Effects of Prostaglandin E₂ Analogue, Enprostil, on Lipid Metabolism in Mice

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ABSTRACT : This study was conducted to investigate the effects of enprostil, a prostaglandin E₂ analogue, on liver triacylglycerol content and factors that regulate liver lipid metabolism in mice. Mice received vehicle or 10 μg enprostil/kg body weight intraperitoneally every 6 h, and were killed at 0, 6, 12, 18 and 24 h after the first injection. Enprostil significantly lowered liver triacylglycerol content after 12 h of the first injection. However, the peroxisomal β-oxidation activity was inconsistent with the result of liver triacylglycerol content, because its activity was lowered by enprostil. In another experiment, the effect of enprostil on lipid metabolism in mice was investigated in a short period. Mice received 10 μg enprostil/kg body weight intraperitoneally, and were killed after 0, 5, 10, 30 and 60 min. After 30 min, malic enzyme activity was significantly increased by the administration of enprostil compared with the activity at 5 min after. No significant changes in liver carnitine palmitoyltransferase and peroxisomal β-oxidation activities were observed. Plasma free fatty acid concentrations were markedly reduced from 5 through 60 min after the administration of enprostil. Consequently, enprostil suppressive effect on liver triacylglycerol concentration might resut from the decreased entry of free fatty acid into the liver.

(**Key Words** : Mouse, Enprostil, Liver, Lipid, Metabolism)

INTRODUCTION

Enprostil, a synthetic dehydro-prostaglandin E₂ (PGE₂) structural analogue, is used as anti-ulcer drug. Its cytoprotective action such as inhibition of gastric acid secretion is well studied clinically. Studies on the cytoprotective action of PGs in the liver have been also done (Bang et al., 1992; Rush et al., 1986; Ruwart et al., 1975; Guarner et al., 1985; Stachura et al., 1981). In these reports, 16, 16-dimethyl PGE₂ (dmPGE₂) can histologically prevent fat accumulation in the liver of rats challenged with carbon tetrachloride (CCl₄) (Ruwart et al., 1975; Stachura et al., 1981).

According to Bjoernsson et al. (1992), PGE₂ inhibited the secretion of very low density lipoprotein (VLDL)-associated triacylglcerol (TG) in primary cultures of rat hepatocytes. This result implies that TG may be accumulated in the liver by PGE₂. However, we found that enprostil reduced the liver TG concentration of the control mice in the similar fashion as observed in the CCl₄ treated mice *in vivo* (Kawamoto et al., 1996). So it was hypothesized that enprostil aside from cytoprotection was involved in lipid metabolism in the liver. Thus, the present study was conducted to investigate the time course effect of enprostil on liver TG content in mice. Furthermore, in order to elucidate the mechanism by

which enprostil reduces the liver TG content, we studied the effects of enprostil on lipid metabolism by measuring the activities of carnitine palmitoyltransferase (CPT), peroxisomal β-oxidation and malic enzyme in the liver, plasma TG and free fatty acid (FFA).

MATERIALS AND METHODS

Experimental Procedure

Experiment 1

Sixty-three female Std:ddY mice [average body weigt (BW), 27g] were starved for 6 h, and were distributed into 9 groups of 7 animals each. Mice were injected intraperitoneally vehicle or 10 μg enprostil/kg BW every 6h, and were given only water during experiment. At 6, 12, 18 and 24 h after the first injection, mice were sacrificed by decapitation. The liver was removed quickly, and TG content was measured. To obtain an initial value, mice were sacrificed in the same manner without any treatment. Enprostil was purchased from Tanabe Seiyaku Co. Ltd., Osaka, Japan.

In order to examine the enzyme activities after fourth enprostil injection, mice received vehicle or 10 μg enprostil/kg BW in the same schedule as described above. Mice were sacrificed by decapitation at 6 h after the last injection.

