EFFECT OF 1,3-DITHIA-2-THIOXO-CYCLOPENT-4-ENE AND ITS DERIVATIVES ON LIVER INJURY INDUCED BY CARBON TETRACHLORIDE AND OROTIC ACID IN RATS

Shinro SADANOBU, Masaya TAKEUCHI* and Masakatsu TEZUKA

Department of Hygienic Chemistry, College of Pharmacy, Nihon University
7-7-1, Narashinodai, Funabashi, Chiba 274, Japan
* Sapporo General Pathology Laboratory Co., Ltd.
3-17, Minami-12, Nishi-18, Chuo-ku, Sapporo, Hokkaido 064, Japan

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ABSTRACT — The protective effect of 1,3-dithia-2-thioxo-cyclopent-4-ene (DT827A) and its two derivatives of 4-phenyl-1,3-dithia-2-thioxo-cyclopent-4-ene (DT827B) and 4-(4-fluorophenyl)-1,3-dithia-2-thioxo-cyclopent-4-ene (DT827C) on liver injury induced by carbon tetrachloride (CCl₄) and orotic acid was studied using male rats. The approximate lethal doses were about 100mg/kg for DT827A-treated animals and more than 800mg/kg for the other two compounds-treated groups. Single oral administration of the three test compounds at the dose levels of 2 and 10 mg/kg 1 hour before CCl₄ exposure revealed a protective effect on the findings of centrolobular necrosis, balloon cells and macrophage infiltration in histopathological findings in livers in the order of DT827B-treated rats > DT827A-treated rats > DT827C-treated rats. Repeated oral administration of the compounds at the dose levels of 2 and 10 mg/kg/day for 10 consecutive days revealed a protective effect against liver injury on the findings of centrolobular necrosis, balloon cells and macrophage infiltration in the order of DT827B-treated rats > DT827A-treated rats ≈ DT827C-treated rats. Simultaneous administration of the compounds at the dose level of 10 mg/kg/day together with a high sucrose diet containing orotic acid for 12 days revealed an inhibitory effect on fatty liver formation in the order of DT827B-treated rats > DT827C-treated rats > DT827A-treated rats. A hepatoprotective potential of the DT827 series compounds was suggested under the conditions of these studies, and DT827B was considered to be the most effective.

KEY WORDS : Hepatoprotective effect, Carbon tetrachloride, Orotic acid, Rat, SH-group

INTRODUCTION

Many studies in animals have been performed to demonstrate protection against liver injury caused by various substances such as cadmium, lead, methyl mercuric chloride, carbon tetrachloride (CCl₄), alcohol and paraquat, through administration of organic substances containing, or potentially having, an SH-functional group such as cysteine (Aikawa et al., 1972; Iida et al., 1978; Sugiyama et al., 1975; Fuji et al., 1967; Kitahara, 1971; Nagasaki et al., 1972); thiosulfate (Yamamoto, 1993); and acetyl cysteine (Valles et al., 1994). In these studies, the authors reported that the administration of such compounds reduced liver injury as judged by biochemical tests, and, in part, histopathology. These reports discussed detoxification activities of amino acids having an SH-group, and focused on their important role in activating enzyme reaction in the detoxification process in the liver. Furthermore, Koga et al. reported

Correspondence : Shinro SADANOBU at the above address.
(24th Southeast Regional Meeting of the Japan Society of Pharmacology) the effect of L-cysteine on liver injury induced by CCl₄ in rats (both single and repeated oral administration) as reflected in changes in the parameters of GOT, GPT, and triglyceride.

We had an opportunity to obtain the series compounds of 1,3-dithia-2-thioxo-cyclopent-4-ene (DT827A) and its two derivatives of 4-phenyl-1,3-dithia-2-thioxo-cyclopent-4-ene (DT827B) and 4-(4-fluorophenyl)-1,3-dithia-2-thioxo-cyclopent-4-ene (DT827C), whose chemical structures are shown in Fig.1. DT827A was synthesized by F. Challenger et al. in 1952 (Challenger et al., 1952), and has been used as an intermediate for the synthesis of other compounds, but its pharmacological effects have not been studied. Since compounds having a dithiol function are widely known as hepatoprotective drugs such as diisopropyl-1,3-dithiol-2-ylidenemalonate (malotilate) (Imaizumi, et al., 1981; Katoh and Sugimoto, 1982; Wakasugi et al., 1985), the studies presented were carried out using the three DT827 series compounds to confirm the hepatoprotective effect of DT827A and to determine whether the two derivatives would have more potential than DT827A in their hepatoprotective effects.

