Relationship between Hepatic Phosphoglucomutase Activity and Oxidative Stress Caused by Dietary Products of Lipid Peroxidation

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Lipid peroxidation products in the diet induce oxidative stress. A hepatic enzyme inactivated in proportion to the oxidative stress was found. The oxidative stress was evaluated by a decrease in the tocopherol level and increases in contents of lipid peroxides and thiobarbituric acid-reactive substances in the serum and liver, when secondary peroxidation products of linoleic acid were fed to rats. The changes in activities of mitochondrial NAD-dependent aldehyde dehydrogenase, succinate dehydrogenase, phosphoglucomutase, and glucose-6-phosphate dehydrogenase in the liver, the activities of which are decreased after the oral dose of lipid peroxidation products, were measured. A marked decrease in the phosphoglucomutase activity was closely related to the increase in oxidative stress.

Products of lipid peroxidation are toxic, but occur in our daily foods. An intake of peroxidation products gives the living body oxidative stress, and causes hepatic dysfunction.¹⁾ We are interested in which enzymatic activities in the liver are closely related to this oxidative stress.

Primary products of the lipid peroxidation, hydroperoxides, may be the most toxic among the peroxidation products, but hydroperoxides in the diet are scarcely incorporated into the body without changing form, because they are decomposed in the digestive tract.²⁾ On the contrary, some parts of secondary peroxidation products are incorporated. When secondary products were administered orally to rats, hexanal and 9-oxononanoic acid, which are the major components in secondary products,³⁾ were detected in the liver.⁴⁾ The dose of secondary products kept thiobarbituric acid (TBA)-reactive substances at a high level in the liver even after disappearance of the products,⁵⁾ and increased the activity of hepatic glutathione peroxidase, which is a detoxifying enzyme for endogenous lipid peroxides.⁶⁾ Therefore, the oxidative stress caused by

dietary products of lipid peroxidation is considered to be due to the components in secondary products such as aldehydes.

Hepatic dysfunctions caused by secondary products in the diet can be summarized as specific inactivations of mitochondrial NAD-dependent aldehyde dehydrogenase⁶⁾ and succinate dehydrogenase,⁷⁾ and depletions of glucose 6-phosphate,⁸⁾ NADPH,⁹⁾ and CoA.¹⁰⁾ The depletion of glucose 6-phosphate was due to the decreases in activities of the synthetic enzymes, phosphoglucomutase and glucokinase.⁸⁾ The depletion was considered to be contributed to more seriously by the decrease in phosphoglucomutase activity, since the substrate, glucose 1-phosphate, was markedly accumulated in the liver.⁷⁾ The depletion of NADPH was partly due to reduction in the supplement from the pentose phosphate cycle, because an activity of the rate-limiting enzyme, glucose-6-phosphate dehydrogenase, was decreased.⁸⁾ SH-compounds such as CoA has been reported to be decomposed by aldehydes,^{11,12)} which come from the secondary products. Thus, the hepatic dysfunction is complicated with decreases in activities of four

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hepatic enzymes, aldehyde dehydrogenase, succinate dehydrogenase, phosphoglucumutase, and glucose-6-phosphate dehydrogenase.

In this study, the correlations between oxidative stress and decreases in activities of the four hepatic enzymes were investigated over the course of four days after secondary products of linoleic acid were administered orally to rats. The close correlation was detected between the decrease in phosphoglucumutase activity and oxidative stress.

Materials and Methods

Secondary peroxidation products of linoleic acid. Linoleic acid (Tokyo Kasei Kogyo Co., Ltd.) was autoxidized at 37°C for 7 days (the peroxide value was about 1600 meq/kg, and the carbonyl value was 480 meq/kg). Secondary peroxidation products were prepared from the autoxidized linoleic acid by silica-gel column and thin-layer chromatographies (the recovery was 33% by weight), and they were characterized as previously described in detail.³⁾ The secondary product fraction consisted of 36% mixture of polymers, 26% epoxyhydroperoxides or endoperoxides, 4.8% 9-oxononanoic acid, 3.7% hexanal, 2.5% nonanedioic acid, 2.4% short-chain carboxylic acids, 0.75% 8-oxooctanoic acid, 0.34% 12-oxododecadienoic acid, and other smaller unidentified compounds. Hydroxy alkenals were also included, and the amounts were estimated roughly to be less than 1%.

