PROTECTIVE EFFECT OF MALOTILATE (DIISOPROPYL 1,3-DITHIOL-2-YLIDENEMALONATE) ON CARBON TETRACHLORIDE -INDUCED LIVER INJURY IN MICE AND RATS

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Abstract.....The protective effect of malotilate was studied on the liver injury induced by carbon tetrachloride (CCl_4) in mice and rats. Plasma GOT and GPT activities were used as indices for the liver injury, and the liver was histopathologically examined. A remarkable suppressing effect on the liver injury was observed when malotilate was orally given 6 hr prior to oral administration of CCl_4 in mice and 3, 6 or 12 hr in rats. Malotilate was also effective in preventing the liver injury caused by intraperitoneal injection of CCl_4 , indicating that the protective effect is not derived from the decreased CCl_4 absorption. The liver injury was suppressed even when malotilate was given 12 or 24 hr prior to oral administration of CCl_4 in mice and 24, 48 or 72 hr in rats. It may be the characteristic of malotilate that the protective effect lasts for a long period. It is supposed that the effect is due not only to the inhibition of the metabolic activation of CCl_4 but also the other action(s) of malotilate.

Key words: Malotilate, carbon tetrachloride (CCl_4), liver injury, protective effect, mouse, rat.

INTRODUCTION

Malotilate (diisopropyl 1,3-dithiol-2-ylidenemalonate) shows the protective (Nakayama et al., 1979) and therapeutic (Imaizumi et al., 1981) effects on fatty liver induced by carbon tetrachloride (CCl_4). This compound has been already known to affect the liver microsomal drug oxidation (Nakayama, 1979) and the liver microsomal electron transport systems (Kato et al., 1980; 1981).

CCl_4 is metabolized by microsomal drug oxidizing systems to form toxic metabolite(s) that combine covalently with cell protein to induce necrosis (Rao and Recknagel, 1969). CCl_4 -induced liver injury is known to be enhanced by the pretreatment with inducers of microsomal drug oxidation such as phenobarbital (Suarez et al., 1972) and reduced by the metabolic inhibitors such as SKF-525A (Reynolds and Moslen, 1974).

In the present paper, the protective effect of malotilate on CCl_4 -induced liver injury was studied. The relationship between the protective effect and the changes of microsomal drug oxidation activity is discussed.

MATERIALS AND METHODS

Chemicals : Malotilate was synthesized and purified by repeated crystallization at Nihon Nohyaku Co., Ltd., Tokyo. The purity was more than 99.5%. CCl_4 (Wako Pure Chemical Industries, Inc., Osaka) and other reagents were of the analytical grade.

Animals and animal treatment : Male dd mice (6 week-old) and male Sprague-Dawley rats (6 week-old) were fed laboratory chow (CE-2, Clea Japan, Inc., Tokyo or F-2, Shizuoka Agricultural Cooperative Association for laboratory Animals, Shizuoka) and tap water ad libitum. Malotilate and CCl_4 were dissolved in olive oil in proper concentrations. The solutions were orally or intraperitoneally administered to mice and rats in volume of 5 ml/kg. Malotilate was orally administered to animals and then CCl_4 was orally or intraperitoneally given selected hours after the administration of malotilate. Animals were sacrificed by cervical dislocation 24 hr after CCl_4 treatment. Blood from the cervical vein was collected into a heparinized tube. Plasma was obtained by the centrifugation at 2,500 rpm for 15 min and liver was rapidly excised.

Analytical method : Plasma glutamic-oxalacetic (p-GOT) and plasma glutamic-pyruvic transaminase (p-GPT) activities were determined by the method of Reitman and Frankel (1957) and were shown in Karmen unit (K. U.).

Histopathological examination : Portion of the liver were fixed in 10% formalin and embedded in paraffin. Tissue sections were prepared and were stained with hematoxylin and eosin (H. E.) for microscopic examination.

Statistics : The significance of the difference between two mean values was assessed by Student's t-test.

RESULTS

Fig. 1 shows the duration of the effect of the pretreatment with malotilate on the changes in p-GPT activities in mice treated with CCl_4 . Malotilate was orally administered at the dose of 250 mg/kg 6, 12, 24 or 48 hr prior to CCl_4 treatment (0.05 ml/kg p. o.). Plasma GPT activities were 13 to 16 K. U. in the control group but elevated more than 1,000 K. U. in all of mice treated with CCl_4 alone. In mice pretreated with malotilate 6 hr prior to the administration of CCl_4 , however, the elevation of p-GPT activities induced by CCl_4 was prevented (15 to 30 K. U.). This preventive effect was also observed when the interval between the treatment with malotilate and the administration of CCl_4 was 12 or 24 hr. In histopathological examination in the mouse liver (Photo. 1-3), the pretreatment with malotilate 6 hr before prevented the centrilobular necrosis, vacuolation of parenchymal cells and infiltration of inflammatory cells induced by CCl_4 . In the liver of mice pretreated 24 hr before, the histopathological aberrations induced by CCl_4 were slighter than those in the liver of mice treated with CCl_4 alone. In

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Fig. 1 Duration of the effect of the pretreatment with malotilate on the changes in p-GPT activities in mice orally given with CCl_4 . The doses of malotilate and CCl_4 were 250 mg/kg p. o. and 0.05 ml/kg p. o., respectively. Each spot represents the value determined in one animal. An arrow indicates that the value is over the scale. For further details, see MATERIALS AND METHODS.