**MATERIALS AND METHODS**

**Materials and Animals**

DT827A, DT827B and DT827C were supplied from the laboratories of SS Pharmaceutical Co., Ltd. (Tokyo, Japan), which synthesized the three compounds. CCl₄ was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and orotic acid and olive oil were obtained from Katayama Chemical Industries Co. (Osaka, Japan). Other chemicals used were of reagent grade.

Male Wistar/ST strain rats purchased from Japan SLC, Inc. (Shizuoka, Japan) were used in this study.

**Acute Toxicity Test**

Five-week-old male Wistar/ST rats were acclimated for one week before being used for this study. Throughout the acclimatization period and test period, MM-3 diet (Funabashi Farm Co., Ltd.; Chiba, Japan) and water were given ad libitum.

Test compounds were suspended in olive oil at a concentration of 1.25% or 5%, and were orally given via a polyethylene tube at the dose levels of 50, 100 and 200 mg/kg for DT827A, and 400 and 800 mg/kg for both DT827B and DT827C. General symptoms were continuously observed for 6 hours following administration, and then once or more per day for the subsequent 7 days. Body weight was measured immediately before administration, and again on day 3 and day 7. Gross pathology was performed immediately on all dead animals and the surviving animals which were sacrificed on day 7. Histopathological examination was performed immediately on all dead animals and some of the surviving animals which were sacrificed on day 7. Tissues examined were liver, kidney, spleen, heart, lung, and any tissue where any abnormality was observed in gross pathology. The tissues were preserved in 10% formalin, embedded in paraffin wax, and were stained

![Fig. 1. Chemical Structures of DT827A, DT827B and DT827C.](image-url)

DT827A: 1,3-dithia-2-thioxo-cyclopent-4-ene, DT827B: 4-phenyl-1,3-dithia-2-thioxo-cyclopent-4-ene, DT827C: 4-(4-fluorophenyl)-1,3-dithia-2-thioxo-cyclopent-4-ene.
with hematoxylin and eosin.

**Acute liver injury induced by CCl₄**

Five-week-old male Wistar/ST rats were acclimatized for one week with diet (MM-3) and water given ad libitum. The rats were divided into 7 groups comprising the CCl₄-treated group (exposed only to CCl₄) and groups which received 2 and 10 mg/kg each of DT827A, DT827B and DT827C.

DT827A, DT827B and DT827C were dissolved or suspended in olive oil at concentrations of 0.5% for the 2 mg/kg-treated group and 2.5% for the 10 mg/kg-treated group, and orally administered to animals using a polyethylene tube one hour before exposure to CCl₄. 1.5 ml/kg of CCl₄ mixed with olive oil (6 ml/kg as the mixture) was administered subcutaneously to all animals. Forty-eight hours after exposure to the CCl₄, the animals were sacrificed by exsanguination under pentobarbital anesthetization. To determine biochemical parameters, the serum activities of the three enzymes, GOT, GPT and Al-p were measured. Liver weights were measured and relative weights were calculated. The liver tissues were fixed for histopathology and the result was presented in the 5 grades.

**Liver injury induced by administration of orotic acid with diet mixture**

Five-week-old male Wistar/ST rats were acclimatized for 2 weeks with water and animal diet without orotic acid, prepared in our laboratory, having the following formulation of sucrose (60%), vitamin-free casein (20%), corn oil (4%), salt mixture (4%), cellulose (10%) and vitamin mixture (1%), ad libitum. The rats were divided into 5 equal groups comprising a high sucrose control group, a high sucrose plus 1% orotic acid-treated group, and 3 groups to which a diet of high sucrose plus 1% orotic acid were fed and furthermore either 10 mg/kg of DT827A, DT827B or DT827C was administered.