Treatment of animals. Male Wistar rats, aged 5 weeks and weighing about 110 g (KY, SPF: Japan SLC Inc.) were fed for 1 week on a fresh diet (containing 3.9 mg tocopherols/100 g) that has been described previously.¹⁰⁾ The rats were separated into 2 groups of 42 rats each. One group of the rats was given 400 mg/rat of linoleic acid orally to serve as controls and the other was given 400 mg/rat of secondary products. Six animals in each group were killed periodically (after 0, 6, 15, 24, 48, 72, and 96 hr). Blood was collected into plastic tubes and the liver was removed. Serum was obtained by centrifugation of blood at 3,000 rpm for 5 min. The liver was homogenized with 10 volumes of a 1.15% solution (w/v) of KCl.

Measurement of oxidative stress. Tocopherol levels in serum and liver homogenate were measured by the method of Taylor *et al.*¹³⁾ Serum and hepatic lipid peroxidation were evaluated with TBA¹⁴⁾ and hemoglobin-methylene blue (HMB) tests.¹⁵⁾ The results of the TBA and HMB tests are expressed as neq of malonaldehyde and as nmol of linoleic acid hydroperoxide, respectively.

Enzymatic analyses. The liver homogenate was cen-

trifuged at $700 \times g$ for 10 min, and the supernatant was recentrifuged at $5,000 \times g$ for 20 min. The pellet was suspended in the original volume of the KCl solution and centrifuged again at the same g . The pellet was suspended in KCl solution and used as the mitochondrial fraction. This supernatant was further centrifuged at $105,000 \times g$ for 60 min and the supernatant is referred to as the cytosolic fraction. The activities of mitochondrial NAD-dependent aldehyde dehydrogenase and succinate dehydrogenase were measured by the methods of Black¹⁶⁾ and Veeger *et al.*,¹⁷⁾ respectively. The activities of cytosolic phosphoglucumutase and glucose-6-phosphate dehydrogenase were measured by the methods of Bergmeyer *et al.*¹⁸⁾ and Glock and McLean,¹⁹⁾ respectively. The protein concentration was measured by the method of Lowry *et al.*²⁰⁾

Statistical analysis. When the *F*-test for homogeneity of variance showed that variances were heterogeneous, Student's *t*-test was used to determine the statistical significance, and a probability level of 0.05 was chosen.

Results

The rats given linoleic acid or secondary products orally showed no abnormalities

Fig. 1. Changes in Tocopherol Level (A), and HMB (B) and TBA (C) Values in Serum Caused by an Oral Dose of Secondary Peroxidation Products of Linoleic Acid (●), Compared to Those Caused by a Dose of Linoleic Acid (○) as Controls.

Asterisks show significant differences between corresponding values.

macroscopically such as diarrhea, hemorrhage, or necrosis.

Then the induction of oxidative stress caused by dietary secondary products was measured by the reduction in levels of tocopherol, and increases in amounts of lipid peroxides and TBA-reactive substances in the serum and liver of the rats. It is generally known that the tocopherol levels reflect oxidative stress in the living body since tocopherols are consumed to protect from endogenous lipid peroxidation. The HMB test can estimate the contents of lipid peroxides, and the TBA test can be complicated by both amounts of lipid peroxides and aldehydes that originate endogenously from lipid peroxides.²¹⁾

