EFFECT OF SODIUM TAURINE N-CARBODITHIOATE ON ACUTE EXPERIMENTAL HEPATIC INJURY INDUCED IN RATS BY CARBON TETRACHLORIDE

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In the previous report (1) from our laboratory, a series of dithiocarbamate derivatives have been demonstrated to have a potent protective effect against experimental hepatic injury induced by carbon tetrachloride (CCl₄) in rats, and relationship between the chemical structures and their biologically activity has been described with respect to plasma transaminase levels.

In this paper, with the purpose of investigating the action mechanism of these dithiocarbamate compounds, sodium taurine N-carbodithioate (TDT) was chosen as a typical dithiocarbamate, and correlations between the hepatoprotective effect of this compound and its administration route, dose, and time were studied in terms of plasma transaminase levels (p-GPT and p-GOT) and microscopical findings of liver cells. In addition, influences of this compound, when administered to rats before or after CCl₄ poisoning, on the developmental or recovery processes of the hepatic injury, were also studied, employing p-GPT levels, liver lipid contents, and histological findings as indices to evaluate the degree of the liver lesions.

MATERIALS AND METHODS

Male Donryu strain rats weighing 130 to 150 g were used throughout all experiments. They were fed on a commercial diet (CLEA, CE-2) and water ad libitum.

Carbon tetrachloride was suspended in 1% gum tragacanth solution with a glass homogenizer, and administered to rats intraperitoneally in doses of 0.1 or 0.3 ml/kg to induce the liver injury.

TDT was given as aqueous solution. Administration route, dose, and time of it were as described in the section of experimental results.

Animals were sacrificed 24 hours after CCl₄ poisoning in most experiments except in that designed to investigate the influence of TDT on the developmental and recovery processes of CCl₄ liver injury.

Plasma samples for the estimation of plasma transaminase levels were obtained from blood collected from rats by cardiac puncture under Nembutal anesthesia just before sacri-
fice. The levels of plasma transaminases were measured according to the method of Reitman and Frankel (2), and expressed in Karmen units. Total liver lipids were extracted with the Folch’s solvent from liver acetone powder, and weighed after complete removal of the solvent. The total lipids thus obtained were then dissolved in an appropriate volume of diethylether for the use in the phospholipid and cholesterol determinations. The phospholipid content was determined by measuring inorganic phosphorus after the HCIO₄-H₂O₂ digestion of an aliquot of the ether solution, and expressed as the amounts of lecithine contained in 100 g of wet liver. Liver cholesterol determination was made on the same ether solution according to the method of Libermann and Burchard (3, 4).

A portion of liver from individual animals was fixed in 10% formalin, and stained with sudan III or hematoxylin and eosin for the microscopical studies.

RESULTS

1. Relationship between administration route or dose of TDT and its biological activity

Fig. 1 summerizes the relationship between administration route or dose of TDT and its efficiency against the elevation of p-GPT levels caused by CCl₄. Rats were given CCl₄ intraperitoneally in a dose of 0.1 ml/kg, and sacrificed 24 hours later. TDT was administered through different routes (i.e.; p.o., s.c., i.v., and i.p.) and with various doses immediately prior to the CCl₄ injection.

As shown in the figure, administration of CCl₄ alone resulted in a marked increase in p-GPT levels. This increase was completely prevented by oral dosages of 5, 10, and 50 mg/kg of TDT, and was partially prevented by oral dosages of 1 and 3 mg/kg, and also by subcutaneous, intraperitoneal, and intravenous dosages of 50 and 200 mg/kg, but it...
was failed to be prevented by intraperitoneal or intravenous dosages of 10 mg/kg, indicating
that the effect of TDT against the elevation of p-GPT levels by CCl₄ was most potent with
oral administration among the four routes of administration tested.

It is very intriguing and quite suggestive for clarifying the action mechanism of TDT
that the biological effect of this compound should be strongest with oral administration,
and that the minimum oral dose that is required for the complete prevention of the increase
in p-GPT levels caused by 0.1 ml/kg of CCl₄ should be so low as 5 mg/kg, its molar ratio
to CCl₄ being quite small (about 1:20).

2. Relationship between administration time of TDT and its biological effect

In the preceding experiment, TDT was given to rats exclusively immediately prior
to CCl₄ injection. It is reasonable, therefore, to regard the observed effect of TDT on
the CCl₄-induced elevation of p-GPT levels as being attributable to a prophylactic pro-
perties of the compound rather than to a therapeutic effect on the damaged liver.