DT827A, DT827B and DT827C were dissolved in olive oil at a concentration of 0.125% and were administered orally to each rat in the respective groups by polyethylene tube twice daily (morning and evening) for 12 successive days. To all the groups except the high sucrose control group, a high sucrose diet plus 1% orotic acid was fed ad libitum for the 12 days. To the high sucrose control group, the high sucrose diet without orotic acid was fed ad libitum. Body weight was measured twice weekly and the animals were sacrificed on day 13 by exsanguination under pentobarbital anesthetization. Biochemical parameters measured for all the sacrificed animals included the enzymes GOT and GPT, triglyceride (TG; Spayd et al., 1978), total cholesterol (T-CHO; Allain et al., 1974) and phospholipid (PL; Takayama et al., 1977, Enzyme Method: Serotec Ltd. Oxford, England). Liver weight was measured and rela-
tive weight was calculated. Liver TG values were obtained from homogenized liver in Folch solution (chloroform : methanol = 2 : 1). Liver tissues were fixed and histopathology results presented in the 5 grades.

Statistical analysis

The Student t-test was used for statistical analysis.

RESULTS

Single dose toxicity of the test compounds

The incidence of death in DT827A-, DT827B- and DT827C-treated animals is shown in Table 1. One animal in the 100mg/kg-treated group of DT827A died one day after administration, and all animals in the 200 mg/kg-treated group were dead by 2 days following administration of the drug. However, all animals in the DT827B- and DT827C-treated groups survived up to 400 mg/kg dosage.

General symptoms of the dead animals in the DT827A-treated group included dacryohemorrhhea, salivation, and decrease of spontaneous movement after one or after several hours following administration, with some animals showing tremor before dying. Some surviving animals showed symptoms similar to those of animals that died together with other symptoms such as piloerection on the day of administration, but no symptoms were apparent 6 hours to 3 days after drug administration. The 100 mg/kg-treated group of DT827A showed decreased body weight three days after the drug administration, but body weight tended to be regained in 7 days. In gross findings of rats that died in all DT827-treated groups, there were indications of injury to digestive organs, but there were no abnormal findings in surviving animals. In histopathological findings of rats that died in the DT827-treated groups, changes were observed in the liver, kidney and mucous membrane of digestive organs, but there were no abnormal findings in surviving animals of all groups except for a few unrelated findings.

Effect of DT827A, DT827B and DT827C on acute liver injury induced by CCl4

Serum GOT and GPT values are shown in Fig. 2. The serum GOT value of the CCl4-treated group was significantly increased, and similar increases were observed in the 2 mg/kg DT827A-treated group. The GOT values of the other DT827-treated groups showed equally moderate increases, but the increases were less than those of the CCl4-treated group. The serum GPT value of the CCl4-treated group increased significantly, and the GPT values of the DT827A-, DT827B- and DT827C-treated groups were significantly less than the CCl4-treated group, and in particular, the DT827 10 mg/kg-treated groups showed no increase in GPT levels. Serum Al-p value of the CCl4-treated group increased significantly, but the values of Al-p in all DT827A-, DT827B- and DT827C-treated groups, while greater than normal, were significantly less than the CCl4-treated group (data not shown).

In liver weight measurements, net weights of the 2 and 10 mg/kg-treated groups of DT827A and 10 mg/kg-treated group of DT827B were increased significantly in comparison with the CCl4-treated group, and relative weights of 2 and 10 mg/kg-treated groups of DT827A were also increased. Liver histopathological findings are shown in Table 2. In the CCl4-treated group, either minimal or mild findings of centrolobular necrosis, balloon cells and macrophage infiltration were observed in all groups as well as either moderate or mild incidence of centrolobular lipid droplets. In the DT827A-treated groups, less centrolobular necrosis and macrophage infiltration were

<table>
<thead>
<tr>
<th>Table 1. Incidence of death after single oral administration of DT827A, DT827B and DT827C to rats.</th>
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</thead>
<tbody>
<tr>
<td><strong>Compound</strong></td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>DT827A</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td>DT827B</td>
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<tr>
<td></td>
</tr>
<tr>
<td>DT827C</td>
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<td></td>
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</tbody>
</table>

