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Elevation of Collagenase Activity in Fatty Liver

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ABSTRACT

Liver collagenase activity increased without liver cell necrosis in non-alcoholic and alcoholic fatty liver induced by feeding rats a choline-deficient diet or alcohol-liquid diet, respectively. The liver hydroxyproline content did not increase during a marked rise in collagenase activity in non-alcoholic fatty liver, but the content gradually increased after collagenase activity diminished. However, the hydroxyproline content increased slowly with a slight concomitant increase in collagenase activity in alcoholic fatty liver. The regulatory mechanism of fibrogenesis in fatty liver is discussed.

Biochemical and histological liver fibrosis is frequently observed in non-alcoholic, non-diabetic patients with fatty liver, in some of whom the disease might progress to fatty cirrhosis of the liver[5]. The mechanism of fibrosis in fatty liver is not well understood, but impaired microcirculation of the sinusoidal space due to its compression by deposited fat[3] has been proposed. In the present study, non-alcoholic fatty liver and alcoholic fatty liver were experimentally produced in rats by feeding them an choline-deficient or alcoholic-liquid diet, and the liver hydroxyproline content and collagenase activity were determined.

Male Sprague Dawley rats (Clea Japan, Inc.), weighing about 200 g each, were kept for a week on a basal diet (CE-2, Clea Japan, Inc.) and were fasted overnight before the experiment. The choline-deficient diet was purchased from Oriental Yeast Co.[7] and given daily to rats for 9 weeks. Drinking water was given *ad libitum* during the experiment. The alcohol-liquid diet[1] was prepared by mixing sodium casein (4.0 g), Kaloreiner (3.0 g), corn salad oil (4.0 g), calaginine (0.2 g), a vitamin mixture (0.5 g), a mineral mixture (0.1 g) and ethanol (Gekkeikan, Okura Shuzo Co., Ltd., a rice wine of 17% ethanol) (5.0 g) and diluted with water to make a 100-ml solution (100 kcal/100 ml). The liquid diet was fed daily for 16 weeks. The liver hydroxyproline content was determined by Rojkind's method[4], and collagenase activity was determined according to Takahashi et al[6]. The liver triglyceride content and serum glutamate pyruvate transaminase (GPT) activity were determined according to routine methods.

The liver collagenase activity increased soon after initiation of the choline-deficient diet. This increase was associated with a marked elevation of liver triglyceride content but without a rise in serum GPT activity (Fig. 1). However, the liver hydroxyproline content did not change up to the 6th week of the experiment while collagenase activity was elevated. The hydroxyproline level began to rise from the 6th week of the experiment, 4 weeks following the peak of collagenase activity (average of 3.1 mU/mg protein). Serum GPT activity did not change at all during the experiment. After initiation of the alcohol-liquid diet, liver hydroxyproline contents increased slowly, and collagenase activity also started to rise concomitantly (Fig. 2). The peak values of both parameters were reached in the 16th week of the alcohol-liquid diet, although the time course was not investigated further. The peak level of collagenase activity was much low-