With the purpose of testing whether TDT would exert a therapeutic action on the
damaged liver as well as the preventing effect, and of testing how long its action would last
following its administration, the compound was given to rats at various time intervals before
and after CCl₄ administration. Moreover, in the above experiment, only the p-GPT
levels were employed as the index by which the severity of the liver damage was estimated,
and by which the effect of TDT was evaluated. However, it is well recognized that no
direct relation is seen between the degree of liver lesions and the serum enzyme levels.
(5-7). The possibility could not be excluded, therefore, that this compound would be
efficacious only against the elevation of serum enzyme levels such as transaminases among
a variety of manifestations resulting from CCl₄ liver lesions.

In this experiment, for fear of such possibility, histological changes of the liver such
as fatty and hydropic alterations of the cells were also examined with the light microscope.
Groups of rats were given orally with 50 mg/kg of TDT, ten times the minimum effective
dose, at 1, 2, 4, 6, 15, and 24 hours prior to, or 1, 3, and 6 hours after the injection of
0.1 ml/kg of CCl₄. They were sacrificed 24 hours after the CCl₄ poisoning. The plasma
transaminase levels were measured, and the degree of fatty and hydropic degenerations
was examined.

The results are shown in Fig. 2 and Table 1. In the groups of rats which received
TDT during the period between 6 hours before and immediately prior to CCl₄ injection,
neither the elevation of plasma transaminase levels nor the histological alteration of hepatic
cells was observed in contrast with the pronounced changes of control, while in the groups
of rats which received TDT 24 hours before and 3 or 6 hours after CCl₄, these biochemical
and histological changes were marked, and left unchanged in the same regions as control.
On the other hand, in the groups treated with TDT 15 hours before or 1 hour after CCl₄,
partial protection was observed in both the biochemical and histological indices.

From these results, it was demonstrated that TDT was effective not only against the
leakage of hepatocellular enzymes which might be due to the CCl₄-induced alterations of
hepatic membrane systems but also against the fatty or hydropic degenerations of the
Carbon tetrachloride was injected i.p. to rats in a dose of 0.1 ml/kg, and TDT was given orally at the time indicated in the figure. Rats were sacrificed to estimate p-transaminase levels 24 hours after the CCl₄ injection. Each bar represents mean of 5-6 rats.

Table 1. Relationship between administration time of TDT and its effect on histological changes of the liver in CCl₄-intoxicated rats.

<table>
<thead>
<tr>
<th>Time of TDT administration</th>
<th>Fatty infiltration</th>
<th>Hydropic changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hr before CCl₄</td>
<td>‡‡‡</td>
<td>‡‡‡</td>
</tr>
<tr>
<td>15</td>
<td>‡‡</td>
<td>‡‡</td>
</tr>
<tr>
<td>6</td>
<td>‡‡</td>
<td>‡‡</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Simultaneously with CCl₄</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>1 hr after CCl₄</td>
<td>‡‡‡</td>
<td>‡‡‡</td>
</tr>
<tr>
<td>3</td>
<td>‡‡</td>
<td>‡‡</td>
</tr>
<tr>
<td>6</td>
<td>‡‡‡</td>
<td>‡‡‡</td>
</tr>
<tr>
<td>CCl₄ alone</td>
<td>‡‡‡</td>
<td>‡‡‡</td>
</tr>
<tr>
<td>Normal</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Hepatic cells which were thought to be caused through another mechanism(8). In addition, it was shown that this compound had no therapeutic effect in spite of possessing a strong preventing activity which persisted no less than 6 hours after its administration.

3. Effect of TDT administered after CCl₄ on the developmental or recovery processes of the liver damage

Though it was demonstrated in the preceding experiment that TDT had no therapeutic effect on the liver injury induced by CCl₄ as far as judged at 24 hours after CCl₄ poisoning, the possibility cannot be excluded that it might exert any influences on the time-
course development of the liver damage or on its recovery process. To test this possibility, time-course changes of hepatic lesions were studied, after CCl₄ poisoning, in terms of p-GPT levels and hepatic contents of total lipids, phospholipids, and cholesterol, and also in terms of the degree of histological alterations of the liver cells.