Photo. 1 Liver section from a mouse treated with CCl₄ alone (0.05 ml/kg p. o.). Centrilobular necrosis and infiltration are evident. Slight vacuolation is observed. (H. E. $\times 40$)

Photo. 2 Liver section from a mouse pretreated with malotilate (250 mg/kg p. o.) 6 hr prior to CCl_4 treatment (0.05 ml/kg p. o.). The appearance is essentially normal. (H. E. $\times 40$)

Photo. 3 Liver section from a mouse pretreated with malotilate (250 mg/kg p. o.) 24 hr prior to CCl_4 treatment (0.05 ml/kg p. o.). The area of the histopathological changes induced by CCl_4 is limited. (H. E. $\times 40$)

rats, malotilate was orally administered in the dose of 50 mg/kg 0, 1, 3, 6, 12, 24, 48, 72 or 96 hr prior to CCl₄ treatment (1 ml/kg p. o.) (Table 1). Plasma GOT and p-GPT activities in the control group were 119 and 44 K. U., respectively. Those activities in the group treated with CCl₄ alone were 17 and 27-fold (1,986 and 1,174 K. U.) higher than those in the control group, respectively. In the groups pretreated with malotilate 3, 6 or 12 hr before, however, p-GOT and p-GPT activities remained in a range of the control level. Both of those activities in the group pretreated with malotilate 0 to 72 hr before were significantly lower than those in the group treated with CCl₄ alone. The histopathological changes in the rat liver induced by CCl₄ were hardly observed in rats pretreated with malotilate 6 hr before and were slighter in rats pretreated 48 hr before than those in rats treated with CCl₄ alone (Photo. 4-6).

Table 2 presents the effect of the pretreatment with malotilate at various doses on the changes in p-GOT and p-GPT activities induced by CCl₄ in rats. Malotilate was orally administered at the dose of 0, 10, 25 or 50 mg/kg 6 or 48 hr prior to CCl₄ treatment (1 ml/kg p. o.). In the group treated with CCl₄ alone, p-GOT and p-GPT activities were 35 and 39-fold (1,904 and 1,081 K. U.) higher than those activities in the control group (55 and 28 K. U.), respectively. The elevation of the transaminase activities induced by CCl₄ was prevented by the pretreatment with more than 10 mg/kg of malotilate 6 hr before. When malotilate was administered at the dose of 50 mg/kg 48 hr prior to CCl₄ treatment, p-GOT and p-GPT activities were 235 and 147 K. U., respectively and the preventive effect of malotilate against the elevation of the transaminase activities was observed.

Table 1. Duration of the effect of pretreatment with malotilate on p-GOT and p-GPT activities after administration of CCl₄ in rats

Treatment	Interval between the treatments (hour)	p-GOT activity (Karmen unit)	p-GPT activity (Karmen unit)
control		119 ± 7	44 ± 3
CCl ₄		1,986 ± 536**	1,174 ± 351*
malotilate + CCl ₄	96	768 ± 168**	232 ± 73*
	72	410 ± 77**	116 ± 18**
	48	256 ± 21**	93 ± 10**
	24	260 ± 34**	106 ± 13**
	12	121 ± 7++	56 ± 2+
	6	124 ± 8++	47 ± 2+
	3	114 ± 5++	45 ± 4+
	1	302 ± 117+	102 ± 55+
	0	231 ± 18**	72 ± 6**

Each value is a mean ± S. E. of 5 rats. Malotilate (50 mg/kg) was orally given at selected intervals before CCl₄ (1 ml/kg p. o.) administration.

Significant difference from control group: * (p < 0.05), ** (p < 0.01).

Significant difference from CCl₄ group: + (p < 0.05), ++ (p < 0.01).

For further details, see MATERIALS AND METHODS.

Photo. 4 Liver section from a rat treated with CCl_4 alone (1 ml/kg p. o.). Centrilobular vacuolation is evident. Necrosis and infiltration are observed. (H. E. $\times 40$)

Photo. 5 Liver section from a rat pretreated with malotilate (50 mg/kg p. o.) 6 hr prior to CCl_4 treatment (1 ml/kg p. o.). The appearance is essentially normal. (H. E. $\times 40$)

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Photo. 6 Liver section from a rat pretreated with malotilate (50 mg/kg p. o.) 48 hr prior to CCl₄ treatment (1 ml/kg p. o.). The area of the histopathological changes induced by CCl₄ is limited. (H. E. $\times 40$)

Table 2. Effect of pretreatment with malotilate at various doses on p-GOT and p-GPT activities after administration of CCl₄ in rats.

