

## Hepatic Collagen and Serum Prolyl Hydroxylase in Rats After Carbon Tetrachloride Poisoning

Kenzo KOYAMA\*, Noriko OMICHI\*, Tatsuya SATO\*  
and Kazuya MAEDA\*\*

*\*Department of Health Science, Osaka Kyoiku University,  
1-6-7 Nagaremach, Hirano-ku, Osaka 547, Japan*

*\*\*Institute of Health Science and Physical Education,  
Osaka City University, Sumiyoshi-ku, Osaka 558, Japan*

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Male rats of Wister-strain, at 4 weeks of age were used at the start of this study. They were divided into two groups; namely, control and carbon tetrachloride (CCl<sub>4</sub>). Rats of CCl<sub>4</sub> group were administered 50% CCl<sub>4</sub> from 5 to 13 weeks of age. The liver sampled and its ratio of lipids to liver weight and collagen content were examined by Forch's and Woessner's methods, respectively. Moreover, concentration of serum lipid and prolyl hydroxylase were examined. The ratio of lipids to liver weight in CCl<sub>4</sub> group reached 2.6 times against that of control group. Concentration of serum lipids, triglyceride and free fatty acid, showed significant difference between two groups. Collagen content of liver ( $\mu\text{g}/\text{mg}$  dry defatted liver $\pm$ SD) was  $2.28\pm 0.47$  and  $3.27\pm 0.48$  in control and CCl<sub>4</sub> groups, respectively. Concentration of prolyl hydroxylase in serum ( $\mu\text{g}/\text{ml}\pm$ SD) was  $0.61\pm 0.13$  and  $2.11\pm 0.23$  in control and CCl<sub>4</sub> groups. The liver of CCl<sub>4</sub> group was falling into fatty liver and fibrosis. From these results, it was suggested that synthesis and secretion of collagen may be affected by the accumulation of neutral fat in liver cell. And we presumed that the increase of prolyl hydroxylase in serum is demonstrated the liver damage.

### Introduction

In human being, the normal liver contains about 5 per cent lipids. By physiological and morbid condition, the ration of lipids to liver weight reaches about 30 per cent. Main factors of appearance of fatty liver are diabetes and excess intake of alcohol etc, in human being [1, 8]. In order to make the fatty liver in the treated animals, investigators have to supply choline deficient foods [3], alcohol [9] or carbon tetrachloride [5]. We observed the changes of the lipids content in liver and serum of rats which were administered carbon tetrachloride.

Collagen is abnormally formed by a disorder in a liver. We have reported that collagen content in liver increases by choline deficient food or alcohol [6, 7].

Collagen has the structure of triple helix, and its stability necessitates the hydroxylation of proline which results in the activity of prolyl hydroxylase. Previous studies reported that the more fibrosis advances in liver and the more prolyl hydroxylase increases in serum [13, 16]. In tissue and serum, prolyl hydroxylase reflects the synthesis of collagen. We attempted to see the changes of collagen content in liver and to see the changes of prolyl hydroxylase in serum of rats which were administered carbon tetrachloride.

### Materials and Methods

Male rats of Wister-strain, at 4 weeks of age were used in this study. They were divided in to two groups; namely, control and carbon tetrachloride ( $\text{CCl}_4$ ).  $\text{CCl}_4$  group was subcutaneously injected 50%  $\text{CCl}_4$  for two times a week. Carbon tetrachloride was diluted with olive oil and the dose of hypodermic injection was 0.1 ml per 100 g body weight of rat. Control group was subcutaneously injected olive oil at same dose. Animals were fed in one small cage. They were housed in cages in a temperature- and light-controlled animal room (at  $23 \pm 1^\circ\text{C}$ ) and were provided with food (Oriental Yeast Co., Osaka). Rats of  $\text{CCl}_4$  group were administered 50%  $\text{CCl}_4$  from 5 to 13 weeks of age.