In this experiment, in order to distinguish possible effect of TDT unequivocally from spontaneous cure, an intraperitoneal dosage of 0.3 ml/kg of CCl₄, which might cause much severer manifestations in the liver than those induced by 0.1 ml/kg of the toxin, was used. Rats were divided into four groups; the first group was served as control, receiving CCl₄ alone, and the second, third, and fourth were treated with TDT in a dose of 50 mg/kg at the times immediately prior to, and 15 and 60 minutes after CCl₄ injection, respectively.

Fig. 3 shows the results of p-GPT levels. The levels of p-GPT in the control group began to increase at 3 hours after CCl₄ injection, and reached the maximum levels 48 hours later, then gradually decreased with the return to the normal levels by 96 hours. In the second group which received TDT immediately prior to CCl₄, no elevation of p-GPT activity was observed both 24 and 48 hours later at which the elevation in control group was most remarkable. On the other hand, in the third and fourth groups which received TDT 15 and 60 minutes after CCl₄, respectively, an apparent increase in p-GPT levels was noted at every experimental period, though the increase at each period was considerably reduced as compared with control. In addition, the patterns of the time-course changes of p-GPT levels in these groups were quite similar to those of control, suggesting that post-administration of TDT could scarcely affect the developmental or recovery processes of CCl₄ liver injury.
Table 2 shows the results of the measurement of liver lipid contents in the same experiment. The time-course changes of total liver lipids following CCl₄ intoxication and the influences of TDT on them resembled the results obtained from the determination of p-GPT. The observed increase by CCl₄ in total liver lipids may be attributed, as generally accepted (9, 10), to the increase of liver neutral fat, especially, triglycerides, since the increases in liver phospholipids and cholesterol were little, or if any, trifling as shown in

### Table 2. Influence of TDT on changes of liver total lipids, cholesterol, and phospholipids through the developmental and recovery processes of CCl₄ liver injury.

Treatment of rats was the same as described in the legend to Fig. 3. Figures in the table represent mean ± standard deviation of 5-6 rats.

<table>
<thead>
<tr>
<th>Time after CCl₄</th>
<th>CCl₄ alone</th>
<th>TDT simultaneously with CCl₄</th>
<th>TDT 15 min after CCl₄</th>
<th>TDT 60 min after CCl₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Normal)</td>
<td>5.5 ± 0.6</td>
<td>0.29 ± 0.04</td>
<td>3.3 ± 0.4</td>
<td>5.8 ± 0.6</td>
</tr>
<tr>
<td>3 hr</td>
<td>5.9 ± 0.5</td>
<td>0.36 ± 0.3</td>
<td>3.1 ± 0.3</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td>6 hr</td>
<td>6.3 ± 0.6</td>
<td>0.37 ± 0.2</td>
<td>3.0 ± 0.2</td>
<td>7.6 ± 0.9</td>
</tr>
<tr>
<td>15 hr</td>
<td>9.0 ± 0.9</td>
<td>0.38 ± 0.5</td>
<td>3.2 ± 0.5</td>
<td>7.6 ± 1.0</td>
</tr>
<tr>
<td>24 hr</td>
<td>10.1 ± 0.7</td>
<td>0.38 ± 0.4</td>
<td>2.8 ± 0.3</td>
<td>8.0 ± 1.5</td>
</tr>
<tr>
<td>48 hr</td>
<td>9.0 ± 0.7</td>
<td>0.50 ± 0.5</td>
<td>2.6 ± 0.5</td>
<td>7.1 ± 1.0</td>
</tr>
<tr>
<td>72 hr</td>
<td>6.0 ± 0.8</td>
<td>0.43 ± 0.4</td>
<td>2.7 ± 0.4</td>
<td>6.3 ± 0.9</td>
</tr>
<tr>
<td>96 hr</td>
<td>5.2 ± 0.7</td>
<td>0.37 ± 0.3</td>
<td>2.8 ± 0.3</td>
<td>5.4 ± 0.4</td>
</tr>
</tbody>
</table>

### Table 3. Influence of TDT on histological changes of the liver through developmental or recovery processes of CCl₄ liver injury. Treatment of rats and expression of histological findings were the same as described in the legend to Fig.4 and Table 1.