* Mortality is presented with the animal numbers of dead/treated.
Fig. 2. Serum GOT and GPT values of rats treated with DT827A, DT827B and DT827C on acute liver injury induced by exposure to CCl₄.
CCl₄ (s.c., 1.5 ml/kg) was exposed 1 hour after administration of the test drugs (p.o.). “Control” represents control values from each individual animal of each group before administration of the test drugs, and “CCl₄ (1.5ml/kg) alone” represents values obtained from the CCl₄-treated group without administration of the test drugs. *, **: Significantly different from values in the CCl₄-treated group, p<0.05 and p<0.01, respectively (n=5).

Table 2. Histopathological findings of rats treated with DT827A, DT827B and DT827C on acute liver injury induced by CCl₄.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Findings</th>
<th>Number of Rats</th>
<th>Centrolobular Necrosis</th>
<th>Balloon Cells</th>
<th>Macrophage Infiltration</th>
<th>Centrolobular Lipid Droplets</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>±</td>
<td>+</td>
<td>++</td>
<td>±</td>
</tr>
<tr>
<td>CCl₄ (1.5ml/kg) alone</td>
<td></td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>DT827A (2mg/kg) + CCl₄ (1.5ml/kg)</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>DT827B + CCl₄</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>DT827C + CCl₄</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>DT827A (10mg/kg) + CCl₄ (1.5ml/kg)</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>DT827B + CCl₄</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>DT827C + CCl₄</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

CCl₄ (s.c.: 1.5 ml/kg) was exposed 1 hour after administration of the test drugs (p.o.). "CCl₄ (1.5ml/kg) alone" represents values obtained from the CCl₄-treated group without administration of the test drugs. Each column represents the number of rats with the corresponding finding (±: minimal abnormality, +: mild abnormality, ++: moderate abnormality).
Fig. 3. Serum GOT and GPT values of rats treated with DT827A, DT827B and DT927C on liver injury induced by repeated exposure to CCl₄.
CCl₄ (s.c., 0.75 ml/kg/day) was exposed every other day for 10 days (5 times), and the test drugs (p.o.) were administered every day for the 10 days. "Control (non-treatment)" represents control values obtained from animals of the non-treatment control group, and "CCl₄ (0.75ml/kg x 5) alone" represents values obtained from the CCl₄-treated group without administration of the test drugs. *: Significantly different from value in the CCl₄-treated group, p<0.05 (n=5).

Table 3. Histopathological findings of rats treated with DT827A, DT827B and DT827C on liver injury induced by repeated exposure to CCl₄.

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<thead>
<tr>
<th>Groups</th>
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<th>Balloon Cells</th>
<th>Macrophage Infiltration</th>
<th>Centrolobular Lipid Droplets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (non-treatment)</td>
<td>5</td>
<td>0 0 0 0</td>
<td>0 0 0 0</td>
<td>0 0 0 0</td>
<td>0 0 0 0</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>CCl₄ (0.75ml/kg x 5) alone</td>
<td>5</td>
<td>4 1 0 0</td>
<td>0 1 3 1</td>
<td>3 2 0 0</td>
<td>0 1 3 1</td>
<td>0 1 3 1</td>
</tr>
<tr>
<td>DT827A (2mg/kg x 10) + CCl₄ (0.75ml/kg x 5)</td>
<td>5</td>
<td>4 1 0 0</td>
<td>0 3 1 0</td>
<td>3 1 0 0</td>
<td>0 0 3 2</td>
<td>0 0 3 2</td>
</tr>
<tr>
<td>DT827B + CCl₄</td>
<td>5</td>
<td>4 0 0 0</td>
<td>0 1 3 1</td>
<td>4 0 0 0</td>
<td>0 2 2 1</td>
<td>0 2 2 1</td>
</tr>
<tr>
<td>DT827C + CCl₄</td>
<td>5</td>
<td>3 0 0 0</td>
<td>1 2 1 1</td>
<td>4 0 0 0</td>
<td>0 2 3 0</td>
<td>0 2 3 0</td>
</tr>
<tr>
<td>DT827A (10mg/kg x 10) + CCl₄ (0.75ml/kg x 5)</td>
<td>5</td>
<td>3 0 0 0</td>
<td>1 3 0 0</td>
<td>3 0 0 0</td>
<td>0 0 5 0</td>
<td>0 0 5 0</td>
</tr>
<tr>
<td>DT827B + CCl₄</td>
<td>5</td>
<td>2 0 0 0</td>
<td>0 0 0 0</td>
<td>1 1 0 0</td>
<td>1 0 4 0</td>
<td>1 0 4 0</td>
</tr>
<tr>
<td>DT827C + CCl₄</td>
<td>5</td>
<td>3 0 0 0</td>
<td>1 1 2 0</td>
<td>2 1 0 0</td>
<td>0 2 3 0</td>
<td>0 2 3 0</td>
</tr>
</tbody>
</table>