All animals were sacrificed for the chemical analysis at 13 weeks of age. About 6 ml of blood was obtained by cardiac puncture using a syringe with needle, and was transferred into a centrifuge tube. The serum was prepared by centrifuging. Concentration of triglyceride, total-cholesterol and free fatty acid in serum were determined by using respectively kit (Wako Chemicals, Osaka). Concentration of rat prolyl hydroxylase (rPH) in serum was determined by using the sandwich enzyme immunoassay kit (Fuji Chemical Industries Ltd., Toyama). The liver was excised, and a column of the liver and the remains were used as the sample of experiment. The remains were homogenated, and about 2g of the liver was used to measure the ratio of lipids to liver weight. The measurement of lipids was performed by Forch's method [2]. The collagen content of the columnar liver was examined by means of measuring their hydroxyproline content, which was performed by Woessner's method [17]. The absorbancy at 560 nm was measured using a spectrophotometer (Hitachi Model 100-10).

### Results

In two groups, body weight and liver weight at 13 weeks of age were shown in Table 1. Body weight was observed significant difference between two groups. However, liver weight does not showed significant difference between two groups.

Table 2 showed water content of liver and the ratio of lipids to liver weight in two groups. Water content ( $\% \pm \text{SD}$ ) of liver was  $63.5 \pm 1.4$  and  $59.3 \pm 2.0$  in control and  $\text{CCl}_4$  groups, respectively. The ratio of lipids to liver weight ( $\% \pm \text{SD}$ ) was  $12.7 \pm 3.2$  and  $33.1 \pm 7.9$  in control and  $\text{CCl}_4$  groups, respectively. Water and lipids content of liver were observed significant difference between two

groups.

Concentration of triglyceride, total-cholesterol and free fatty acid (FFA) in serum were shown in Table 3. In serum, concentration of triglyceride and FFA indicated significant difference between two groups.

The standard curve in hydroxyproline can be expressed with a formula of  $Y=0.088X+0.030$ . Fig. 1 showed hydroxyproline content of liver ( $\mu\text{g}/\text{mg}$  dry defatted liver  $\pm\text{SD}$ ) was  $2.28\pm0.42$  and  $3.27\pm0.48$  in control and  $\text{CCl}_4$  groups, respectively. Hydroxyproline content of liver showed significant difference between two groups.

Concentration of prolyl hydroxylase in serum ( $\mu\text{g}/\text{ml}\pm\text{SD}$ ) was  $0.61\pm0.13$  and  $2.11\pm0.23$  in control and  $\text{CCl}_4$  groups, respectively, which was shown in Fig. 2. Content of rPH in serum indicated significant difference between two groups.

Table 1 Body weight and liver weight in two groups.

group	n	Body weight (g)	Liver weight (g)
control	5	$444.6\pm23.7$	$21.9\pm3.0$
chronic $\text{CCl}_4$	7	$394.1\pm23.4^{**}$	$24.3\pm3.4$

Values are means $\pm$ SD.  $^{**}$ ;  $P<0.01$

Table 2 Water content of liver and the ratio of lipids to liver weight in two groups.

group	n	Percent of wet tissue	
		water	Lipids
control	5	$63.5\pm1.4$	$12.7\pm3.2$
chronic $\text{CCl}_4$	7	$59.3\pm2.0^{**}$	$33.1\pm7.9^{**}$

Values are means $\pm$ SD.  $^{**}$ ;  $P<0.01$

Table 3 Concentration of triglyceride, total-cholesterol and free fatty acid (FFA) in serum.

group	n	triglyceride ( $\text{mg}/\text{dl}$ )	total-cholesterol ( $\text{mg}/\text{dl}$ )	FFA ( $\text{mEq}/\ell$ )
control	5	$96.1\pm20.1$	$96.8\pm17.9$	$2.78\pm1.80$
chronic $\text{CCl}_4$	7	$48.2\pm12.8^{**}$	$94.0\pm 9.7$	$1.29\pm0.30^*$

Values are means $\pm$ SD.  $^{***}$ ;  $P<0.05$ ,  $P<0.01$

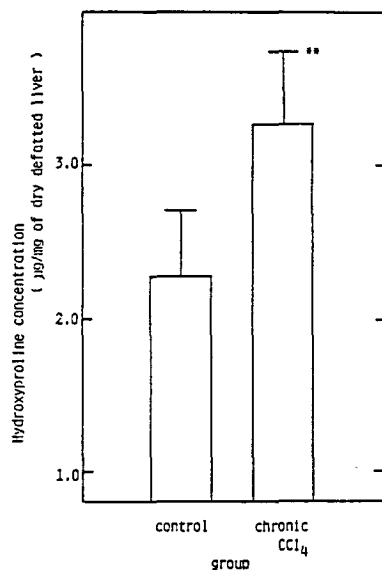


Fig. 1 Hydroxyproline concentration in dry defatted liver of two groups.