<table>
<thead>
<tr>
<th>Time in hours after CCl₄</th>
<th>Control</th>
<th>TDT simultaneously with CCl₄</th>
<th>TDT 15 min after CCl₄</th>
<th>TDT 60 min after CCl₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fatty inflit.</td>
<td>Hydropic changes</td>
<td>Fatty inflit.</td>
<td>Hydropic changes</td>
</tr>
<tr>
<td>3</td>
<td>±</td>
<td>−</td>
<td>±</td>
<td>−</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>±</td>
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<tr>
<td>15</td>
<td>++</td>
<td>±</td>
<td>++</td>
<td>±</td>
</tr>
<tr>
<td>24</td>
<td>+++</td>
<td>±</td>
<td>+++</td>
<td>±</td>
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<tr>
<td>48</td>
<td>+++</td>
<td>±</td>
<td>+++</td>
<td>±</td>
</tr>
<tr>
<td>72</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>±</td>
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<tr>
<td>96</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Normal</td>
<td>− ±</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>
the Table 2. Table 3 summerizes the results of histological studies made on the same liver. After CCl₄ intoxication, the fatty and hydropic changes of the liver parenchymal cells became most conspicuous at 15 hours, and persisted till 48 hours later. These histological changes were not observed even at 24 and 48 hours if TDT was given simultaneously with CCl₄, but they were seen apparently when TDT was given 15 or 60 minutes after CCl₄.

From all data described above, it may be concluded that post-administration of TDT can scarcely affect the developmental or recovery processes of CCl₄ injury, though it diminishes, to some extent, the degree of the lesions at each period.

DISCUSSION

As shown in Fig. 1, oral administration of TDT was most potent among the four routes of administration tested in protecting the liver from toxic action of CCl₄. This phenomenon seems, at a glance, to be unreasonable, but would be properly explained by the presumption that the hepatoprotective effect may not be produced by TDT itself but by a compound or compounds converted from TDT in the gastrointestinal tracts. To ascertain this possibility, we are now investigating the degradation and metabolism of TDT when administered to rats and rabbits via various routes. From the fact that TDT failed to protect the liver from the toxicity of CCl₄ by post-administration in spite of showing a strong protective effect in cases of previous or simultaneous administrations, it may be concluded that this compound acts at an early step or steps of the pathogenetic process of CCl₄ liver injury. This conclusion was further supported by the fact that TDT administered at 15 or 60 minutes after CCl₄ injection could not exert any influences on the developmental or recovery processes of the liver injury.

The minimum oral dose of TDT required to prevent the liver completely from the toxic effect of 0.1 ml/kg CCl₄ was quite small (5 mg/kg), the relative molar ratio of TDT to CCl₄ being 1:20. This fact suggested us that TDT might exert its hepatoprotective effects indirectly via some factors or mediators. It has been well recognized that the effects of CCl₄ upon the liver are modified to the direction of either aggravation or alleviation by numerous factors (11). For instances, hormonal and alimentary imbalances which cause the mobilization of fat to the liver, as a general rule, aggravate the lesions, and to the contrary, the imbalances which counteract the fat mobilization attenuate or suppress the effects of the toxin on the liver (12). Therefore, we examined, using adrenalectomized rats, alloxan diabetic rats, and starved rats, the possibility that TDT might produce the hepatoprotective action against CCl₄ indirectly through its action on these factors. The results obtained showed that TDT could prevent the liver from the toxic action of CCl₄ in these hormonally and metabolically imbalanced rats as well as in intact rats (13) (unpublished).

Taking these unpublished data into account, all the results reported in this paper seem to suggest strongly that the action mechanism of TDT by which it protects the liver from CCl₄, would not be the indirect action via hormonal or metabolic factors, but be the
direct action on the liver itself, where it inhibits the early pathogenic process of CCl₄ injury.

SUMMARY

Taurine N-carbodithioate (TDT) protected the liver of rats from the toxic action of carbon tetrachloride (CCl₄) by preventing both the increase in plasma transaminase levels and the histological alterations of the liver parenchymal cells.

This compound showed more remarkable effect by the administration through mouth than through parenteral routes (i.e.; i.v., i.p., and s.c.), and when given to rats prior to or simultaneously with CCl₄ injection, it protected the liver, but when given after CCl₄ poisoning, it did not. Moreover, TDT administered to rats after CCl₄ intoxication did neither delay the onset of the hepatic injury nor accelerate the recovery process.

Following conclusions may be drawn from these results:

1. The observed effect of TDT on CCl₄ liver injury may not be due to TDT itself but be due to its metabolites or degradation products.

2. The action site of TDT (or of its metabolites or degradation products) would be located at the early stage of the pathogenetic process of CCl₄ liver injury.

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