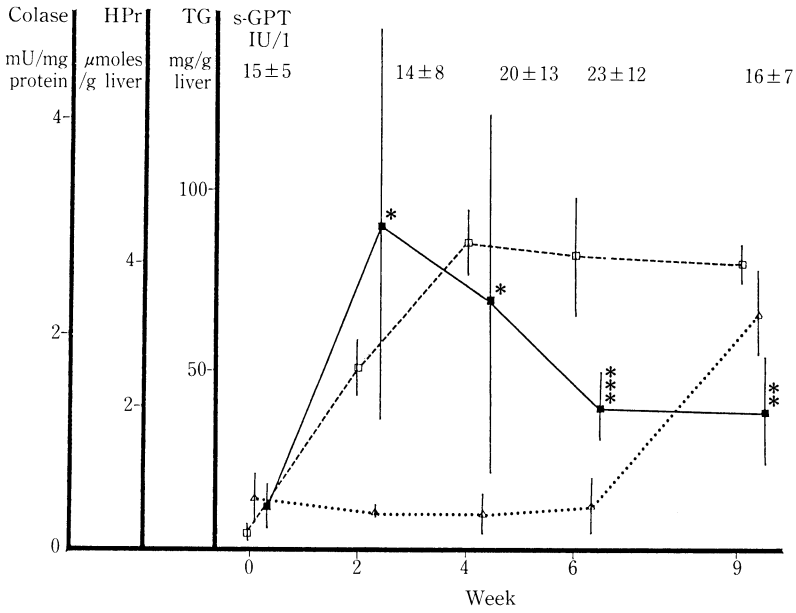

Fig. 1. Time course of the liver hydroxyproline content, collagenase activity, triglyceride content and serum GPT activity following initiation of a choline-deficient diet to rats. □---□; Triglyceride (TG), △---△; hydroxyproline (HPr) and ■---■; collagenase (Colase). All the results were expressed as mean ± standard deviation of the mean, and significance of deviation from the control (0 time) was calculated by Student's t-test. Vertical lines express the standard deviation of the mean. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.01$.

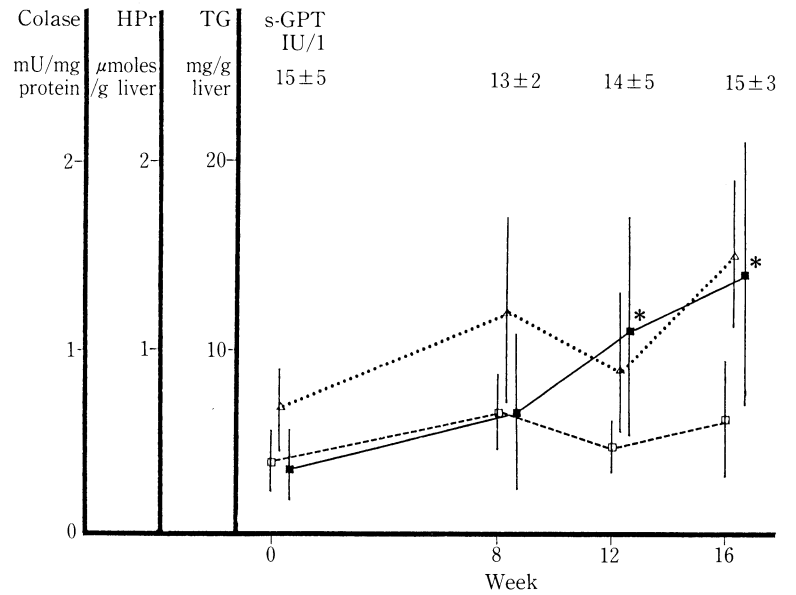

Fig. 2. Time course of the liver hydroxyproline content, collagenase activity, triglyceride content and serum GPT activity following initiation of an alcohol-liquid diet to rats. Other details are described in the legend to Fig. 1.

er in the alcohol group (average of 1.8 mU/mg protein) than in the choline-deficient group. Only slight and insignificant fatty liver was observed under the present experimental conditions in the alcohol-liquid diet group. A rise in the serum GPT was not observed during the entire test

period in the alcohol group.

In the case of non-alcoholic fatty liver, the early elevation of collagenase activity might result in the degradation of newly synthesized collagen, so that no increase in the collagen content was observed. However, when collagenase activity began to decrease following the peak, the collagen contents gradually increased. However, collagen deposition as well as a slight rise in collagenase activity was observed in alcoholic fatty liver. This result indicates that the rise in collagenase in alcoholic fatty liver is not enough for the degradation of newly synthesized collagen; i.e., the capacity for collagen degradation was not sufficiently greater than the capacity for collagen production under the present conditions. These changes were not accompanied with liver cell necrosis nor with inflammation, and thus the mechanisms of the stimulated production of collagenase in the liver are not explained. Okazaki et al[2] have reported that liver fibrosis can progress only under the predominance of collagen production over collagen degradation by collagenase.

The mechanism of enhanced fibrogenesis with the development of fatty liver and in the absence of a hepatotoxin is under investigation.

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