CCl₄ (s.c.; 0.75 ml/kg/day) was exposed every other day for 10 days (5 times), and the test drugs (p.o.) were administered every day for the 10 days. "Control (non-treatment)" represents control values obtained from animals of the non-treatment control group, and "CCl₄ (0.75ml/kg x 5) alone" represents a value obtained from the CCl₄-treated group without administration of the test drug. Each column represents the number of rats with the corresponding finding (±: minimal abnormality, +: mild abnormality, ++: moderate abnormality, +++: marked abnormality).
observed in the 2 mg/kg-treated group, and fewer balloon cells were observed in the 10 mg/kg-treated group. In the DT827B-treated groups, improvements of the above-mentioned findings were apparent in both the 2 and 10 mg/kg-treated groups. In the DT827C-treated groups, some toxic findings due to exposure to CCl₄ were still observed in 2 cases of the 2 mg/kg-treated group, but were less in 3 cases of the same group, and the protective effect was apparent in the 10 mg/kg-treated group. Similar levels of centrolobular lipid droplets were observed in all groups.

**Effect of DT827A, DT827B and DT827C on liver injury induced by repeated exposure to CCl₄**

In body weight changes of the rats during the experimental period, there were no significant differences among the non-treatment group, the CCl₄-treated group, and DT827-treated groups.

Serum GOT and GPT values are shown in Fig. 3. The serum GOT values in the CCl₄-treated group increased 10-fold or more. The values in the 10 mg/kg-treated groups of DT827A and DT827B were significantly less, but were not in the 2 mg/kg-treated groups of these compounds. In the 2 and 10 mg/kg-treated groups of DT827C, the GOT values were significantly less than the CCl₄-treated group. The serum GPT value in the CCl₄-treated group increased 10-fold or more. In the 10 mg/kg-treated groups of DT827A, DT827B and DT827C, the values were significantly less than the CCl₄-treated group. Serum Al-p values in the CCl₄-treated group increased slightly in comparison with the non-treatment group, and they were similar to those of the DT827-treated groups.

In liver weight there was no statistical difference between the three DT827-treated groups and the CCl₄-treated group.

Histopathological findings in the liver are shown in Table 3 (non-treatment control: Photo 1). In all animals of the CCl₄-treated group,
Photo 2. Liver from a male rat of the CCl4-treated group showing centrolobular necrosis (±), balloon cells (++), macrophage infiltration (±), and centrolobular lipid droplets (++). The rat received 0.75 ml/kg/day of CCl4 every other day for 10 days (5 times). Hematoxylin and eosin staining, ×66.

Photo 3. Liver from a male rat of the DT827A 10mg/kg-treated group showing centrolobular lipid droplets (++). The rat received 0.75 ml/kg/day of CCl4 every other day for 10 days (5 times) and 10 mg/kg/day of DT827A for the 10 days. Hematoxylin and eosin staining, ×66.
Photo 4. Liver from a male rat of the DT827B 10mg/kg-treated group showing centrallobular lipid droplets (+). The rat received 0.75 ml/kg/day of CCl₄ every other day for 10 days (5 times) and 10 mg/kg/day of DT827B for the 10 days. Hematoxylin and eosin staining, ×66.

