

Effect of Phospholipid on Lipid Metabolism in Experimental Fatty Liver

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(Received November 20, 1974)

Effect of phospholipid on fatty liver induced by orotic acid, carbon tetrachloride, or ethionine has been studied. Results are summarized as follows:

1. Orotic acid induced a marked accumulation of liver glycerides, and a decreased concentration of liver phospholipid and protein, and of serum beta-lipoprotein. These lesions were restored by administration of phospholipid.

2. In rats given carbon tetrachloride or ethionine, liver lipid accumulation and a decreased concentration of serum beta-lipoprotein were partially restored by administration of phospholipid, but a decreased protein content in the liver was unaffected.

These results indicate that administration of phospholipid is effective in counteracting the depression of serum beta-lipoprotein and liver lipid accumulation by orotic acid, carbon tetrachloride, or ethionine. Phospholipid appears to have an important role in normal release of beta-lipoprotein.

Fatty liver can be induced in rats by the administration of a large variety of chemicals.^{2,3)} It has been shown that, despite the multiplicity of etiologic factor, formation of fatty liver is related to one or a few basic and general mechanisms.⁴⁻⁷⁾ In the case of fatty liver induced by carbon tetrachloride⁸⁻¹²⁾ or ethionine,¹³⁻¹⁸⁾ as well as those due to puromycin^{19,20)} or white phosphorus intoxication,²¹⁾ the primary lesion appears to be an inhibition in the synthesis of protein moiety of beta-lipoprotein. Similarly, fatty liver induced by orotic acid seems to result from a block in the release of beta-lipoprotein from the liver.^{22,23)} However, our previous results²⁴⁾ and others²⁵⁾ have shown that orotic acid has little or no effect on the incorporation

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of ^{14}C -labeled amino acid into liver protein. Roheim, *et al.* found that serum apoprotein ($d > 1.21$), which combined with lipids in the liver to form betalipoprotein,²⁶⁾ was unaffected by orotic acid.^{27,28)} They suggested that a failure in the binding of apoprotein with lipids in rats given orotic acid produces lipid accumulation in the liver. Previously, it was found that the incorporation of methionine[methyl- ^{14}C] into liver phosphatidylcholine and serum beta-lipoprotein was significantly decreased by orotic acid²⁴⁾ and that a decreased release of beta-lipoprotein in rats given orotic acid was restored by the addition of phosphatidylcholine into the incubation medium. These findings indicate that impaired phosphatidylcholine synthesis may be responsible for a decreased release of beta-lipoprotein and, as a result induce lipid accumulation in the liver. If so, administration of phospholipid may cure orotic acid-induced fatty liver. The purpose of this study was to determine if phospholipid has the ability to restore fatty liver induced by orotic acid, ethionine, and carbon tetrachloride.

Experimental

Rats used in all the experiments were females (180 to 210 g) of Wistar strain and were supplied with food and water freely. In the initial study, rats were fed a semisynthetic diet (18 per cent casein, 72.8 per cent sucrose, 2.0 per cent corn oil, 2.2 per cent vitamin mixture, and 5.0 per cent salts.)²⁹⁾ for 2 days. To these diet 1 per cent orotic acid was added during the next 7 days. Intact rats continued on the indicated diet. Rats in both groups were injected intraperitoneally, daily, with 0.4 ml of 0.9 per cent sodium chloride solution containing 40 mg of ox brain phospholipid throughout the experimental period. At the end of this period, animals were sacrificed by heart puncture under ether anesthesia.

Liver lipids were extracted once with 80 per cent ethanol once with 100 per cent ethanol, twice with chloroform-ethanol mixture (1:1, by vol.), and once with ether. Glycerides were hydrolyzed in 0.5 N alcoholic potassium hydroxide for 30 min at 70° and liberated glycerol was enzymically determined by the method of Eggstein.³⁰⁾ Lipid phosphorus was estimated by the method of Chen, *et al.*³¹⁾ after digestion with perchloric acid. Protein was determined according to Lowry, *et al.*³²⁾ Serum beta-lipoprotein was separated according to precipitation method with mepesulfate (sodium salts of sulfated methyl polygalacturonate methyl glycoside) as described by Florsheim, *et al.*³³⁾ Lipids in serum beta-lipoprotein were extracted by the procedure of Folch, *et al.*³⁴⁾ Analysis of phospholipid and protein was carried out as described above.

