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Protective Action of Desoxypodophyllotoxin on D-Galactosamine-induced Liver Lesion in Rats[1)]

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Pretreatment of rats with desoxypodophyllotoxin for 4 d (day −3 to day 0) has been found to inhibit the increase of serum transaminase activities at 24 h after D-galactosamine injection (day 0) in a dose-dependent manner and to prevent histological deterioration of the liver. In the case of pretreatment with desoxypodophyllotoxin, serum transaminase activities were lower than those of the control group until 24 h after D-galactosamine injection, but rather higher than the control levels afterwards. Successive treatment with desoxypodophyllotoxin for 6 d (day −3 to day 2) markedly inhibited the increase of serum enzyme activities induced by D-galactosamine up to the end of the experiment (day 4). Concerning the mechanism of the liver-protective action of desoxypodophyllotoxin, it is suggested that the stabilizing effect of desoxypodophyllotoxin on the plasma membrane may play an important role in protecting the liver from lesions caused by D-galactosamine.

Keywords——desoxypodophyllotoxin; D-galactosamine; hepatotoxicity; liver-protective effect; rat

During the course of our studies to substantiate the liver-protective actions of traditional crude drugs and to clarify active principles in the drugs, we previously isolated desoxypodophyllotoxin from "asunaro-yo", *Thujopsis dolabrata* leaves, as the liver-protective principle against carbon tetrachloride (CCl_4) hepatotoxicity in mice.[2)]

Desoxypodophyllotoxin is known as one of the major principles of podophyllin, which has been used as a cathartic and cholagogue.[3)] Although several reports have been published on the cytological, biochemical and pharmacological actions of podophyllin,[3)] the mechanism of the liver protective action of desoxypodophyllotoxin has not been reported.

In the present work, therefore, the effects of desoxypodophyllotoxin on D-galactosamine-induced liver damage[4)] in rats have been evaluated, since the experimental liver lesions are now recognized to be rather like those of viral hepatitis in humans from both the morphological and functional point of view.[5)] Further, we have tried to examine the mechanism of the liver-protective action of desoxypodophyllotoxin.

Materials and Methods

Materials——Desoxypodophyllotoxin was isolated from podophyllin (from *Podophyllum hexandrum* Royle, E. Merck). Other chemicals were obtained from usual commercial sources.

Measurement of the Activity of the Serum Transaminases——Five male rats of the Wistar strain (weighing 160—180 g) were employed per group. Pretreatment with desoxypodophyllotoxin was effected by administering a suspension in physiological saline solution containing 3% gum arabic subcutaneously once daily for 4 d (day −3 to day 0), with D-galactosamine (500 mg/kg) in the saline solution being injected intraperitoneally at 3 h after the last treatment with desoxypodophyllotoxin (day 0). For successive treatments, desoxypodophyllotoxin was given for 2 more days after D-galactosamine injection (day −3 to day 2). Blood was obtained from cardiac puncture or by tail cutting at 24 h or periodically after D-galactosamine injection. Serum glutamic-oxalacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) activities were measured according to the method of Karmen[6)] using an auto-analyzer.

Histological Examination——The livers tested were 1) those obtained from rats at 27 h after the last dosing in the desoxypodophyllotoxin treatment (5 mg/kg/d, day −3 to day 0), 2) those obtained from rats at 24 h after the D-galactosamine injection (day 0), and 3) those obtained from rats at 24 h after the D-galactosamine injection (day 0) with the desoxypodophyllotoxin pretreatment (5 mg/kg/d, day −3 to day 0). Tissues from the livers were fixed with 10% formalin, processed in the usual way, stained with hematoxylin and eosin, and examined microscopically.

Statistical Analysis——The data are given as mean±S.E. and statistical significance was evaluated by one-way analysis of variance.

Results

Effect of Desoxypodophyllotoxin on Normal Liver

Desoxypodophyllotoxin at the dose of 5 mg/kg/d for 4 d produced no changes in SGOT and SGPT levels at 27 h after the last dosing (95±5 and 8±2 IU/l, respectively) as compared with those of normal animals (90±13 and 12±3 IU/l, respectively). On histological examination, the livers of the desoxypodophyllotoxin-treated group (5 mg/kg/d for 4 d) showed no appreciable changes in comparison with those of normal animals except that slight nodular regeneration was observed in the peripheral portion of the acini in the former (Fig. 1).