Figure 1 shows the changes with time in serum levels of tocopherol, of lipid peroxides, and of aldehydes after rats were orally administered linoleic acid or its secondary products. In the control group, given linoleic acid, these values remained unchanged with

time. In the group given secondary products, the level of tocopherol decreased significantly between 6 and 24 hr after the dose and recovered to the normal level 48 hr after the dose (Fig. 1-A). The HMB value in serum was increased 15 hr after the dose of secondary products, reached a maximum at 24 hr, and returned to the normal level 72 hr after the dose (Fig. 1-B). The TBA value was also elevated 15 hr after the dose, remained at a high level between 24 and 72 hr, and returned to the normal level 96 hr after the dose (Fig. 1-C). Figure 2 shows the changes in these parameters in the liver. The reduction in tocopherol level appeared later than that in the serum, at 15 hr

Fig. 2. Changes in Tocopherol Level (A), and HMB (B) and TBA (C) Values in Liver Caused by an Oral Dose of Secondary Peroxidation Products of Linoleic Acid (●), Compared to Those Caused by a Dose of Linoleic Acid (○) as Controls.

Asterisks show significant differences between corresponding values.

Fig. 3. Changes in Activities of Hepatic Enzymes between Corresponding Caused by an Oral Dose of Secondary Peroxidation Products of Linoleic Acid (●), Compared to Those by the Dose of Linoleic Acid (○) as Controls.

Asterisks show significant differences between corresponding values.

after the dose (Fig. 2-A). The increase in the HMB value in the liver was detected earlier than that in the serum (Fig. 2-B), but the change in the TBA value was similar to that in the serum (Fig. 2-C). Thus, the changes in levels of tocopherol were the mirror image of those in the HMB value in both serum and liver. The changes in TBA values were delayed a little as compared to the changes in the other parameters.

Figure 3 shows the changes in enzymatic activities in terms of the dose of linoleic acid or secondary products. In the group with linoleic acid as a control, the enzymatic activities did not change significantly with time, and were in the same range as those in the rats dosed with saline solution as shown previously.⁶⁻⁹ In contrast with the dose of secondary products phosphoglucomutase activity was reduced 6 hr after the dose, reached a minimum between 24 and 48 hr, and then recovered 72 hr after the dose. Glucose-6-phosphate dehydrogenase activity was reduced similarly to phosphoglucomutase activity, but it recovered 96 hr after the dose. The activity of succinate dehydrogenase was low for a short period between 6 and 15 hr after the dose, and

that of aldehyde dehydrogenase was low between 15 and 24 hr after the dose.

To identify the correlations between oxidative stress and the changes in enzymatic activities, the enzymatic activities from the group given secondary products were plotted against the corresponding values of tocopherol, HMB, or TBA, and the correlation coefficients were calculated (Table I). The changes in phosphoglucomutase activity were closely correlated with both serum and hepatic parameters for oxidative stress, *i.e.* with serum tocopherol levels (coefficient: 0.875), hepatic tocopherol levels (0.949), serum HMB values (-0.877), hepatic HMB values (-0.993), serum TBA values (-0.957), and hepatic TBA values (-0.850). The changes in aldehyde dehydrogenase activity gave similar correlations. Glucose-6-phosphate dehydrogenase activity was closely correlated with serum and hepatic TBA values. Succinate dehydrogenase activity was not obviously related to the oxidative stress.

Discussion

This study demonstrates that the decrease in hepatic phosphoglucomutase activity closely correlated with the oxidative stress caused by dietary products of lipid peroxidation. The decrease in aldehyde dehydrogenase activity also gave similar correlation. However, the significant changes in phosphoglucomutase activity lasted for a longer period than those in aldehyde dehydrogenase activity (Fig. 3). It is, therefore, apparent that phosphoglucomutase activity might be useful as a marker of oxidative stress. The values for hepatic phosphoglucomutase activity of between 70.0 and 83.5 nmol/min/mg protein, obtained here from the group given linoleic acid, were normal and values below 62.7 nmol/min/mg protein were indicative of oxidative stress in the rats.