Experiment 2

Thirty-five female Std:ddY mice (average BW 27 g) were starved for 24 h before enprostil administration, and were distributed into 5 groups of 7 animals each.

Mice were injected intraperitoneally 10 μg enprostil/kg BW. At 5, 10, 30 and 60 min after enprostil injection, mice were sacrificed by decapitation. The trunk blood was collected for the measurements of plasma TG and FFA concentrations. The liver was removed quickly, and enzyme activities were measured. One group of mice was also sacrificed in the same manner without any treatement for the initial value.

Analysis of liver TG, plasma TG and FFA concentrations

Liver was homogenized in chloroform-methanol solution (v/v 2:1) to extract the crude fat. Blood was collected in the heparinized tube and centrifuged immediately for plasma separation. The sample was kept frozen at −20℃ until the assay. The concentrations of liver and plasma TG and FFA were measured using the commercial kits (Triglyceride Test-Wako and NEFA Test-Wako, Wako Pure Chemicals, Osaka, Japan).

Analysis of liver enzyme activities

Liver was homogenized in ice-cold sucrose medium (0.25 M sucrose in 3 mM HEPES, pH 7.4, and 1 mM EDTA), and then centrifuged at 400 × g for 10 min.

The pellet was resuspended in the medium, centrifuged and added to the first supernatant. Mitochondrial and peroxisomal-enriched fraction was prepared by recentrifuging the supernatant at 14,000 × g for 30 min and used for the assay of CPT and peroxisomal β-oxidation. The pellet was resuspended in the medium and centrifuged at 100,000 × g for 60 min. The resulting supernatant was used for the assay of malic enzyme. The activities of enzymes were estimated as described in Takada et al. (1994). Protein concentration was determined according to the method of Lowry et al. (1951).

Statistical Analysis

Date were subjected to ANOVA by using the general linear models procedures (SAS 1985). When significant treatment effects were found, Duncan's multiple range test was used to determine the significance of differences between treatment groups. Results were considered significant at p < 0.05.

RESULTS

Experiment 1

Time course changes of TG content in the liver after first, second, third or fourth injection of enprostil are shown in figure 1. Liver TG contents in mice received vehicle were gradully increased in the course of time. Enprostil significantly lowered liver TG contents after 12, 18 and 24 h of the first injection.

The activities of malic enzyme, CPT and peroxisomal β-oxidation in the liver are shown in figure 2. There were no significant differences between vehicle- and enprostil-injected mice in malic enzyme and CPT activities. Peroxisomal β-oxidation concentration was significantly lowered by enprostil.

Experiment 2

Time course changes of the activities of malic enzyme, CPT and peroxisomal β-oxidation in the liver after enprostil administration are shown in figure 3. After a temporary suppression, the activity of malic enzyme increased from 5 to 30 min after enprostil injection, and subsequently decreased to 60 min. No significant changes were observed in CPT and peroxisomal β-oxidation. Hepatic TG concentration was not changed during 60 min (data not shown).

Time course changes of the plasma concentrations of FFA and TG after enprostil administration are presented in figure 4. Both FFA and TG concentrations were reduced remarkably from 5 through 60 min after the injection of enprostil.
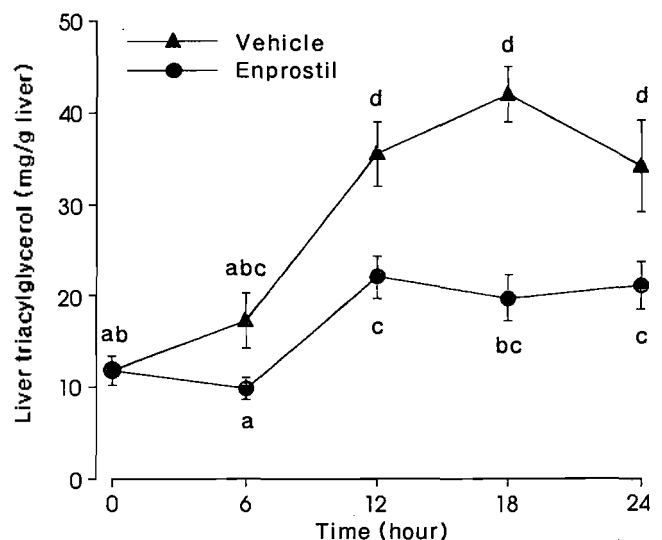