\*\*; Difference from control group :  
P<0.01.

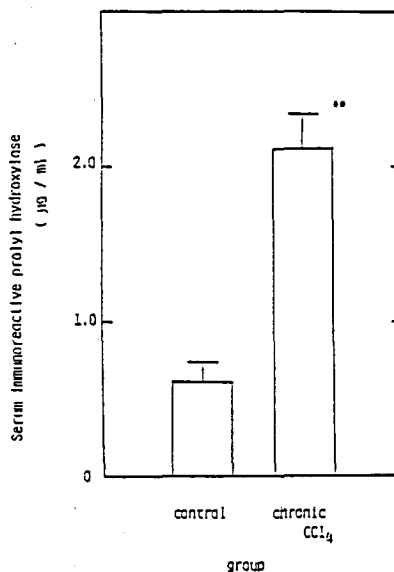


Fig. 2 Serum immunoreactive prolyl hydroxylase content in two groups.

\*\*; Difference from control group :  
P<0.01.

### Discussion

In this study, we administered rats carbon tetrachloride. Water content of liver decreased and the ratio of lipids to liver wet weight remarkably increased in CCl<sub>4</sub> group, which showed 59.3% and 33.1%, respectively (cf. Table 2). The liver in CCl<sub>4</sub> group was falling fatty liver, and appearances of liver was slightly observed hypertrophy and clearly yellows. Concentration of triglyceride and FFA in serum showed significant difference between two groups (cf. Table 3). Concentration of triglyceride and FFA in serum of CCl<sub>4</sub> group decreased in comparison with that of control group. Heimberg et al. [4] have reported that the appearance of fatty liver results from inhibition of the transfer of triglyceride from liver to blood, because of the barrier of triglyceride from liver to blood, because of lipoprotein. We conform to their views, but we considered that the transfer of lipids from liver to blood is influenced by the administered dose of CCl<sub>4</sub>.

Lieber et al. [9] have reported that fatty liver is shifted to liver cirrhosis. Rojkind et al. [14] have reported that collagen content of liver cirrhosis goes up to about ten times in comparison with that of normal liver. Hydroxyproline content of liver showed significant difference between two groups (cf. Fig. 1). Hydroxyproline content of liver in CCl<sub>4</sub> group reached 1.43 times against that in control group. Secertion of collagen was stimulated by ballooning and the accumu-

lation of fat in liver cell [12]. Therefore, we presumed that synthesis and secretion of collagen is affected by the accumulation of neutral fat in liver.

In this study, prolyl hydroxylase of serum in CCl<sub>4</sub> group reached 3.5 times against that in control group (cf. Fig. 2). Muragaki [10] has reported that an close relationship is recognized between hepatic prolyl hydroxylase activity and serum immunoreactive prolyl hydroxylase, and that prolyl hydroxylase in serum can be used as an early indicator for liver fibrosis. It is considered that the liver of CCl<sub>4</sub> group is falling into fibrosis. The decrease of body weight in CCl<sub>4</sub> group may be due to the liver damage, fatty liver or fibrosis (cf. Table 1). It is generally known that prolyl hydroxylase is key enzyme of the synthesis of collagen [11]. Liver fibrosis is influence by the formation and decomposition of collagen, and in progress of fibrosis the former exceeds the latter [15]. We presumed that the increase of prolyl hydroxylase in serum is demonstrated the transfer from the excessive accumulation of lipids to fibrosis cirrhosis in liver.

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#### 四塩化炭素投与ラットの肝コラーゲンと血清プロリルヒドロキシラーゼについて

(小山健蔵・大道乃里江・佐藤達也・前田如矢)

Wister系雄ラットを4週齢からこの実験に用いた。それらは、対照群と四塩化炭素投与群 (CCl<sub>4</sub>群) との2群に分けた。CCl<sub>4</sub>群は、5から13週齢まで週2回四塩化炭素を投与した。CCl<sub>4</sub>群は肝臓の脂質の割合とコラーゲン量および血清プロリルヒドロキシラーゼ量 (PH量) が、対照群に比べ著しく増加することが示唆された。肝障害は、血清PH量を測定することで把握されることが示唆された。