Effects of OP 2507, a Stable Analogue of Prostaglandin I₂, on Carbon Tetrachloride-Induced Liver Damage in Starved Rats

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IRITA, K., SAKAI, H., YAMAKAWA, M., NAWATA, H., YOSHITAKE, J. and TAKAHASHI, S. *Effects of OP 2507, a Stable Analogue of Prostaglandin I₂, on Carbon Tetrachloride-Induced Liver Damage in Starved Rats.* Tohoku J. Exp. Med., 1996, **178** (3), 279-285 — It has been reported that vasodilatory prostaglandins have cytoprotective effects against various types of liver damage. We investigated the effects OP 2507, a stable analogue of prostaglandin I₂, on carbon tetrachloride-induced liver damage in starved rats. Intraperitoneal administration of OP 2507 at 1,500 µg/kg lessened both an increase in serum alanine aminotransferase activity and an inhibition of starvation ketosis, both of which were induced by carbon tetrachloride. At lower doses, however, OP 2507 not only failed to ameliorate the carbon tetrachloride-induced changes, but it actually exaggerated them. Although the deterioration of carbon tetrachloride-induced liver damage by lower doses of OP 2507 was not statistically significant, it seems possible that OP 2507 has dual effects on carbon tetrachloride-induced liver damage. While none of the three agents cimetidine, reduced glutathione and deferoxamine, prevented increase in serum alanine aminotransferase activity induced with lower dose OP 2507, allopurinol had a tendency to prevent the increase, indicating that lower doses of OP 2507 may promote a reaction catalyzed by xanthine oxidase. We propose that both the co-administration of prostaglandins and other potentially hepatotoxic drugs, and the administration of prostaglandins to patients with drug-induced liver damage should be done carefully. ——— OP 2507; prostaglandin I₂; carbon tetrachloride; liver

Carbon tetrachloride (CCl₄) has been shown to induce liver damage by its biotransformation into reactive intermediates, which initiate lipid peroxidation (Recknagel et al. 1989). The possible involvement of prostaglandins in CCl₄-induced liver damage has been suggested, and the effects of prostaglandin I₂ (PGI₂) and its analogues have been studied by several groups (Guarner et al.

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1983; Marinovich et al. 1987; Bursch et al. 1989). The results, however, are controversial: the cytoprotective effects of PGI₂ seem to be dependent on the stage of liver damage (Guarner et al. 1983), and iloprost, a stable analogue of PGI₂, have been reported either effective (Bursch et al. 1989) or ineffective in lessening liver damage (Marinovich et al. 1987).

OP 2507, a stable analogue of PGI₂, has been shown to have cytoprotective effects against liver damage induced by ischemia (Sikujara et al. 1983) and hypoxia (Araki and Lefer 1980; Irita et al. 1990). In this study, the effects of OP 2507 on CCl₄-induced liver damage in starved rats were investigated.

MATERIALS AND METHODS

Animals

All animal experiments were conducted in accordance with the Kyushu University guidelines for the care and the use of laboratory animals. Seven week old, male Wistar rats weighing about 200 g were used. Rats were starved to enhance CCl₄-induced liver damage, and to allow the inhibition of starvation ketosis as a marker for liver damage (Nakajima et al. 1982). The rats were divided into 2 groups according to feeding conditions. In group 1, the rats had free access to food before CCl₄ was injected, but were kept starved thereafter. In group 2, food deprivation was started 24 hr before CCl₄ was injected, and continued until blood samples were taken. Water was offered ad libitum to each group. A one ml/kg dose of CCl₄ dissolved in olive oil (50% solution) was injected intraperitoneally. The control rats were given olive oil, 0.5 ml/kg. OP 2507 (0.5, 5, 50, 500 µg/kg in each group) was administered intraperitoneally 30 min before CCl₄ was injected. In the other subgroup, OP 2507 (500 µg/kg) was administered three times 30 min before, 8 and 16 hr after CCl₄ was injected. The activity of rats injected with 500 µg/kg OP 2507 decreased for about 30 min. Masuda et al. (1986) reported that subcutaneous administration of 3 mg/kg OP 2507 showed a protective effect against cerebral anoxia in mice until 6 hr after its administration.

In a separate experiment, cimetidine (100 mg), reduced glutathione (GSH, 500 mg), deferoxamine (500 mg), or allopurinol (500 mg) was also administered three times intraperitoneally 30 min before, 8 and 16 hr after the CCl₄ injection to rats which had either received or not received a dose of OP 2507, 50 µg/kg. Cimetidine suppresses the biotransformation of CCl₄, GSH reduces lipid peroxidation as well as supplying amino acids (Okabe et al. 1993), and deferoxamine reduces lipid peroxidation. Allopurinol inhibits endothelial xanthine oxidase activity.