In the following experiment, female rats were fasted for 12 hr before receiving carbon tetrachloride or ethionine. Ethionine was given intraperitoneally with 100 mg of ethionine in saline solution (25 mg/ml) per 100 g body weight in four divided doses at 0, 3, 6, and 9 hr. Carbon tetrachloride intoxication was produced by forced feeding of the mixture of carbon tetrachloride and sesame oil (1:1, by vol.) at a dose of 0.5 ml of the mixture per 100 g body weight. In carbon tetrachloride- and ethionine-treated groups 40 mg of ox brain phospholipid was given intraperitoneally, 3 times at 6, 24, and 48 hr before rats were killed. The animals were sacrificed 24 hr after treatment with carbon tetrachloride or the initial administration of ethionine. Liver lipids and protein, and serum beta-lipoprotein were determined as described above.

Results

Effect of Phospholipid on Orotic Acid-induced Fatty Liver

When rats were fed a semisynthetic diet supplemented with 1 per cent orotic acid, liver glycerides increased 523 per cent of that in intact rats (Fig. 1). This glyceride accumulation in the liver was restored by the administration of phospholipid. Administration of phos-

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phospholipid had no effect on liver glyceride content in intact rats. The data in Fig. 2 indicate that the content of liver phospholipid and protein in rats given orotic acid was depressed to 65.1 and 70.2 per cent of that in intact rats, respectively, and this depression was also restored by the administration of phospholipid. As shown in Fig. 3, low level of serum beta-lipoprotein were found in rats given orotic acid. As in liver lipids and protein, administration of phospholipid restored the depression of serum beta-lipoprotein. In intact rats, the concentration of serum beta-lipoprotein was not changed by the administration of phospholipid.

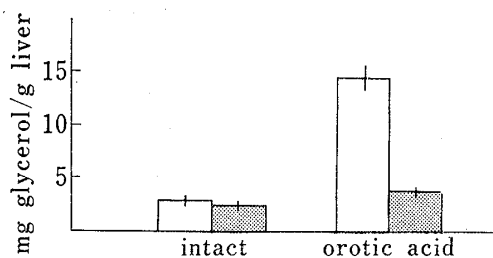


Fig. 1. Effect of Phospholipid on Liver Glyceride Content in Orotic Acid-fed Rats

Histogram shows the content of liver glycerides in control and phospholipid-treated rats. Vertical bar represent the mean \pm standard error.

Rats were fed semisynthetic diet with orotic acid (orotic acid) for 7 days, while intact rats were fed semisynthetic diet alone (intact).

□: control ▨: phospholipid

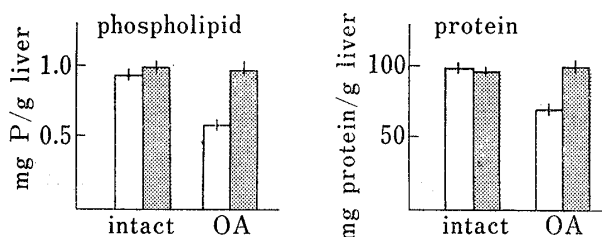


Fig. 2. Effect of Phospholipid on Liver Phospholipid and Protein Content in Orotic Acid-fed Rats

Histogram shows the content of liver phospholipid and protein in control and phospholipid-treated rats. Vertical bar represents the mean \pm standard error. For experimental condition, see the legend to Fig. 1. Orotic acid (OA) was added where indicated at a level of 1 per cent in the diet.

□: control ▨: phospholipid

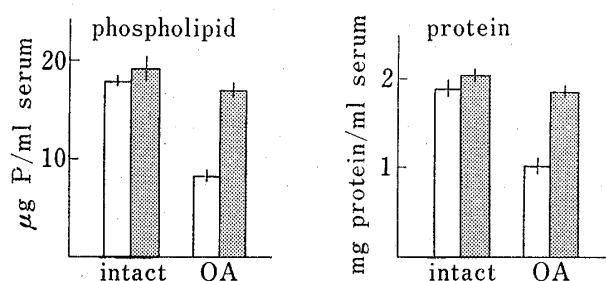


Fig. 3. Effect of Phospholipid on Serum Beta-lipoprotein in Orotic Acid-fed Rats

Histogram shows the concentration of serum beta-lipoprotein in control and phospholipid treated rats. Vertical bar represents the mean \pm standard error.

