents of
cytochrome $b_5$ in all groups of any pantethine level were also increased by the small-dose AL, and were also decreased by the large-dose AL. However, unlike cytochrome P-450, a significant decrease of cytochrome $b_5$ content in each group of any pantethine level was not observed even by the large-dose AL in comparison with non-AL and the small-dose AL. This stability in the change of cytochrome $b_5$ content was also observed in previous experiments (13, 14).

Changes in enzyme activities of electron transfer system

The increase of enzyme activities by the small-dose AL and the decrease of enzyme activities by the large-dose AL were more or less observed in the enzymes of the electron transfer system in each group of all the pantethine levels (Figs. 5, 6 and 7).

The activity of NADPH-cyt.c reductase in the pantethine-deficient non-AL group was significantly (0.01 < $p$ < 0.025) higher than that of the other non-AL groups, and the level of increase of the enzyme activity by the small-dose AL in the pantethine-deficient group was also significantly higher than those of the pantethine-normal ($p < 0.001$) and -sufficient ($0.001 < p < 0.005$) groups.

The activity of NADH-cyt.c reductase in the pantethine-deficient non-AL group was significantly higher than that of the normal group ($0.01 < p < 0.025$), and the increase of the enzyme by the small-dose AL in the pantethine-deficient group was also significantly ($p < 0.001$) higher than that of the other groups.

No difference in the NADH-ferricyanide reductase activities by non-AL was observed between all the groups of any pantethine level. In the small-dose administration of AL, the enzyme activity in the pantethine-deficient group showed a higher level than that of both the pantethine-normal and -sufficient groups, but it was not significantly different.

Changes in drug-metabolizing enzyme activity

The drug-metabolizing enzyme activities in all the groups of any pantethine level were increased by the small-dose AL, and were decreased by the large-dose AL, as shown in Figs. 8 and 9.

No difference of the aminopyrin-N-demethylase activities by non-AL was observed between all the groups of any pantethine level. The enzyme activity in the pantethine-deficient group showed a higher level than that in the pantethine-normal group, and the enzyme activities in the pantethine-deficient and -normal groups were significantly ($0.01 < p < 0.025$) higher than that in the pantethine-sufficient group, which was the same level as that of non-AL groups in all the pantethine levels.

In non-AL, the S-9 activity in the pantethine-deficient group was significantly higher than that of the pantethine-normal ($0.01 < p < 0.025$) and -sufficient ($0.001 < p < 0.005$) groups. In the small-dose AL, the activity in the pantethine-deficient group rose significantly ($0.001 < p < 0.005$) compared with that of the pantethine-normal and -sufficient groups. In the large-dose AL, the activity in the

Fig. 5. Changes in NADPH-cyt. c reductase in electron transfer system after AL administration. □, Non-AL; ☐, 0.2 ml AL/100 g body weight for 5 successive days; ☒, 0.4 ml AL/100 g body weight for 5 successive days.

Fig. 6. Changes in NADH-cyt. c reductase in electron transfer system after AL administration. □, Non-AL; ☐, 0.2 ml AL/100 g body weight for 5 successive days; ☒, 0.4 ml AL/100 g body weight for 5 successive days.

Fig. 7. Changes in NADH-ferricyanide reductase in electron transfer system after AL administration. □, Non-AL; ☐, 0.2 ml AL/100 g body weight for 5 successive days; ☒, 0.4 ml AL/100 g body weight for 5 successive days.
DISCUSSION

We have previously investigated the effects of both the dose period (13) and the dose levels (14) of AL on the drug-metabolizing system in the liver of rats fed on a diet containing a normal amount of pantethine. The characteristic changes of cytochrome P-450 and $b_5$ contents, the enzyme activities of the electron transfer system, and the activities of drug-metabolizing enzymes were discussed in detail in the previous papers (13, 14).

In the present study, we also recognized in any pantethine level that a small pantethine-deficient group was significantly ($0.001 < p < 0.005$) higher than that of the pantethine-normal and -sufficient groups.
dose of AL increased the contents and the activities of drug-metabolizing enzymes, and that a large dose of AL decreased them. Therefore, it has become clear that the effects of AL dose levels on the drug-metabolizing system change essentially in the same way, in any pantethine levels.

In this paper, we focus on and discuss the difference in induction levels of the drug-metabolizing systems between pantethine-deficient, -normal, and -sufficient groups. No difference was observed in the contents of cytochrome P-450 and $b_5$ both by non-AL and by a small dose of AL in any pantethine level (Figs. 3 and 4). However, even under the condition of non-AL, the activities of NADPH-cyt.c reductase and NADH-cyt.c reductase rose in the pantethine-deficient group rather than in the pantethine-normal and -sufficient groups (Figs. 5 and 6). Their induction levels in the pantethine-deficient group, moreover, rose by a small dose of AL in comparison with the other groups. The reason that, in spite of no difference in cytochrome P-450 content, the drug-metabolizing activities rose in the pantethine-deficient group rather than in the other groups might have been attributable to the rise of these enzyme activities in the electron transfer system.

As mentioned in the introduction, it has been reported (1–4) that pantethine has an antioxidative effect against lipid peroxide in vivo. Although pantethine per se does not have a reducing property, it seems reasonable that the absorbed pantethine would be reduced to pantetheine via the redox system in vivo, and the resultant pantetheine would have depressed lipid peroxidation in vivo by the reducing property originating from its sulfhydryl group. In the rats fed on a diet deficient in both pantothenic acid and its related compounds, because of the decrease of radical scavengers, the disposition of active oxygen or lipid peroxide yielded from the in vivo redox system would not be able to proceed smoothly, and thereby in vivo lipid peroxidation would be more accelerated. The rise of drug-metabolizing activity in the pantethine-deficient non-AL group may be a reasonable response of the system against in vivo elevated accumulation of lipid peroxide. Presumably, symptoms such as alopecia or scleroderma observed in the pantethine-deficient non-AL group would appear as results of in vivo lipid peroxidation. The drug-metabolizing activities in the pantethine-deficient group increased more by a small dose of AL in comparison with those of the other groups. These experimental facts suggest that, in an AL dose level of the extent that the body weight did not decrease, the less the scavenger such as pantetheine, the more the drug-metabolizing activities rise.

A large dose of AL decreased the drug-metabolizing activity in any pantethine level. In this experiment, the rats in all the pantethine levels were severely damaged by a large dose of AL. Under such a drastic condition, it was difficult to observe the difference of reduction in the drug-metabolizing system because the system was injured markedly in any pantethine level. However, the survival period of the rats in the pantethine-sufficient group was longer than that of the pantethine-normal group, and the cytochrome P-450 content of the pantethine-sufficient group showed a higher level than that of the pantethine-normal group. In addition, we also found in the preliminary experiments that the contents and the activities of enzymes of the
drug-metabolizing system in the liver of rats administered AL were well maintained in the pantethine-sufficient level diet group rather than in normal level diet group, when male Wistar rats were fed on commercial feeds (Clea Japan Inc., Tokyo, type CE-2 which contains 3.0 mg pantothenic acid per 100 g diet) and drinking water containing 0 mg/\%(normal level diet) or 125 mg/\% pantethine (sufficient level diet). These observations suggested that the pantethine-sufficient diet may effectively protect the drug-metabolizing system from damage by AL.

In conclusion, it seems likely that pantethine relieves the effect of both a small dose of AL and a large dose of AL on the drug-metabolizing system in rat liver. Further investigations will be necessary to clarify the mechanism of these effects.

REFERENCES