Blood samples from the right atrium were taken under anesthesia with diethyl ether 24 hr after the CCl₄ injection. The CCl₄ was purchased from Nakarai Chemical (Kyoto). OP 2507 was supplied by Ono Pharmaceutical Co. (Osaka), and was dissolved in normal saline.

Assays

Serum alanine aminotransferase (ALT) activity was measured with an Ektachem DT60 Analyzer (Eastman Kodak, Rochester, NY, USA). Serum ketone body concentrations were determined spectrophotometrically using the Ketone Test Sanwa (Sanwa Chemical Lab., Nagoya).

Statistical analysis

All results are expressed as mean $\pm$ S.D. Statistical analysis of the data was performed using the Student's *t*-test. $p < 0.05$ was considered statistically significant.

RESULTS

CCl₄ induced an increase in serum ALT activity; the increase was greater in group 2 than in group 1 ($p < 0.05$, Fig. 1). OP 2507 at 1,500 μ g/kg significantly lessened the CCl₄-induced increase in serum ALT activity in group 2. OP 2507 at doses less than 50 μ g/kg accelerated the increase in serum ALT activity in both groups, although the changes were not significant. It should be noted that extraordinarily high ALT activity occurred at lesser doses of OP 2507. OP 2507 in any amount did not affect serum ALT activity in rats injected with oil alone (data not shown).

The CCl₄-induced changes in hepatic function were assessed by the inhibition

Fig. 1. The effects of OP 2507 on serum ALT activity in rats exposed to CCl₄. OP 2507 (0.5, 5, 50, 500 μ g/kg in each subgroup) was administered intraperitoneally 30 min before CCl₄ was injected. In the other subgroup, OP 2507 (500 μ g/kg) was administered three times: 30 min before, 8 and 16 hr after CCl₄ was injected (OP 2507, 1,500 mg 3 $\times$). Blood samples were taken 24 hr after the CCl₄ injection. The error bars show mean $\pm$ S.D. The shaded areas indicate $\pm$ S.D. of the serum ALT activity in rats injected with CCl₄ alone in each group. * $p < 0.05$.

TABLE 1. *The effects of OP 2507 on serum total ketone body concentrations in rats exposed to CCl₄*

Condition		Group 1	Group 2
Oil alone		1,426 ± 550 (20)	2,452 ± 1,021 (19)
CCl ₄		567 ± 226* (20)	840 ± 214* (22)
CCl ₄ + OP 2507	0.5 µg/kg	727 ± 469 (20)	1,075 ± 500 (20)
	5.0	440 ± 172† (20)	744 ± 170 (20)
	50	875 ± 661 (21)	927 ± 498 (19)
	500	735 ± 348 (10)	985 ± 428 (20)
	1,500 (3 ×)	1,008 ± 346† (10)	1,285 ± 201† (9)

The same blood samples obtained in Fig. 1. were examined for serum total ketone body concentrations. Unit, µM. Mean ± s.d. * $p < 0.05$ vs. oil alone; † $p < 0.05$ vs. CCl₄ alone. Numbers of rats are shown in parentheses.

of starvation ketosis (Table 1). Starvation increased total ketone body concentration in serum, which was higher in group 2 than in group 1 ($p < 0.05$). CCl₄ inhibited starvation ketosis in both groups. OP 2507 at 1,500 µg/kg partly counteracted this inhibition in both groups. OP 2507 at doses less than 500 µg/kg did not affect the CCl₄-induced inhibition of starvation ketosis with the exception that OP 2507 at 5 µg/kg significantly accelerated the inhibition of starvation ketosis in group 1.

In order to examine the mechanism(s) of the deleterious effect of OP 2507, rats were pretreated with cimetidine, GSH, deferoxamine, or allopurinol prior to injection with CCl₄ in the presence or in the absence of OP 2507, 50 µg/kg (Table 2). None of cimetidine, GSH nor deferoxamine efficiently lessened low dose OP 2507-induced augmentation of the increase in serum ALT activity by CCl₄. Only allopurinol lessened low dose OP 2507-induced augmentation of serum ALT

TABLE 2. *The effects of antioxidants on serum ALT activities in rats exposed to CCl₄ in the presence or absence of OP 2507*

Condition		Without OP 2507	With OP 2507
CCl ₄ alone		3,827 ± 3,066 (22)	5,222 ± 4,587 (22)
CCl ₄ +	Cimetidine 300 mg 3 ×	745 ± 769* (10)	1,137 ± 575† (10)
	+ GSH 1,500 mg 3 ×	1,437 ± 1,359* (10)	1,722 ± 1,451† (10)
	+ Deferoxamine 1,500 mg 3 ×	944 ± 792* (10)	1,268 ± 976† (10)
	+ Allopurinol 150 mg 3 ×	1,989 ± 792* (15)	1,611 ± 2,669† (10)

Food deprivation was started 24 hr before the injection of CCl₄, and continued until blood samples were taken. OP 2507 (50 µg/kg) was administered 30 min before the injection of CCl₄. Antioxidants were administered three times intraperitoneally 30 min before, 8 and 16 hr after the CCl₄ injection. Unit, IU/liter. Mean ± s.d. * $p < 0.05$ vs. CCl₄ without OP 2507; † $p < 0.05$ vs. CCl₄ with OP 2507. Numbers of rats are shown in parentheses.

activity ($1,611 \pm 2,669$ vs. $1,987 \pm 792$ IU/liter), although it was not statistically significant.