Rats were fed semisynthetic diet alone (intact) and orotic acid-supplemented diet (OA).

□: control ▨: phospholipid

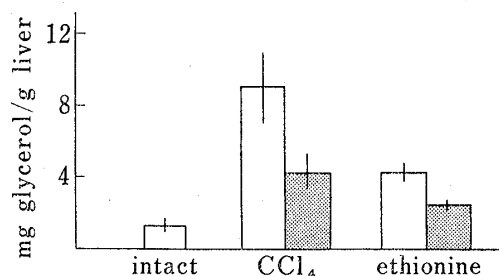


Fig. 4. Effect of Phospholipid on Liver Glyceride Content in Rats Given Carbon Tetrachloride or Ethionine

Histogram shows the content of liver glycerides in control and phospholipid treated rats. Vertical bar represents the mean \pm standard error.

Carbon tetrachloride was given orally with 0.5 ml of carbon tetrachloride-sesame oil(1:1) per 100 g body weight (CCl₄), while ethionine (100 mg/100 g body wt.) was given intraperitoneally in four divided doses at 0, 3, 6, and 9 hr (ethionine). At 24 hr the animals were killed by bleeding from the heart.

□: control ▨: phospholipid

Effect of Phospholipid on Carbon Tetrachloride- and Ethionine-induced Fatty Liver

Liver glycerides in rats given carbon tetrachloride or ethionine were increased about seven- and three-fold, respectively, as compared with that in intact rats (Fig. 4). Administration of phospholipid appears to restore the liver glyceride accumulation in rats given carbon tetrachloride, but a significant difference was not observed. On the other hand, liver lipid accumulation in rats given ethionine was partially restored by the administration of phospholipid. The content of liver phospholipid in rats given carbon tetrachloride was slightly decreased, but administration of phospholipid had no effect (Fig. 5). In rats given ethionine,

the content of phospholipid was the same as that in intact rats. The content of liver protein was decreased in both rats given carbon tetrachloride and ethionine (Fig. 5). This depression was unaffected by the administration of phospholipid in both groups. These results differ from the case of orotic acid-induced fatty liver (Fig. 2). The concentration of serum beta-lipoprotein in rats given carbon tetrachloride or ethionine was also decreased (Fig. 6). The depression of phospholipid moiety of beta-lipoprotein in rats given ethionine and protein moiety in both groups were restored by the administration of phospholipid, but the depression of phospholipid moiety in rats given carbon tetrachloride was not changed.

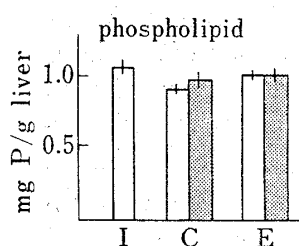


Fig. 5. Effect of Phospholipid on Liver Phospholipid and Protein in Rats Given Carbon Tetrachloride or Ethionine

Histogram shows the content of liver phospholipid and protein in control and phospholipid treated rats. Vertical bar represents the mean \pm standard error.

Rats were administered: none (I: intact), carbon tetrachloride (C), or ethionine (E).

□: control ▨: phospholipid

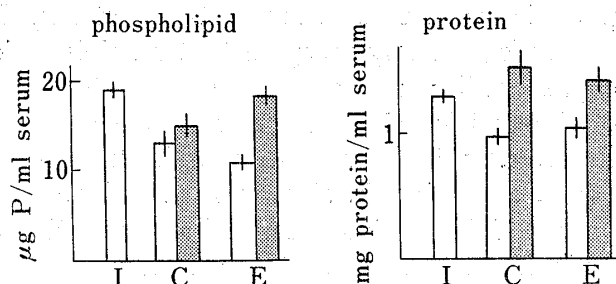


Fig. 6. Effect of Phospholipid on Serum Beta-lipoprotein in Rats Given Carbon Tetrachloride or Ethionine

Histogram shows the concentration of serum beta-lipoprotein in control and phospholipid treated rats. Vertical bar represents the mean \pm standard error.

