Effect of Carbon Tetrachloride Intoxication on the Type Conversion of Xanthine Dehydrogenase into Xanthine Oxidase in Rats

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Abstract
The conversion of xanthine dehydrogenase(type D) into xanthine oxidase(type O) was significantly increased in serum and liver of all CC14 treated rats on the necrosis and early cirrhosis stage of liver tissue. In the pretreatment of prednisolone, the ratio of type O per type O+D showed the decreasing tendency in serum, but the significant decrease in liver. In vitro, the conversion of liver xanthine oxidase from type D into type O was markedly increased by following preincubation with lysosomal fraction. The type conversion of xanthine oxidase may be caused by proteolytic enzymes in lysosome.

Keywords
Xanthine oxidase(type O), Xanthine dehydrogenase(type D), CC14, Prednisolone, Lysosome.

In this study, we observed the type conversion of the enzyme in both liver and serum of rats after administration of CC14. Concomitantly, the effects of prednisolone on the conversion of xanthine dehydrogenase into oxidase in CC14 intoxicated rats and the activity of lysosomal marker enzyme, acid phosphatase were investigated. In addition, the effect of lysosomal fraction in rat liver on the type conversion of the enzyme was measured in vitro.

EXPERIMENTAL METHODS

Animals and CCl4 treatment
Male Sprague-Dowley rats weighing 210 to 230 g fed a standard diet as described in Table I for one month, and were subcutaneously injected 0.15 ml of 50% (v/v) CC14 per 100g body weight once in three days. Control rats were given olive oil only. Water was provided ad libitum. Rats were sacrificed two days after the last injection. The animals were divided into six groups, each containing 6 rats.

Treatment of prednisolone
The rats were divided into four groups. Group I received 0.15 ml of 50% (v/v) CC14 subcutaneously. The second treatment of CC14 was done once more after 18 hr and then the animals were sa-
Table I. Composition of experimental diet (g/kg diet).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
</tr>
<tr>
<td>Corn starch</td>
<td>674.36</td>
</tr>
<tr>
<td>Corn oil</td>
<td>64.85</td>
</tr>
<tr>
<td>Vitamin A and D mixture (a)</td>
<td>10.2</td>
</tr>
<tr>
<td>Vitamin E and K mixture (b)</td>
<td>2</td>
</tr>
<tr>
<td>Water soluble vitamin mixture (c)</td>
<td>3</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt; (d)</td>
<td>1</td>
</tr>
<tr>
<td>Salt mixture (e)</td>
<td>40</td>
</tr>
<tr>
<td>α-Cellulose</td>
<td>20</td>
</tr>
</tbody>
</table>

* 4081 Kcal

a) Vitamin A & D mixture: 51,000 unit of A and 5,100 unit of D dissolved in 100 ml of corn oil.  
b) Vitamin E & K mixture: 5g of a-tocopherol and 0.2g of menadion dissolved in 200 ml of corn oil.  
c) Water soluble Vitamin mixture contained (mg): choline chloride 2000, Thiamine hydrochloride 10, Riboflavin 20, Nicotinic acid 125, Pyridoxine 10, Ca-pantothenate 100, Biotin 0.05, Folic acid 4, Inositol 500, p-aminobenzonic acid 100.  
d) Vitamin B<sub>12</sub>: 5mg of Vitamin B<sub>12</sub> dissolved in 500 ml of distilled water.  
e) Salt mixture: contained (g): CaCO<sub>3</sub> 300, Potassium phosphate dibasic 322.5, MgSO<sub>4</sub> 102, Ca-phosphate monobasic 75, NaCl 167.5, Ferric citrate 27.5, KI 0.8, ZnCl<sub>2</sub> 0.25, CuSO<sub>4</sub>·5H<sub>2</sub>O 0.3, MnSO<sub>4</sub> 5, Molybdic acid 0.2.

crificed 24 hr later. Group II was injected with 0.75 mg prednisolone per 100g of body weight intramuscularly an hour before administration of CCl<sub>4</sub>. Group III was given the same dose of prednisolone only. Control group received olive oil only.

**Preparation of crude hepatic xanthine oxidase**

The animals were killed by exsanguination of the abdominal aorta. The liver was exhaustively perfused with cold 0.25 M sucrose through the portal vein. The liver of rat was rapidly removed and homogenized in ice-cold 0.25 M sucrose. Homogenates (20% w/v) were centrifuged at 700g for 10 min. The supernatants obtained were again spun at 15,000g for 30 min. The postmitochondrial fractions were used for the assay of both xanthine oxidase and acid phosphatase activities. The process of tissue preparation and the assay of enzyme activities with its preparation were rapidly accomplished.

**Separation of lysosomal fraction**

Separation of lysosome from rat liver was performed by some modification of the method of Toncsev et al. The liver was homogenized in ice-cold 0.25 M sucrose. Homogenate (10% w/v) was centrifuged at 800g for 15 min at 4°C. The supernatant was centrifuged at 16,000g for 30 min. The pellet was washed twice with sucrose solution and centrifuged. The precipitate was frozen and thawed. The frozen and thawing procedures were repeated and then the precipitate was dialyzed. Identification of lysosomal fraction was confirmed by determining the activity of lysosomal marker enzyme, acid phosphatase in its preparation using the method of Bessey-Lowry-Brock.

**Enzyme assay**

Xanthine oxidase in both liver and serum was determined at 30°C by the UV method of Della Corte et al. or colorimetric method of Yoon.

The xanthine oxidase activity was aerobically determined by measuring the rate of uric acid formation using 100 mM xanthine as substrate, while the combined dehydrogenase-oxidase activity (type D+O) was done using the same assay with buffer supplemented with both 100 μM xanthine and 670 μM NAD<sup>+</sup> or 33/2 M methylene blue. Enzyme levels are expressed as n mole of uric acid formed/min/mg of protein. The conversion of xanthine dehydrogenase into oxidase type was indicated as the percent ratio of O/D+O (total type).

For in vitro test of conversion of liver xanthine dehydrogenase into oxidase, the supernatant for enzyme assay was promptly prepared with a minimum of manipulation from rat liver.

Lysosomal suspension (16, 81 mg of protein/ml) was mixed with freshly prepared supernatant of rat liver at the 1:1 or 1:2 ratio. After the mixture was preincubated at 37°C for 10 min, the enzyme assay was performed as described above. The protein content was determined by the method of Lowry et al.

**Preparation of the tissue for light microscopy**

Liver tissues were fixed in 10% neutral formalin, made on paraffin embedded tissue and hematoxylin-eosin stain. With these preparations, histological studies were carried out.

**RESULTS**

**Light microscopic finding of rats liver treated with CCl<sub>4</sub>**

The lobules and portal tracts of control liver were intact. From 3 to 6 days of CCl<sub>4</sub> treatment, fatty hepatocytes randomly distributed in central zone of