DISCUSSION

Marinovich et al. (1987) reported that prior use of PGI₂ lessened enzyme leakage from rat hepatocyte suspension exposed to carbon tetrachloride, but a stable analogue of PGI₂, iloprost, did not. Bursch et al. (1989) showed that the CCl₄-induced liver damage examined by means of enzyme leakage, lipid peroxide accumulation and histological changes was partially reduced by continuous, intraperitoneal administration of iloprost, which was started 2.5–4 hr before the CCl₄ injection. Guarner et al. (1983) obtained interesting results: PGI₂ at the dosage of 10 μ g/kg administered intraperitoneally 30 min before and 6 hr after injection with CCl₄ ameliorated liver damage in rats. However, PGI₂ administered 8 hr after injection with CCl₄ at the same dosage deteriorated liver damage. They also observed that PGI₂ was effective in lessening enzyme leakage in rat hepatocyte culture, when added 30 min after exposure to CCl₄, but was not effective, when added 180 min after exposure to CCl₄, indicating that the cytoprotective effects of PGI₂ on the CCl₄-induced liver damage depends on the stage of liver damage.

The present results suggest that OP 2507 possibly has deleterious effects on CCl₄-induced liver damage in vivo besides its cytoprotective effects at a higher dosage. Our results, however, seem to be incompatible with those of Guarner et al. (1983), because a preventive administration of low doses of OP 2507 did not affect, or rather accelerated CCl₄-induced liver damage in vivo, and a higher, repeated dosage lessened the damage. Several possible factors might account for this difference. First, the drugs tested were different; they used PGI₂, but we used OP 2507. OP 2507 has been reported to be more stable than PGI₂ (Masuda et al. 1986), and might be expected to induce more reliable reactions as a PGI₂ agonist. Secondly, the administration route of CCl₄ was different. They injected CCl₄ directly into the stomach, while we injected it intraperitoneally. OP 2507 has been reported to induce vasodilation with a potency of 1/20 compared with PGI₂ (Terawaki et al. 1988). Because OP 2507 has a vasodilatory effect, its presence in the peritoneal cavity promotes the absorption of CCl₄ applied intraperitoneally. This possibility, however, seems questionable, because increasing the OP 2507 concentration did not necessarily increase the damage. Thirdly, the severity of the liver damage might be different. Because different effects of OP 2507 with different doses were seen in both group 1 and 2, this possibility also seems to be questionable.

Our findings can be interpreted as follows: (i) the single administration of the low doses of OP 2507 in the initial stage of the CCl₄-induced liver damage may make the damage worse, and (ii) the administration of the high dose of OP 2507 through the acute phase of the CCl₄-induced liver damage may ameliorate it.

The mechanisms for the amelioration by PGI₂ or OP 2507 are unknown, but membrane stabilization (Araki and Lefer 1980), an increase in intracellular cAMP (Sikujara et al. 1983), an increase in blood flow to the splanchnic region (Smith et al. 1978), an inhibition of platelet aggregation (Moncada and Vane 1979), and improvement of mitochondrial function (Irita et al. 1990) have all been suggested. Recently, Fujiwara et al. (1995) has shown that beraprost, another PGI₂ analog, administered intravenously at 10–100 µg/kg attenuates liver injury caused by dimethylnitrosamine or endotoxin more efficiently than that caused by CCl₄. They suggested that the beneficial effect of beraprost was due to its fibrin clot-clearing action. The mechanisms for the deterioration caused by OP 2507 are also unknown at present. The experiment using antioxidants suggested that OP 2507 did not affect the rate of biotransformation of CCl₄ nor of lipid peroxidation, but possibly promoted a reaction catalyzed by xanthine oxidase. As discussed above, absorption of CCl₄ from the peritoneal cavity could be increased in the presence of OP 2507. The other possibility is that exogenously administered prostaglandins affect the balance between endogenous prostaglandins, resulting in a defect in the endogenous cytoprotective mechanism.

Prostaglandins are now widely used as therapeutic agents. Although the clinical relevance of the present findings is unclear at this point, both the co-administration of prostaglandins and other potentially hepatotoxic drugs, and the administration of prostaglandins to patients with drug-induced liver damage should be done carefully.

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