Photo 5. Liver from a male rat of the DT827C 10mg/kg-treated group showing centrallobular lipid droplets (+). The rat received 0.75 ml/kg/day of CCl₄ every other day for 10 days (5 times) and 10 mg/kg/day of DT827C for the 10 days. Hematoxylin and eosin staining, ×66.
either mild or minimal findings of centrolobular necrosis and macrophage infiltration were observed as well as either mild to marked balloon cells and centrolobular lipid droplets (Photo 2). In the DT827A-treated groups, a diminution in the incidence of balloon cells was observed in the 2mg/kg-treated group and an even greater diminution in other effects, such as centrolobular necrosis, was observed in the 10 mg/kg-treated group (Photo 3). In the DT827B-treated groups, the histopathological findings were similar to the CCl_4-treated group at 2 mg/kg, but the protective effect was significant at 10 mg/kg in terms of centrolobular necrosis, balloon cells and macrophage infiltration (Photo 4). In the DT827C-treated groups, protection was observed at 2 mg/kg and 10 mg/kg in some tissues as evidenced by less centrolobular necrosis (Photo 5).

Effect of DT827A, DT827B and DT827C on liver injury induced by orotic acid

After the third day of administration, body weights of the DT827A-treated groups were less than those in the orotic acid-treated group. The body weights in the DT827B-treated groups were somewhat lower than that of the orotic acid-treated group at 3 and 6 days after the start of administration, but were not significantly less after 9 days. The body weights of the DT827C-treated group were not significantly lower than that of the orotic acid-treated group.

Serum GOT and GPT values are shown in Fig. 4. The serum GOT values of the orotic acid-treated group were significantly increased in comparison with the sucrose control group. In comparison with the orotic acid-treated group, the values of the DT827A-treated group were somewhat less, and those of the DT827B- and DT827C-treated groups were significantly less. The serum GPT values of the DT827A-treated group were somewhat less in comparison with the orotic acid-treated group; the values of the DT827B-treated group were significantly less; and those of the DT827C-treated group were not significantly changed from the control. However, serum TG values of the DT827A-treated group were not significantly changed in

Fig. 4. Serum GOT and GPT values of rats treated with DT827A, DT827B and DT827C on liver injury induced by repeated exposure to orotic acid.

High sucrose animal diet containing 1% orotic acid was fed to the animals for 12 days, and the test drugs (p.o.) were administered every day for the 12 days. "Control (High sucrose diet)" represents control values obtained from animals of the high sucrose control group, and "Orotic Acid (High sucrose diet + 1% orotic acid)" represents values obtained from the orotic acid-treated group without administration of the test drugs. *, **: Significantly different from values in the orotic acid-treated group, p<0.05 and p<0.01, respectively (n=8).
comparison with the orotic acid-treated group, whereas the values of DT827B- and DT827C-treated groups increased, but not significantly. The T-CHO values of the orotic acid-treated group were significantly less than the sucrose control group, and the values of the DT827A- and DT827B-treated groups were similar to the orotic acid-treated group, whereas the values of the DT827C-treated group tended to be closer to the sucrose control value. The PL values of the orotic acid-treated group decreased significantly in comparison with the sucrose control group. There was no statistical difference in the values between the three DT827-treated groups and orotic acid-treated group.

The relative liver weights of the three DT827-treated groups all increased in comparison with the orotic acid-treated group. Liver TG values are shown in Fig. 5. The liver TG values of the DT827B-treated group decreased significantly in comparison with the orotic acid-treated group, whereas a tendency toward decrease and a tendency toward increase were observed in the DT827C and DT827A-treated groups, respectively. Histopathological findings in the liver are shown in Table 4. In the orotic acid-treated group, lipid droplets were observed (1 "marked", 4 "moderate" and 3 "mild"). The following observations of lipid droplets were recorded: in the DT827A-treated group, 2 "moderate", 5 "mild" and 1 "minimal"; in the DT827B-treated group, 1 "moderate", 2

![Liver TG values of rats treated with DT827A, DT827B and DT827C on liver injury induced by repeated exposure to orotic acid.](image)

**Fig. 5.** Liver TG values of rats treated with DT827A, DT827B and DT827C on liver injury induced by repeated exposure to orotic acid.