Figure 1. Time course changes in triacylglycerol content of the liver of mice intraperitoneally administered vehicle or enprostil (10 μg/kg body weight). The mice were sacrificed at 0, 6, 12, 18 and 24 h after the first injection of enprostil. Vertical bars represent SEM. Means not having the same letter are significantly different at p < 0.05. Number of animals used was 7 per treatment.
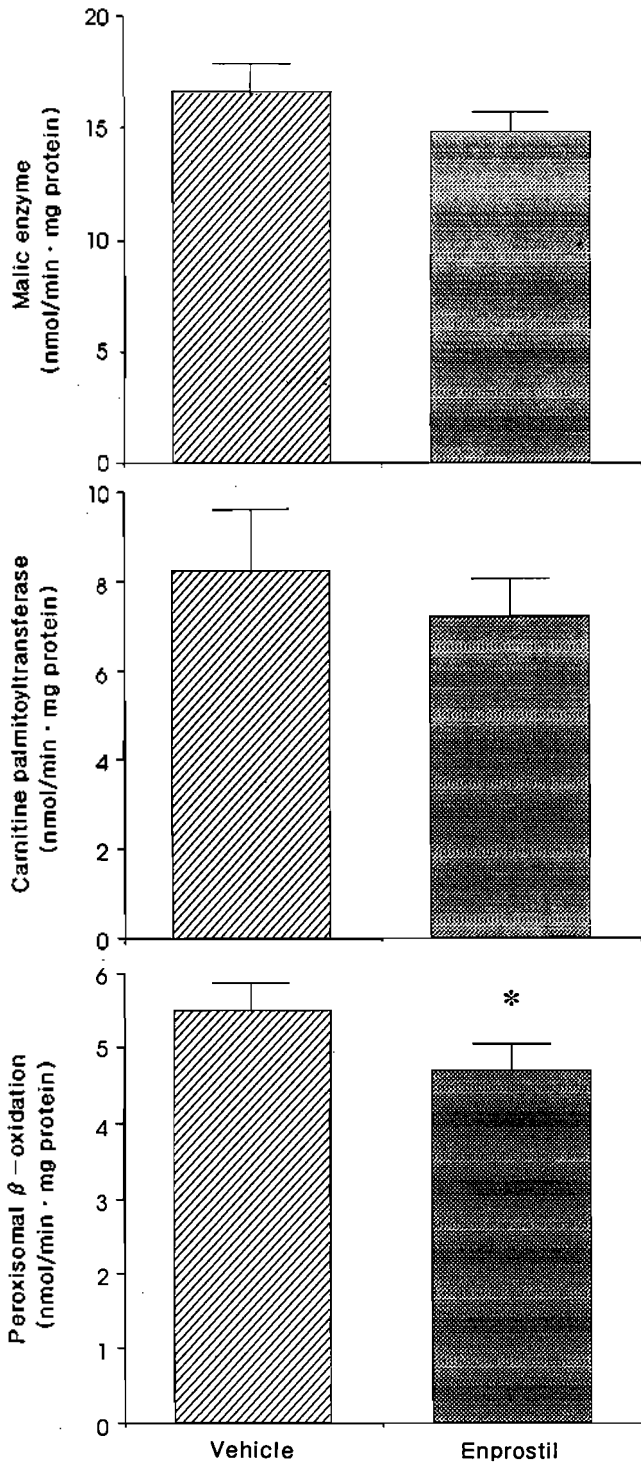