Effect of Desoxypodophyllotoxin on Liver Damage induced by D-Galactosamine

1) Dose-dependency of Liver Protective Effect of Desoxypodophyllotoxin——A single administration of D-galactosamine (500 mg/kg) to rats caused a remarkable increase of SGOT and SGPT activities after 24 h (Table I). Pretreatment with desoxypodophyllotoxin at doses of 0.6, 1.3, 2.5 and 5.0 mg/kg/d for 4 d inhibited the D-galactosamine-induced increase of SGOT and SGPT activities in a dose-dependent manner at 24 h after the hepatotoxin dosing (Table I). Histological examinations were carried out on the livers of rats treated with D-galactosamine alone and with D-galactosamine after the desoxypodophyllotoxin administration (5 mg/kg/d, day −3 to day 0) at 24 h after the D-galactosamine injection. In the group treated with D-galactosamine alone, tiny and focal necrosis, proceeding to granulocytic infiltration, was observed in hepatic tissues. Other liver cells showed single necrosis, forming Mallory bodies. Fatty degeneration containing droplets in the cytoplasm was also observed (Fig. 2). In the

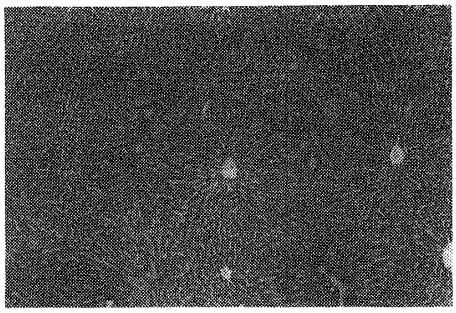

Fig. 1. Section from the Liver of a Rat treated with Desoxypodophyllotoxin alone

Magnification, ×100.

Table I. Effect of Desoxypodophyllotoxin on D-Galactosamine-induced Increase of Serum Transaminase Activity in Rats

Dose (mg/kg/d)	No. of rats	SGOT		SGPT	
		IU/l	%	IU/l	%
0	5	2921±788	100	1640±521	100
0.6	5	1821±479	62	1188±350	72
1.3	5	1073±429	37	594±257	36
2.5	5	900±116[a)]	31	535± 73	33
5.0	5	502±168[a)]	17	224±152[a)]	14

Desoxypodophyllotoxin was given *s.c.* (day −3 to day 0). D-Galactosamine (500 mg/kg) was dosed at 3 h after the last injection of desoxypodophyllotoxin and 24 h thereafter transaminase activity was determined.
a) Significantly different from the control, $p<0.05$.

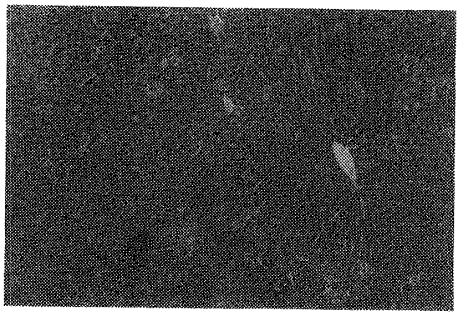

Fig. 2. Section from the Liver of a Rat treated with D-Galactosamine
Magnification, ×100.

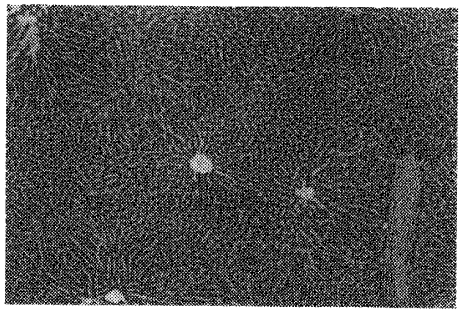

Fig. 3. Section from the Liver of a Rat treated with Desoxypodophyllotoxin and D-Galactosamine
Magnification, ×100.

group pretreated with desoxypodophyllotoxin (5 mg/kg/d, day −3 to day 0), focal necrosis was not observed and instead binucleated liver cells were found (Fig. 3).