High sucrose animal diet containing 1% orotic acid was fed to the animals for 12 days, and the test drug (p.o.) was administered every day for the 12 days. "Control (High sucrose diet)" represents control values obtained from animals of the high sucrose control group, and "Orotic Acid (High sucrose diet + 1% orotic acid)" represents values obtained from the orotic acid-treated group without administration of the test drugs. *: Significantly different from value in the orotic acid-treated group, p<0.05 (n=8).

**Table 4.** Histopathological findings of rats treated with DT827A, DT827B and DT827C on fatty liver induced by repeated exposure to orotic acid.

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<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Lipid Droplets</th>
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</thead>
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<tr>
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<td></td>
<td>-</td>
</tr>
<tr>
<td>Control (High sucrose diet)</td>
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<td>0</td>
</tr>
<tr>
<td>Orotic Acid (High sucrose diet + 1% orotic acid)</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>DT827A (10mg/kg x 12) + Orotic acid (1%)</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>DT827B + Orotic acid (1%)</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>DT827C + Orotic acid (1%)</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

High sucrose animal diet containing 1% orotic acid was fed to the animals for 12 days, and the test drug (p.o.) was administered every day for the 12 days. "Control (High sucrose diet)" represents control values obtained from animals of the high sucrose control group, and "Orotic Acid (High sucrose diet + 1% orotic acid)" represents values obtained from the orotic acid-treated group without administration of the test drugs. Each column represents the number of rats with the corresponding finding (-: negative, ±: minimal abnormality, +: mild abnormality, ++: moderate abnormality, +++: marked abnormality).
"mild" and 5 "minimal"; and in the DT827C-treated group, 1 "marked", 1 "moderate", 4 "mild" and 2 "minimal".

DISCUSSION

Animal studies using experimental liver injury induced by various substances such as CCl₄, ethionine, orotic acid and ethyl alcohol are carried out as models in experiments to assess the potential protective effect of substances in humans (Lindstrom et al., 1977; Castro et al., 1972; Glavela et al., 1977; Standerfer et al., 1955; and Liber et al., 1970). The primary causative factor in liver injury induced by CCl₄ is the free radical ·CCI₃ which is generated during an activating process of CCl₄ metabolism by cytochrome-P450 in the liver endoplasmic reticulum. The free radical induces lipid peroxidation, which is the primary cause of the liver injury. Such injury may extend to the mitochondria or cell membrane (Recknagel et al., 1989 and McLean et al., 1965), as well as inducing fatty liver and necrosis of liver cells (Eliakin, 1974; Dinman et al., 1963). When orotic acid is administered in large amounts to rats, biosynthesis or excretion of lipoprotein in the liver (particularly very low density lipoprotein: VLDL) is inhibited, followed by liver injury such as fatty liver (Hay et al., 1988).

This study was performed as an additional contribution to published studies, using the DT827A and its two related compounds. Single oral administration of the three compounds was carried out to determine the approximate lethal doses of the compounds. Compounds DT827B and DT827C appeared to be less acutely toxic than DT827A, but there was no difference among the three compounds in general symptoms. The approximate lethal doses were about 100 mg/kg for DT827A-treated animals, and more than 800 mg/kg for DT827B- and DT827C-treated animals. Accordingly, the maximum experimental dose level was established as 10 mg/kg (one-tenth of DT827A lethal dose) and in consideration of dose levels employed in other similar pharmacological studies.

Treatment with DT827A, DT827B and DT827C at dose levels of 2 mg/kg and 10 mg/kg showed a protective effect on acute liver injury induced by single CCl₄ exposure. The protective effects were observed in serum enzyme activities, particularly, in GPT values, and 10 mg/kg was more effective than 2 mg/kg. However, if the changes in the three values of GOT, GPT and AL-P are considered together, the three compounds show similar protective effects. In histopathological studies of the liver, the three DT827 compounds showed protective effects on centrolobular necrosis, balloon cells and macrophage infiltration induced by CCl₄, and the order of effectiveness was DT827B-treated group > DT827C-treated group > DT827A-treated group. However, a protective effect on centrolobular lipid droplets was not detected in this study.