Figure 2. Effect of intraperitoneally administered vehicle or enprostil (10 μg/kg body weight) on the activities of malic enzyme, carnitine palmitoyltransferase and peroxisomal β-oxidation in the liver. The mice were treated with vehicle or enprostil at 0, 6, 12 and 18 h and were sacrificed at 24 h after the first injection of enprostil. Vertical bars represent SEM. Number of animals used was 9 or 10 per treatment.
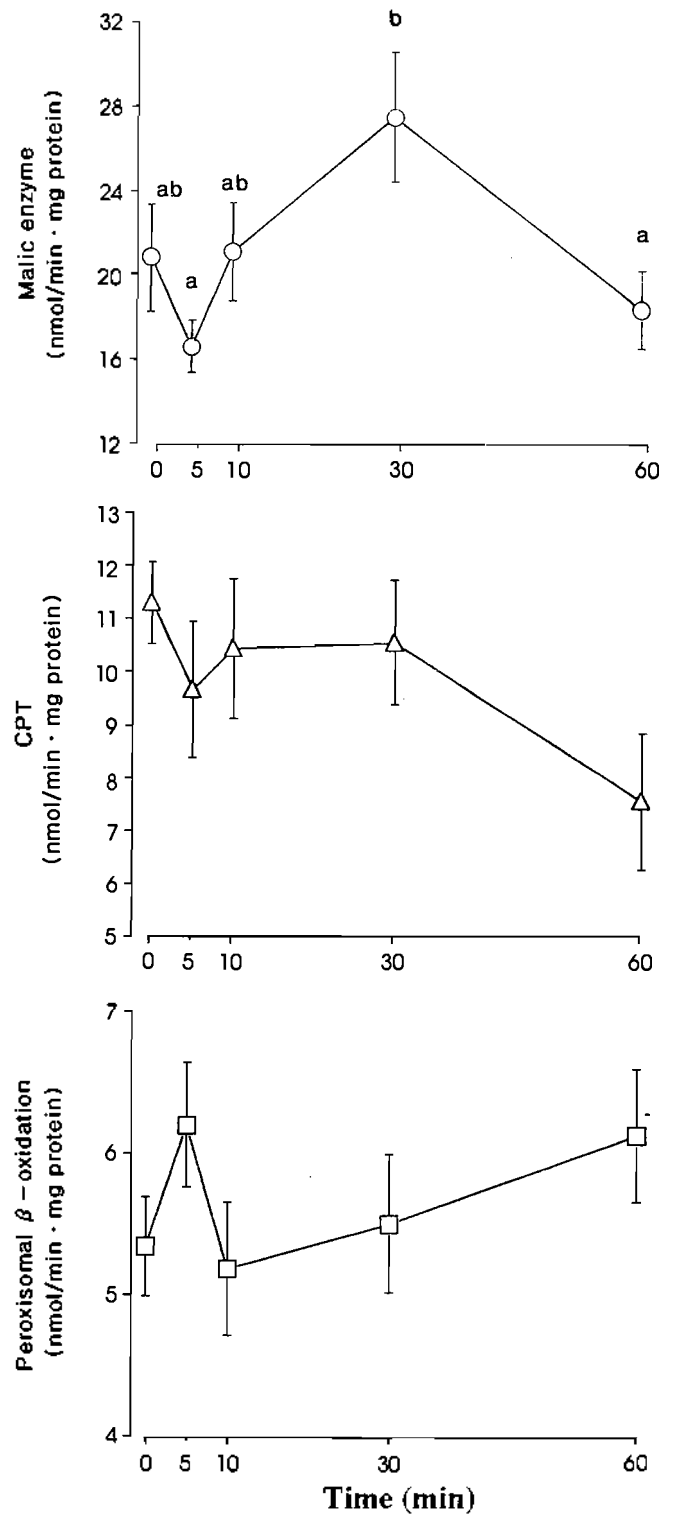
*Significantly different from the control p < 0.05.