Rats were administered: none (I: intact), carbon tetrachloride (C), or ethionine (E).

□: control ▨: phospholipid

Discussion

The present study showed that infiltration of glycerides in the liver, and depression of liver phospholipid and protein, and of serum beta-lipoprotein in rats given orotic acid were observed, and these lesions can be restored by the administration of phospholipid (Fig. 1 to 3). Witting³⁵⁾ reported that liver lipid accumulation induced by orotic acid was inversely related to dietary polyunsaturated fatty acid as in the choline-deficient rat.³⁶⁾ In the present study, it seems possible that polyunsaturated fatty acid from the phospholipid is released by phospholipase. However, the mechanism of the effect of polyunsaturated fatty acid is little known in detail. On the other hand, it was demonstrated that liver lipid accumulation induced by orotic acid was restored by adenine sulfate²⁹⁾ but not by lipotropic factors, such as folic acid, cobalamine, methionine, or choline.³⁷⁾ Our previous results have shown that, when rats fed a semisynthetic diet with supplemented orotic acid, the incorporation of methionine [methyl-¹⁴C] into liver phosphatidylcholine and serum beta-lipoprotein was significantly decreased. Therefore, it can be understood that methionine had no effect on orotic acid-induced fatty liver. In addition, the depression of liver phosphatidylcholine synthesis may be due to the depression of adenine nucleotide. However, phospholipid has a curative effect on fatty liver induced by orotic acid. This curative effect of phospholipid is probably due to its effect on the release of beta-lipoprotein from the liver.

Liver necrosis has been observed in carbon tetrachloride-poisoned rats.³⁸⁾ This differs from fatty liver induced by ethionine or orotic acid. However, carbon tetrachloride-induced fatty liver is due to decreased synthesis of protein moiety in beta-lipoprotein⁸⁻¹²⁾ as in ethionine-

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induced fatty liver.¹³⁻¹⁸⁾ These lesions may result from free radical, such as $\text{CCl}_3\cdot$, and $\text{CCl}\cdot$.³⁹⁻⁴¹⁾ since cystamine, a well-known antidote against radiation injury, can restore carbon tetrachloride-induced liver injury.^{8,38)} In the present study, administration of phospholipid tended to only slightly restore liver lipid accumulation after carbon tetrachloride intoxication (Fig. 4). These results suggest that phospholipid does not play a major role in hepatotoxic effect of carbon tetrachloride. However, administration of phospholipid restored the depression of serum beta-lipoprotein, especially the protein moiety of lipoprotein (Fig. 6). In addition, Sakai found that the incorporation of labeled choline into serum phospholipid increased at 38 hr after carbon tetrachloride intoxication.⁴²⁾ From these findings, phospholipid seems to be an important factor in the release of accumulated lipids from the liver.

It is generally accepted that administration of ethionine induces a rapid decrease in the content of liver adenosine triphosphate⁴³⁾ which is followed, in turn, by inhibition of synthesis of ribonucleic acid⁴⁴⁾ and of protein,^{13,14,45,46)} and as a result, causes lipid accumulation in the liver.⁴⁷⁻⁴⁹⁾ These lesions can be restored by administration of adenine, adenosine triphosphate, adenosine, inosine, or methionine.^{47,48,50-52)} In the present study, administration of phospholipid partially restored accumulation of liver lipids in rats given ethionine (Fig. 4). As in the case of carbon tetrachloride-induced fatty liver, this may be due to its effect on the release of beta-lipoprotein from the liver, since administration of phospholipid resulted in a return of the concentration of serum beta-lipoprotein to normal (Fig. 6). Robinson, *et al.* observed a depression *in vivo* in the incorporation of orthophosphate [^{32}P] into liver and serum phospholipid in rats given ethionine.⁵³⁾ However, the present results showed that administration of phospholipid did not affect the decreased protein content in the liver (Fig. 5). Accordingly, it would appear that a decreased phospholipid synthesis is not a primary action on pathogenesis of fatty liver induced by ethionine.

Thus, fatty liver induced by orotic acid, carbon tetrachloride, and ethionine can be restored by the administration of phospholipid. These results suggest that phospholipid is an important factor in normal release of beta-lipoprotein.

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