In Fig. 2, the peak of TBA value appeared later than the peak of HMB in the liver. The HMB test is more specific for lipid peroxides than the TBA test.²¹ The TBA test can also detect lipid peroxides, but is more specific for

Table I. CORRELATION COEFFICIENTS BETWEEN PARAMETERS FOR OXIDATIVE STRESS AND ACTIVITIES OF HEPATIC ENZYMES^a

Enzyme	Oxidative parameter		
	Serum	HMB	TBA
	tocopherol	HMB	TBA
Phosphoglucomutase	0.875	-0.877	-0.957
	0.949	-0.993	-0.850
Aldehyde	0.906	-0.776	-0.858
dehydrogenase	0.868	-0.883	-0.805
Glucose-6-phosphate	0.564	-0.749	-0.859
dehydrogenase	0.806	-0.591	-0.893
Succinate	0.606	-0.432	-0.468
dehydrogenase	0.395	-0.483	-0.457

^a The enzymatic activities of Fig. 3 were plotted against the corresponding values of Fig. 1 or 2, and then the correlation coefficients were calculated by linear regression from their 42 analyses each.

aldehydes than for peroxides.²²⁾ We therefore considered that endogenous lipid peroxides were produced, and then some of them were detoxified with time²³⁾ and the rest was decomposed mainly to aldehydes, which were accumulated in the liver.²¹⁾ The decrease in phosphoglucomutase activity correlated with both the HMB and TBA values, but the change in glucose-6-phosphate dehydrogenase activity was related only to the TBA value (Table I). It seems likely that phosphoglucomutase was inactivated following the production of endogenous lipid peroxides and the accumulation of aldehydes, and glucose-6-phosphate dehydrogenase was injured specifically by aldehydes accumulated in the liver.

We have also detected decreases in hepatic phosphoglucomutase activity after intraperitoneal doses of pro-oxidative drugs, carbon tetrachloride, paraquat, phenobarbital, and benzo[a]pyrene, which induce the peroxidation of endogenous lipids.²⁴⁾ Moreover, the enzymatic activity was decreased in response to intraperitoneal doses of methyl linoleate hydroperoxides, its secondary products, hydrogen peroxide, or methyl 9-oxononanoate.²⁴⁾ These results indicate that the enzyme is also inactivated by endogenous peroxidation products.

On the other hand, 20 $\mu\text{g}/\text{mg}$ protein of methyl linoleate hydroperoxides added to hepatic cytosol *in vitro* inactivated the enzyme by 20%, but 20 μg of the secondary products did not.²⁵⁾ It is suggested that hepatic phosphoglucomutase is inactivated by lipid peroxides, not by aldehydes, which are the major components in secondary products. However, because it is difficult to imagine that such a large amount of lipid peroxides (20 $\mu\text{g}/\text{mg}$ protein) could be accumulated in the liver,^{21,26,27)} we have doubts about this suggestion and are studying the inactivation mechanism further.

In regard to the inactivation mechanism of hepatic phosphoglucomutase, there are three ideas; (a) the enzymatic activity was regulated *in vivo* with accumulation of bisphosphate compounds of glycolytic intermediates,²⁸⁾ or

by the reduction in activity of glycogen phosphorylase, which is a rate limiting enzyme in glycogenolysis. (b) An injury to biomembranes caused by peroxidation products resulted in leakage of intracellular phosphoglucomutase. (c) The synthetic system of the enzyme was disturbed by peroxidation products. When secondary products were given orally to rats, no accumulation of bisphosphate compounds or change in activity of glycogen phosphorylase was detected,⁸⁾ and no leakage of phosphoglucomutase into blood was observed (data not shown). Therefore, the ideas (a) and (b) can be neglected. Then, we have been investigating the possible mechanism remaining, (c), using parenchymal hepatocytes. The current results suggest that peroxidation products can inhibit the induction of phosphoglucomutase by dexamethasone.

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