The Protective Effects of (4R)-Hexahydro-7,7-Dimethyl-6-Oxo-1,2,5-Dithiazocine-4-Carboxylic Acid (SA3443), a Novel Cyclic Disulfide, on Chemically-Induced Acute Liver Injury

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Received August 3, 1990 Accepted December 6, 1990

ABSTRACT—The effects of SA3443, a novel cyclic disulfide compound, on acute liver injuries induced by carbon tetrachloride (CCl₄), D-galactosamine and DL-ethionine were studied in rats or mice. SA3443 (100–300 mg/kg, p.o.) significantly suppressed the increases of serum transaminase activity and liver triglyceride content in the CCl₄ or DL-ethionine-induced model. Furthermore, SA3443 (300 mg/kg, p.o.) clearly reduced the formation of hepatic lipid peroxide in CCl₄-treated rats. These results indicate that SA3443 protects the liver against acute liver injury.

It is well-known that the administration of carbon tetrachloride (CCl₄), ethionine and galactosamine lead to various forms of liver damage (1, 2). These chemically-induced liver injuries include membrane fragility, enzyme leakage and pathological degeneration of the hepatotoxic mechanism (2). These chemically-induced liver injury models have therefore been utilized to screen and investigate many hepatoprotective agents.

(4R)-Hexahydro-7,7-dimethyl-6-oxo-1,2,5-dithiazocine-4-carboxylic acid (SA3443) is a novel cyclic disulfide compound that was developed as an anti-hepatitis agent. In pharmacological studies, SA3443 was found to be effective in preventing immunologically-induced liver injuries in models (3). These findings suggest that SA3443 would be useful as a protective agent in the treatment of liver diseases. The purpose of the present study was to clarify the hepatoprotective effects of SA3443, using three classical models, CCl₄-, ethionine- and galactosamine-induced acute liver injuries in rats or mice.

Male Wistar rats weighing 200–240 g were used for the CCl₄ and D-galactosamine-induced liver injury model, and male BALB/c mice weighing 20–25 g were used for the ethionine model. To induce liver injuries using CCl₄ and galactosamine, the animals were fasted for 18 hr before intoxication. CCl₄ (5 ml/kg) in the form of a 5% (v/v) solution in olive oil was given to rats by intraperitoneal (i.p.) injection. D-Galactosamine (400 mg/kg) was given by i.p. injection. SA3443 suspended in a 1.0% (w/v) methylcellulose solution was administered p.o. at doses of 100 and 300 mg/kg 30 min before CCl₄ or galactosamine intoxication. The animals were killed 24 hr after the injection of CCl₄ or galactosamine, and blood samples were taken. The transaminase activities were then measured according to the method used by Karmen (4). The liver weight was measured at the time of killing. DL-
Ethionine (1 g/kg) was given by i.p. injection in the form of a 10% (w/v) solution in saline daily for three successive days. SA3443 was administered by p.o. administration at doses of 100 and 300 mg/kg 30 min before ethionine treatment. Twenty-four hours after the last injection of ethionine, serum transaminase activities and triglyceride content were measured. The liver was perfused with saline through the portal vein, excised and homogenized in ten volumes of the same solution. The hepatic triglyceride content was then measured. Hepatic triglyceride was extracted using the procedure described by Folch et al. (5), and its content determined by an assay kit (Determiner TG-S555). To examine the effect of SA3443 on lipid peroxidation, the assay of lipid peroxide levels in CCl₄-treated rat liver was carried out according to the procedure described by Ohkawa et al. (6) as follows: 10% (w/v) liver homogenate was mixed with sodium dodecyl sulfate, acetate buffer (pH 3.5) and an aqueous solution of thiobarbituric acid (TBA). After heating at 95°C for 60 min, the red pigment produced was extracted using an n-butanol-pyridine mixture and then estimated by its absorbance at 532 nm. The results were taken as the TBA value. TBA was expressed as nmoles of malondialdehyde per mg of protein for the homogenate samples. Results were expressed as the mean ± S.E.M. Significance of the difference was analyzed by Dunnett's t-test and Student's t-test.

Serum GOT and GPT activities were remarkably increased at 24 hr after the CCl₄ injection. SA3443 was found to inhibit increases in serum GOT and GPT activity at the doses of 100 and 300 mg/kg in a dose-dependent manner (Table 1). The inhibitory effect of SA3443 was significant at the dose of 300 mg/kg. This effect was also histopathologically supported (data not shown). In order to confirm the protective effects of SA3443 on the cell membrane, the lipid peroxidation products in liver homogenates prepared from CCl₄-intoxicated rats were determined. Similar to findings described in the literature (7), a preliminary study showed the level of lipid peroxide to be significantly increased 6 to 12 hr after injection of CCl₄ (data not shown). As shown in Table 1, SA3443, at a dose of 300 mg/kg, significantly reduced the lipid peroxide at 9 hr after CCl₄-intoxication. In body and liver weight, there were no significant differences between the groups in CCl₄-treated animals (data not shown).