Figure 3. Time course changes of malic enzyme activity, carnitine palmitoyltransferase and peroxisomal β-oxidation in the liver of mice intraperitoneally administered enprostil (10 μg/kg body weight). The mice were sacrificed at 0, 5, 10, 30 and 60 min after the injection of enprostil. Vertical bars represent SEM. Means not having the same letter are significantly different at p < 0.05. Number of animals used was 6 or 7 per treatment.
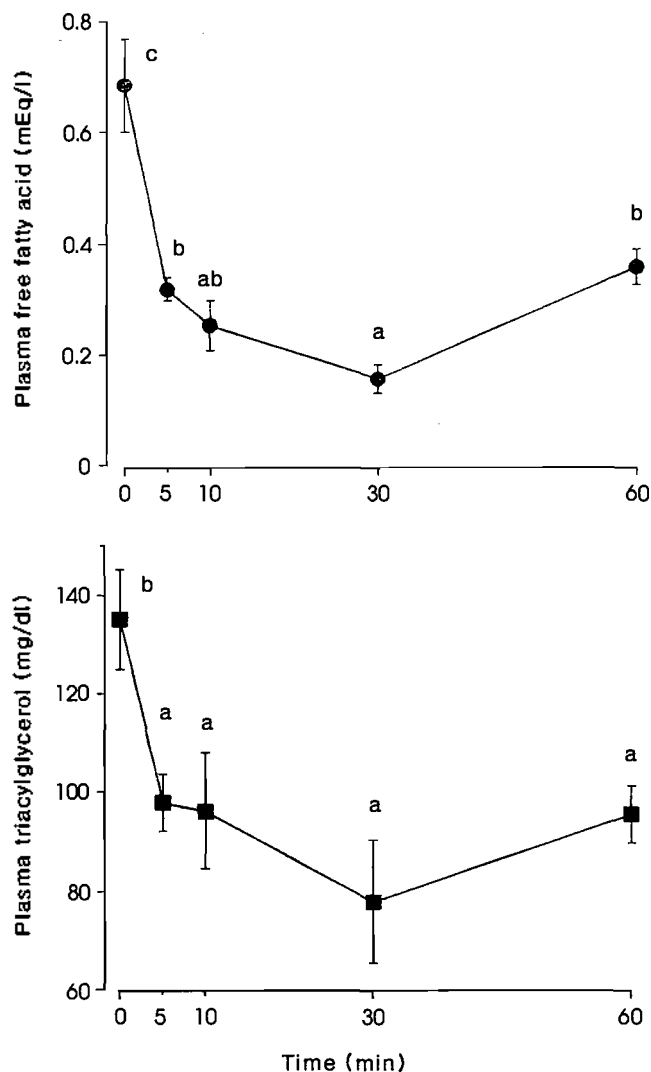
Figure 4. Time course changes of plasma free fatty acid and triacylglycerol concentrations in mice intraperitoneally administered enprostil (10 μg/kg body weight). The mice were sacrificed at 0, 5, 10, 30 and 60 min after the injection of enprostil. Vertical bars represent SEM. Means not having the same letter are significantly different at $p < 0.05$. Number of animals used was 7 per treatment.