Treatment	Interval between the treatment (hour)	Dose of malotilate (mg/kg)	p-GOT activity (Karmen unit)	p-GPT activity (Karmen unit)
control			55 $\pm$ 5	28 $\pm$ 3
CCl ₄			1,904 $\pm$ 553**	1,081 $\pm$ 386*
malotilate+CCl ₄	6	50	82 $\pm$ 5**	43 $\pm$ 3*
		25	61 $\pm$ 6+	35 $\pm$ 2
		10	82 $\pm$ 4**	45 $\pm$ 2**
malotilate+CCl ₄	48	50	235 $\pm$ 95†	147 $\pm$ 50**
		25	1,054 $\pm$ 550*	1,014 $\pm$ 723
		10	3,976 $\pm$ 2,564*	2,484 $\pm$ 1,433

Each value is a mean $\pm$ S. E. of 5 to 10 rats. CCl₄ was orally given at the dose of 1 ml/kg. Significant difference from control group: * (p<0.05), ** (p<0.01). Significant difference from CCl₄ group: + (p<0.05), ++ (p<0.01). For further details, see MATERIALS AND METHODS.

Fig. 2 presents the effect of the pretreatment with malotilate (0, 100 or 250 mg/kg p. o.) 6 hr before on the change in p-GPT activities induced by CCl_4 (0.05 ml/kg) given intraperitoneally to mice. Plasma GPT activities were 20 to 40 K. U. in the control group and elevated more than 1,000 K. U. in the most of mice to which CCl_4 was intraperitoneally given. However, p-GPT activities in mice pretreated with malotilate were 14 to 37 K. U. The elevation of p-GPT activities induced by CCl_4 given intraperitoneally to mice was also prevented by the pretreatment with malotilate.

Fig. 2 Effect of the pretreatment with malotilate on the changes in p-GPT activities in mice orally or intraperitoneally given with CCl_4 . Malotilate was orally administered 6 hr prior to CCl_4 treatment (0.05 ml/kg). Each spot represents the value determined in one animal. An arrow indicates that the value is over the scale. For further details, see MATERIALS AND METHODS.

DISCUSSION

As shown in the present study, the elevation of plasma transaminase activities was well correlated with the histopathological changes by CCl_4 in the liver (Fig. 1, Table 1 and Photo. 1-6). The results of the biochemical and histopathological examinations indicate that the protective effect of malotilate on CCl_4 -induced liver injury lasts for 24 hr in mice and 72 hr in rats.

Marchand et al. (1970, 1971) have reported that the protective effect of SKF-525A on CCl_4 -induced liver injury is due not only to the inhibition of microsomal drug oxidation but also to decreasing the gastrointestinal absorption of CCl_4 . As shown in Fig. 2, however, malotilate exerted a protective effect on liver injury even when CCl_4 was injected intraperitoneally. This result suggests that the protective action of

malotilate is not mediated through the decrease in CCl₄ absorption.

The liver injury by CCl₄ is considered to be due to the metabolic activation of CCl₄ to form the covalent binding to cellular protein (Rao and Recknagel, 1969) and is known to be enhanced by inducers of microsomal drug oxidation such as phenobarbital (Suarez et al., 1972). Katoh et al. (1981) have reported that malotilate inhibits microsomal drug oxidation 4 hr after the treatment and induces it after 24 hr to 48 hr in the liver of rats. When malotilate is given to rats several hours prior to the administration of CCl₄, it probably inhibits the metabolic activation of CCl₄ and consequently suppresses the liver injury. Malotilate was effective, however, even when CCl₄ was given 24 hr or more later at which the metabolic activation is rather induced or not inhibited by malotilate. It is characteristic of malotilate that it, unlike inducers such as phenobarbital, suppresses CCl₄-induced liver injury in spite of the induction of microsomal drug oxidation.

Suarez et al. (1975) have described that 3-methylcholanthrene prevents CCl₄-induced liver injury by the way of stabilization and induction of a lipid peroxidation inhibitor that presents in the soluble fraction of liver homogenate. Some antioxidants such as vitamin E (Benedetti et al., 1974; Pesh-Imam and Recknagel., 1977) and zinc (Chvapil et al., 1973) were also found to inhibit the peroxidative decomposition of lipid and reduce CCl₄-induced liver injury. Egashira et al. (1982) indicated that malotilate prevents the lipid peroxidation induced by CCl₄. Furthermore, Imaizumi et al. (1981) have reported that malotilate exerts a therapeutic effect on CCl₄-induced fatty liver by repairing the protein synthesis lowered by CCl₄ in liver microsomes. The protective effect may be due not only to inhibiting the metabolic activation of CCl₄ but also to inducing the inhibitory factor(s) of the lipid peroxidation and/or acting on the protein synthesis.

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