As shown in Fig. 1, the serum GOT and GPT activities in mice were remarkably increased at 24 hr after the last injection of ethionine. The treatment with SA3443 (100 and 300 mg/kg) significantly suppressed the increase in serum GOT and GPT activity in a dose-dependent manner. The triglyceride content in the livers of mice injected with

<table>
<thead>
<tr>
<th>Groups</th>
<th>GOT (U/L)</th>
<th>GPT (U/L)</th>
<th>Lipid peroxide (nmol MDA/100 mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41 ± 4 (15)</td>
<td>16 ± 2 (15)</td>
<td>62.7 ± 7.9 (4)</td>
</tr>
<tr>
<td>CCl₄</td>
<td>6304 ± 432* (15)</td>
<td>4259 ± 391* (15)</td>
<td>82.7 ± 8.8* (8)</td>
</tr>
<tr>
<td>CCl₄ + SA3443, 100 mg/kg</td>
<td>5183 ± 283 (15)</td>
<td>3301 ± 330 (15)</td>
<td>78.1 ± 10.9 (7)</td>
</tr>
<tr>
<td>CCl₄ + SA3443, 300 mg/kg</td>
<td>4250 ± 531* (15)</td>
<td>2578 ± 265* (15)</td>
<td>62.7 ± 11.5* (8)</td>
</tr>
</tbody>
</table>

CCl₄ (5 ml/kg) was given i.p. as a 5% (v/v) solution in olive oil. SA3443 was given p.o. 30 min before CCl₄-intoxication. A sample of blood was taken 24 hr after intoxication for the estimation of serum transaminase activity. The level of lipid peroxide in the liver was determined 9 hr after CCl₄-intoxication. Each value represents the mean ± S.E. The number of animals is indicated in parentheses. *: Statistically significant differences between the control and CCl₄-treated group, P < 0.01. #: Statistically significant differences between the CCl₄ and CCl₄ + SA3443-treated group, P < 0.05.
ethionine was approximately three times higher than that of the control. SA3443 significantly reduced the accumulation of hepatic triglyceride content at the doses of 100 and 300 mg/kg (Fig. 1). On the other hand, the serum triglyceride content was decreased by the ethionine-intoxication. The treatment with SA3443 (300 mg/kg) significantly suppressed the decrease of serum triglyceride levels (Fig. 1).

Twenty-four hours after the galactosamine injection, a remarkable increase in serum transaminase activity was observed. However, the models treated with SA3443 did not show a significant decrease in serum transaminase activity (data not shown).

The present study showed that SA3443 can prevent acute liver injury induced by carbon tetrachloride and ethionine. The treatment with SA3443 suppressed increases in serum transaminase activity in these liver injuries, and it inhibited the accumulation of hepatic triglyceride in the ethionine-induced model. These results suggest that SA3443 has a hepatoprotective effect. As reported by Nakata et al., SA3443 was also found to be a protective compound in immunological hepatic injuries (3). These findings indicate that SA3443 could be an effective drug in the treatment of liver diseases.

Several nonspecific protective mechanisms against hepatotoxins have been reported. They are as follows: the stimulation of hepatic regeneration, which makes the liver resistant to damage by hepatotoxins (8) and activation of the functions of the reticuloendothelial system (9) or the biosynthesis of protein (10). The exact mechanism by which SA3443 exerts its hepatoprotective effect is unknown. However, since SA3443 can prevent the production of lipid peroxide induced by CCl₄, we consider the hepatoprotective effect of SA3443 to be in part linked to the elimination of CCl₄-derived free radicals. In fact, it has been reported that thiol and certain disulfide compounds, including cystamine and tiopronin, appeared to act as free radical scavengers, and that these compounds showed protective
effects on CCl₄-induced liver injury (11–13). Based on these findings, we suggest that SA3443 can protect some functions of the hepatocytes, such as membrane enzyme activity, by acting as a free radical scavenger.

It was reported that ethionine-hepatotoxicity leads to a disturbance in the biosynthesis of S-adenosylmethionine and to a deficiency in adenosinetriphosphate (14, 15). This hepatotoxin leads to the inhibition of plasma membrane protein synthesis, thus provoking cell membrane defects and enzyme leakage. In our experiments, we showed that treatment with SA3443 prevented the leakage of transaminase and improved steatosis in liver injury induced by ethionine. Thus, the protective effect of SA3443 may derived in part from protection of the membrane.

In conclusion, SA3443, a novel cyclic disulfide compound, clearly prevented acute liver injuries induced by CCl₄ and ethionine.

Acknowledgments
We would like to thank Mr. M. Fukumoto, Mr. H. Tanioka and Ms. C. Setoguchi for their excellent technical assistance.

REFERENCES