DISCUSSION

Histological studies have indicated that dmPGE$_2$ can prevent fat accumulation in the liver of rats challenged with CCl$_4$ (Ruwart et al., 1975; Stachura et al., 1981). In previous study, we chemically determined the effect of enprostil on TG content in the liver after the CCl$_4$ challenge and confirmed that enprostil reduced TG content compared with the control (Kawamoto et al., 1996). Although livers of rats treated with CCl$_4$ showed severe centrilobular necrosis, those of the control rats as

well as of the animals receiving dmPGE$_2$ only were normal (Stachura et al., 1981).

On the other hand, enprostil was effective to decrease liver TG content for the mice without CCl$_4$ treatments (Kawamoto et al., 1996). This result suggests that enprostil might have the effect on lipid metabolism which is separated from its cytoprotection in the liver. Thus, the present study was done to clarify in detail the effect of enprostil on lipid metabolism under a CCl$_4$ nonchallenged condition.

A marked increase in liver TG content was found in mice received vehicle (figure 1), and this phenomenon would be due to the effect of starvation in agreement with the findings of others (Herrera et al., 1975; Menahan and Sobocinski, 1983). The present study clearly showed that enprostil strongly prevented liver TG accumulation by starvation after 12 h of the first enprostil injection (figure 1). This result implied that a single administration of enprostil did not fully suppressed TG accumulation in the liver. To decrease TG content in the liver frequency of enprostil administration was required more than two times. According to Menahan and Sobocinski (1983), hepatic TG in mice increased markedly during fasting but declined in rats. There may be a case that the effect of enprostil on liver TG content would be different among species. On the other hand, the reduction of TG content in the liver is caused mainly by: (1) increased oxidation of FFA in the liver, (2) decreased intrahepatic synthesis of FFA, (3) increased release of the lipoproteins, and (4) decreased arrival of FFA from outside of the liver. At 6 h after the fourth enprostil injection, however, remarkable changes of enzyme activities that explain the mechanism of decreased TG content were not observed (figure 2). Particularly, the value for peroxisomal β-oxidation activity was rather lowered than enhanced by enprostil. Smith et al. (1989) reported that a half life of plasma enprostil by the oral administration (8 μg enprostil/kg BW) was approximately 3 h in mice. Accordingly, time course effects of enprostil in a short period were examined in a separate experiment.

From 10 to 60 min after enprostil administration, the activity of malic enzyme, lipogenic enzyme, was significantly increased compared to the control (figure 3). The activity of CPT, the rate-limiting enzyme of mitochondrial fatty acid oxidation in the liver (McGarry and Foster, 1980), tended to decrease from 30 min after enprostil injection. These results were contrary to the causes of the reduction of liver TG. It was suggested that the liver TG suppression by enprostil was not responsible for the decrease of FFA synthesis and the increase of FFA oxidation in the liver.

Plasma TG concentrations were significantly suppressed following the administration of enprostil (figure 4). This result was consistent with the report for diabetic humans (Davis et al., 1989; Reaven et al., 1988). Additionally, Reaven et al. (1988) reported that secretion of TG in very low density lipoprotein fell in response to enprostil treatment. Furthermore, VLDL-associated TG secretion from primary culture of rat hepatocyte was inhibited by enprostil (Bjornsson et al., 1992). These results suggested that the reduction of TG in the liver was suppressed by enprostil, but several administrations of enprostil reduced hepatic TG content (Experiment 1) and no changes were observed by single administration of enprostil over a short period (Experiment 2). Since enprostil reduced hepatic TG content during 1 h in Japanese quail (Murai et al., 1996), there may be species difference in effects of enprostil.

Plasma TG lowering effect by enprostil would be caused by the decrease of lipoprotein secretion as a consequence of small TG pool in the liver. The reason for this would be explained by plasma FFA concentration. The concentration of plasma FFA decreased by an average of more than 50% from 5 to 60 min after enprostil administration (figure 4). This result suggests that the entry of FFA into the liver would be decreased. PGE_2 is produced in adipose tissue and is a potent antilipolytic agent *in vivo* by decreasing the formation of cAMP (Axelrod et al., 1976). Enprostil shows a high degree of EP_3 receptors selectivity which is involved in the inhibition of cAMP formation (Coleman et al., 1994). It is probable that the antilipolytic action in adipocytes may be associated with the clearance of FFA in the plasma. Consequently, the lowered FFA concentration by enprostil may be one of the reasons for the suppression of TG in the liver.

In conclusion, several challenges of enprostil lowered the liver TG in mice under the condition that TG accumulation is induced in the liver such as fasting. Further studies remain to be investigated in relation to species differences and feeding conditions.

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