Plasma Prolidase in the Rat: No Index of Liver Fibrosis

To the Editor:

A recent paper (1) focused attention on plasma prolidase (proline dipeptidase, EC 3.4.13.9) activity as a possible marker of early stage of fibrosis. So far, at least to our knowledge, no reliable markers of liver fibrosis in plasma have been described hitherto, although such a marker would be very helpful in clinical as well as experimental evaluation of liver fibrosis. For that reason we investigated whether plasma prolidase activity showed a correlation with the extent of liver fibrosis in the rat. If that was the case then one could not expect correlation with leakage from liver cells as reflected in greater alanine aminotransferase (EC 2.6.1.2) activity in plasma. No such correlation was found in humans (1). Liver fibrosis was documented by proline hydroxylase (procollagen-proline,2-oxoglutarate 4-dioxygenase, EC 1.14.11.2) activity and hydroxyproline concentration. Proline hydroxylase is an important enzyme in the synthesis of collagen. Hydroxyproline is a characteristic component of collagen.

We measured plasma prolidase and liver proline hydroxylase activities as described elsewhere (1, 2). Liver proline hydroxylase activity is expressed as radioactivity (TH2O) released from [3H]procollagen after incubation with liver homogenate. Other variables mentioned below were measured by standard clinical chemical methods.

Liver fibrosis was induced in rats with CCl4 in mineral oil. Each rat received two 0.1-mL doses of CCl4 per 100 g body weight each week. In the period between the two doses, as well as at the start and the end of the experiment, blood was collected by orbital puncture. Approximately one week after the last dose of CCl4 the rats were killed and liver tissue was sampled for hydroxyproline concentration and proline hydroxylase activity measurements. The time course of the experiments and the number of animals used are indicated in Figure 1.

We found rat plasma prolidase activity to be very similar in its characteristics to the human enzyme (1), except that hemolysis of up to 1% of the erythrocytes did not significantly influence the enzyme activity in the rat plasma.

After three and five weeks of treatment with CCl4 liver collagen had increased progressively as indicated by hydroxyproline concentrations: 660 (SEM 240) μg per gram of liver and 1760 (SEM 140) μg per gram of liver, respectively. The mean concentration in livers of untreated rats was 240 (SEM 40) μg per gram of liver. In all groups n = 4.

At these stages active increased collagen synthesis occurred as shown by proline hydroxylase activity in the livers: 38 (SEM 6) dpm/mg of protein per minute and 63 (SEM 10) dpm/mg of protein per minute, respectively. The corresponding value for untreated rats was 22 (SEM 4). In all groups n = 4.

However, plasma prolidase activity after both three and five weeks of CCl4 treatment was the same as the activities measured in the untreated animals at the start of the experiments (Figure 1).

In between three and five weeks, plasma prolidase activity paralleled plasma alanine aminotransferase activity. These are the periods during CCl4 treatment in which there very likely is profound leakage from liver cells.

We conclude that plasma prolidase activity, at least in the rat, is very much influenced by liver cell leakage and does not seem to reflect active stages of liver fibrosis.

References


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