2) **Time-course of the Liver Protective Effect of Pretreatment with Desoxypodophyllotoxin**——SGOT and SGPT activities of the rats treated with D-galactosamine alone and with D-galactosamine after the desoxypodophyllotoxin pretreatment were measured periodically. As shown in Fig. 4, both SGOT and SGPT values of the D-galactosamine-treated group reached maximum values at 24 h after dosing of the hepatotoxin and recovered to approximately within normal limits at 96 h after the hepatotoxin treatment. SGOT and SGPT values of the desoxypodophyllotoxin-pretreated group (5 mg/kg/d, day −3 to day 0) were kept at low levels until 24 h after D-galactosamine dosing, but attained higher levels subsequently than those of the control group. Maximum values were reached at around 48 h after the hepatotoxin injection.

3) **Time-course of Liver Protective Effect of Successive Treatments with Desoxypodophyllotoxin**——SGOT and SGPT activities of rats treated with desoxypodophyllotoxin for a further

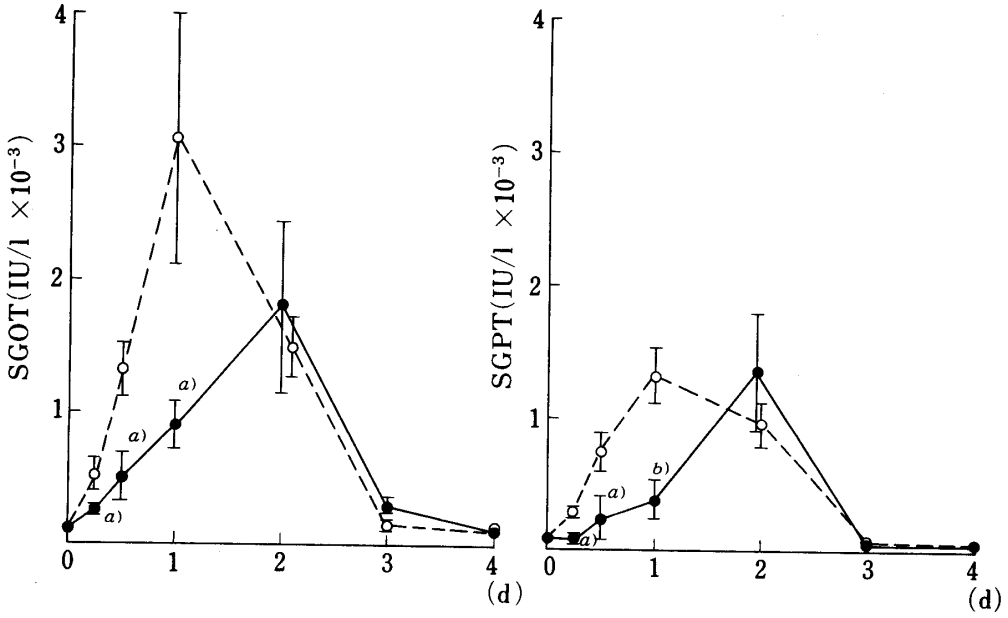

Fig. 4. Effect of Pretreatment with Desoxypodophyllotoxin on Serum Transaminase Activity in Rats given D-Galactosamine

——○——: control, ——●——: desoxypodophyllotoxin (5 mg/kg/d, day −3 to day 0), $n=5$.
Significantly different from the control, *a*) $p<0.05$ or *b*) $p<0.01$.

