Decreased activity of hepatic alkaline protease in rats with carbon tetrachloride-induced liver cirrhosis

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To investigate the cause of accumulation of oxidised proteins in the livers of rats with carbon tetrachloride (CCL_4) induced liver cirrhosis, the activity of alkaline protease (a high molecular weight, multisubunit cysteine proteinase) was determined in the cirrhotic livers. A significant decrease (P<0.05) in the activity of hepatic alkaline protease was observed in the cirrhotic rats. Decreased activity of alkaline protease in the liver of cirrhotic rats may contribute to the accumulation of the oxidised proteins in the liver.

Carbon tetrachloride is a well known hepatotoxin and CCL_4/rat model of cirrhosis is the widely used animal model for the study of mechanism of development of human alcoholic cirrhosis. Free radicals have been implicated in the pathogenesis of human alcoholic liver injury and CCL_4-induced liver injury in the rats. In both cases, peroxidation of membrane lipids plays an important role. The administration of CCL_4 to the rats enhances hepatic protein oxidation and results in the accumulation of oxidised proteins in the liver. Oxidative damage to hepatocellular proteins may contribute to the pathogenesis of CCL_4 induced liver injury. The cause of the accumulation of oxidised proteins in the liver of CCL_4 treated rats is not known.

Protein oxidation marks proteins for degradation. Normally, the oxidised proteins do not accumulate in the liver as they are rapidly degraded by the enzymes of the non-lysosomal pathway. Three non-lysosomal proteases have been found to degrade oxidised proteins. One such protease is alkaline protease which is a high molecular weight, multisubunit cysteine proteinase.

The importance of the protease activity is highlighted by the fact that the accumulation of oxidised proteins in the brains of old gerbils varied inversely with the amount of alkaline protease activity. The present study is an attempt to examine whether there is any alteration in the activity of alkaline protease in the livers of rats with CCL_4-induced cirrhosis.

Adult male Wistar rats weighing 150-175 g were used. Cirrhosis was induced in the rats by the combined chronic administration of CCL_4 vapours and phenobarbitone in drinking water as per the method of McLean et al., with modifications. Phenobarbitone treated rats served as controls. Briefly, the rats were divided into two groups. Group I served as the experimental group and group II as control.

Group I: The rats in this group were administered phenobarbitone in drinking water at the concentration of 500 mg/l 10 days prior to the first dose of CCL_4 and throughout the treatment period. The rats were exposed to CCL_4 vapours passed at the rate of 4 l/min for 10 min, twice a week for 12 consecutive weeks.

Group II: The rats in this group were administered phenobarbitone alone.

The rats in both the groups were sacrificed after overnight fast and the livers were excised; a part of the liver was fixed in formalin for histological assessment and an another part homogenised and used for the assay of protein carbonyl content and alkaline protease activity as described by Levine et al. and Tanaka et al. respectively. The mean values of control and test groups were statistically compared with Student’s t test.

The rats that were treated with CCL_4 showed micronodular cirrhosis (Fig. 1a). The livers of
Fig. 1—(a) Liver of a rat treated with CCl₄/phenobarbitone for 12 weeks. The liver shows micronodular cirrhosis. The architecture of the liver is distorted with the formation of nodules (Foot’s reticulin, 40X), FS—Fibrous septa, N—Nodule. (b) Histology of the liver of control (phenobarbitone treated) rat showing normal architecture (H&E, 200X). PT—Portal tract.

Phenobarbitone treated rats showed normal architecture (Fig. 1b).

The results show that the accumulation of oxidised proteins in the livers of cirrhotic rats is accompanied by a decrease in the activity of alkaline protease.

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<th>Parameter</th>
<th>Control (n=6)</th>
<th>Cirrhosis (n=8)</th>
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<tbody>
<tr>
<td>Protein carbonyl content (nmol/mg protein)</td>
<td>5.12±0.39 **</td>
<td>14.12±1.02 **</td>
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<tr>
<td>Alkaline protease activity (U/mg protein)</td>
<td>215.17±23.1</td>
<td>74.74±24.7 **</td>
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Nearly three fold increase in protein carbonyl content and a 20% decrease in the activity of alkaline protease was observed in the livers of cirrhotic rats (Table 1). The decrease in alkaline protease activity may contribute to the accumulation of oxidised proteins in the livers of rats with CCl₄-induced cirrhosis.

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References