In liver injury induced by repeated administration of CCl₄ (exposed second day for 10 days; 5 times in total), biochemical parameters such as GOT and GPT values in the CCl₄-treated group showed a significant increase in comparison with those of the non-treatment group, and histopathological findings of the group showed changes in centrolobular necrosis, macrophage infiltration, balloon cells and centrolobular lipid droplets at a range of ± to +++; they suggest production of liver injury in the 10-day study period. The 10 mg/kg-treated groups of DT827A, DT827B and DT827C showed protective effects on both GOT and GPT values. In these groups, centrolobular necrosis, balloon cells and macrophage infiltration incidence decreased as observed in histopathological findings, but no effect on centrolobular lipid droplets was detected. Considering these results, the order of the protective effect of the three DT827 compounds administered at 10 mg/kg was considered to be DT827B-treated group > DT827A-treated group ≈ DT827C-treated group.

The protective effect of the three compounds was studied under the condition of a high sucrose diet containing orotic acid fed to rats for 12 days. By the twelfth daily administration, marked to mild lipid acid were observed in the liver, suggesting production of fatty liver. The production of fatty liver was considered to be due to both inhibition of excretion of VLDL caused by the disorder of assem-
ably of lipoprotein in the endoplasmic reticulum and acceleration of lipid synthesis in liver cells caused by the high sucrose diet (Martin et al., 1982). Fatty liver also developed in the sucrose control group, which had to be considered in interpreting the experimental results. In the DT827-treated groups, histopathological findings suggested various degrees of lessening of lipid droplet formation. The TG values in livers were significantly decreased in the DT827B group with the order of protective effect being DT827B-treated group > DT827C-treated group > DT827A-treated group. In the groups in which the inhibitory effect on fatty liver was observed, the TG values in blood tended to increase in comparison with the orotic acid group, and the effect was most apparent in the DT827B-treated group. It was considered that this result may be due to improvement on lowering of VLDL levels. A tendency of decrease in both GOT and GPT was observed in both the DT827B- and DT827C-treated groups. However, since the two enzyme activities were slightly increased in the orotic acid control group and no hepatocyte injury was observed in histopathological findings, it was considered that the variations of the two biochemical parameters had little significance.

It is speculated that a possible mechanism of the protective properties of DT827A, DT827B and DT827C may be similar to that of the SH-group containing disopropyl 1,3-dithiol-2-yidenemalane (malotilate). Malotilate is reported as being effective in protection against liver injury induced by repeated exposure to CCl₄, as reflected in improvements in levels of the enzyme parameters of serum transaminases and triglycerides. Katoh et al. reported that the effect of malotilate may be due to any or all of 1) inhibition of CCl₄ activation, 2) inhibition of lipid peroxidation, and 3) acceleration of liver function (Katoh et al., 1982). Our DT827 studies showed relief in liver necrosis caused by exposure to CCl₄ dose-dependently in all of the three DT827-treated groups, and particularly in the DT827B-treated group. It suggests a possible role of DT827 series compounds to inhibit lipid peroxidation. In the effect on liver injury induced by orotic acid, the liver TG value of the DT827B-treated group significantly decreased in comparison with both the sucrose control group and orotic acid-treated group, although the values of the DT827A- and DT827C-treated groups did not decrease. This suggests a possible mechanism of DT827B to accelerate liver function by improving accumulation of TG in the liver.

In consideration of these results, the three DT827 compounds were recognized as having hepatoprotective potential with possible mechanism of inhibition of lipid peroxidation and, for DT827B, acceleration of liver function. From global viewpoints of safety and effectiveness, DT827B was believed to be the most safe and effective among DT827A, DT827B and DT827C.

In pharmacological studies on malotilate, both vacuolation and fibrosis are observed in liver by 5-week repeated exposure to CCl₄ (0.5ml/kg; twice a week) (Katoh et al., 1982), which are more severe liver injuries than those observed in our repeated administration study. A longer period of exposure to CCl₄ has therefore to be considered for our further studies.

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