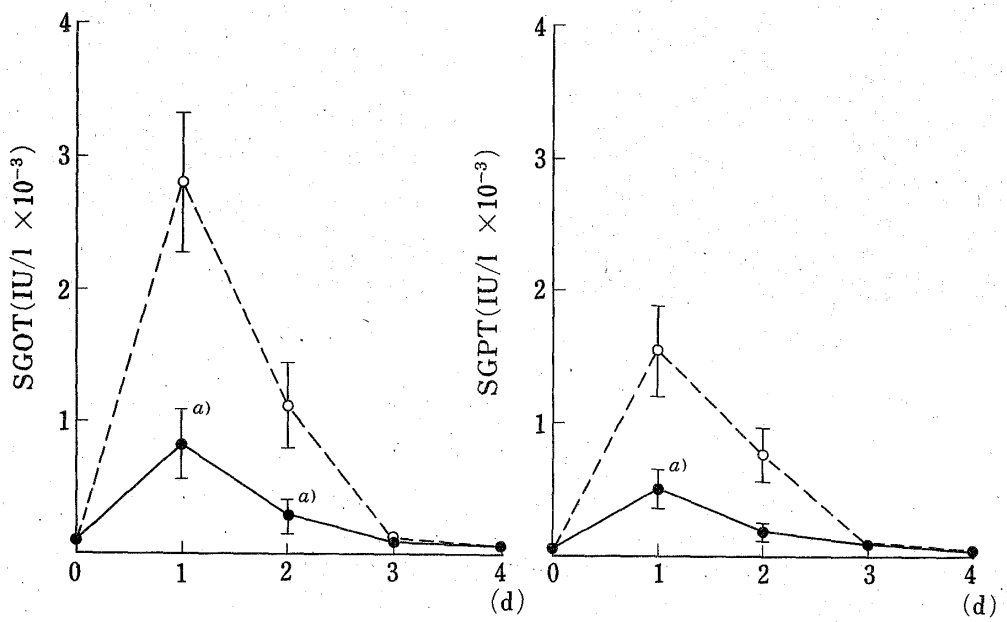

Fig. 5. Effect of Successive Treatment with Desoxypodophyllotoxin on Serum Transaminase Activity in Rats given D-Galactosamine

—○—: control, —●—: desoxypodophyllotoxin (5 mg/kg/d, day −3 to day 2), $n=5$.
a) Significantly different from the control, $p<0.05$.

2 d after the D-galactosamine injection were measured periodically. As shown in Fig. 5, the increase of SGOT and SGPT activities of the rats treated with desoxypodophyllotoxin successively (5 mg/kg/d, day −3 to day 2) was inhibited until 96 h after the D-galactosamine injection, and a significant reduction was observed at 24 and 48 h after the hepatotoxin administration.

Discussion

Administration of desoxypodophyllotoxin alone resulted in no significant changes in biochemical and morphological parameters as compared with those of normal rats. A single administration of D-galactosamine produced a remarkable increase of SGOT and SGPT values, and caused focal necrosis and disarrangement of liver cells at 24 h after the hepatotoxin injection. However, in rats pretreated with desoxypodophyllotoxin before D-galactosamine injection, neither necrosis nor inflammatory infiltration was observed, and an increase of SGOT and SGPT activities was inhibited at 24 h after D-galactosamine dosing. Desoxypodophyllotoxin has thus been concluded to exhibit a protective action against D-galactosamine-induced liver damage.

Pretreatment with desoxypodophyllotoxin was observed to reduce transaminase levels until 24 h after the D-galactosamine administration, but unexpectedly rather increased them compared with control group levels at around 48 h after the hepatotoxin dosing (Fig. 4). Therefore, an explanation of this unexpected feature in the time-course was sought. When desoxypodophyllotoxin was administered for 2 d after D-galactosamine injection in addition to the pretreatment, and SGOT and SGPT activities were measured periodically, both enzyme activities remained at low levels up to the end of the experiment (day 4) as shown in Fig. 5, indicating that D-galactosamine-induced liver damage was prevented by the successive treatment with desoxypodophyllotoxin up to 96 h after the hepatotoxin treatment. On the basis of the above finding, it is concluded that the effective period of desoxypodophyllotoxin is rather short as compared with the toxic period of D-galactosamine, so that SGOT and SGPT activities of the rats pretreated with desoxypodophyllotoxin were reduced at the initial stage

but increased afterwards to those of the control group. As indicated in Fig. 5, when rats received desoxypodophyllotoxin successively after D-galactosamine injection, increases of these enzyme activities were apparently inhibited over the whole experimental period.

D-Galactosamine is known to induce a selective depletion of uridine phosphate and UDP-sugars in the liver with a consequent inhibition of synthesis of nucleic acids, glycoproteins and glycolipids, which are regarded as the first steps in the sequence of events leading to hepatocellular necrosis.[4,8] The early appearance of the alteration of the plasma membrane parallels the increase of the activities of SGOT and SGPT, supporting the existence of a specific correlation between the early alteration in plasma membrane and the induction of D-galactosamine-induced liver damage.[9] While glycyrrhizin and saikosaponin are also known to prevent D-galactosamine-induced liver damage, the stabilizing effects of these drugs on the plasma membrane are thought to play an important role in protecting the liver from the damage caused by D-galactosamine.[10,11] It is thus suggested that the liver-protective effect of desoxypodophyllotoxin is elicited at least partly by the stabilizing action on the plasma membrane.

References and Notes

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