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Trace Elements (Cu, Fe, Zn) in Several Tissues of CCl_4-Induced Chronic Hepatitis Rats

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It is well known that trace elements importantly act in the body (Schroeder and Nason 1971) and they are maintained at steady levels by means of absorption and excretion. If this mechanism was broken, various damages will occur.

Liver injury induced by CCl_4 inhalation is used as the models of chronic hepatitis. We used rats, induced CCl_4 inhalation and measured their hydroxyproline contents as the index of chronic liver fibrosis. And we tried to find when a part of homeostasis is broken, how the trace elements in the organs would appear.

METHODS AND MATERIALS

Twenty-four male Wistar-strain rats approximately 6 weeks old (body weight 250-280 g) were obtained from Nihon Clea Co. Ltd. (Osaka). After breeding them for one week, they were divided into 2 groups: control and CCl_4 treated groups. The CCl_4 treated group inhaled CCl_4 gas for 15 min. a day, 3 times a week, for 6 weeks. The rats were anesthetized, and their blood was sampled from vena cava. The control group was bred without CCl_4 gas treatment. Feed (CE-2, Nihon Clea Co. Ltd.) and tap water for drinking were given ad libitum, and their metal contents were shown in Table 1.

Table 1. Metal content in the feed and tap water

		Cu	Fe	Zn
Feed (μg/g)	mean	16.495	464.450	43.600
	S.E.	4.405	50.950	25.200
Tap Water (ppm)	mean	7.600	239.260	1.958
	S.E.	2.026	73.438	0.121

Each value is obtained from 6 samples

Each part of the liver, the kidney and the skin were ashed by low temperature plasma asher (ASH-302, Hitachi Co. Ltd., Tokyo). We measured their dry weight, and then 1 ml of conc. HNO_3 was added. After dissorbed on a hot plate, it was adjusted to 10.0 ml with distilled water. Each 1 ml of blood, which was measured for hematocrit, or serum were added to 1 ml of conc. HNO_3, then we did the same procedure as above. Nails were washed by hot distilled water, acetone and ether, and each procedure was repeated three times. After drying in the desiccator, they are measured accurately, and dissolved in 0.5 ml of mixed acid solution which consists of HNO_3, $HClO_4$ and H_2O_2 (6:2:1). The sample is adjusted to 1.0 ml by adding water.

Metals in each sample were assayed with the Zeeman-effect atomic absorption spectrophotometer (170-70, Hitachi Co. Ltd.).

Hydroxyproline contents were assayed with the method of Woessner (1961).

RESULTS AND DISCUSSION

Table 2 shows serum enzyme activity and hydroxyproline contents.

Table 2. Serum enzyme activity and hydroxyproline contents in the tissues

	AlP(KA u)	LDH(u)	s-GOT(u)	GPT(u)
Control group	16.0 ± 0.2	538 ± 18	96 ± 2	61 ± 3
CCl_4 group	36.3** ± 1.5	836** ± 27	677** ± 25	105** ± 36

	Hydroxyproline Contents (μg/mg)	
	Liver	Abdominal Wall
Control group	0.63 ± 0.03	9.24 ± 1.30
CCl_4 group	1.76** ± 0.11	17.47** ± 1.22

**P < 0.01 (mean ± S.E.)

Enzyme activity in serum increased in the CCl_4 treated group, and hydroxyproline contents in liver and abdominal wall increased, too. Therefore, it was

thought that the CCl_4 treated group showed the symptoms of chronic hepatitis. Then, we measured the metal contents in each organ. In the CCl_4 treated group as compared with the control one, Cu contents statistically increased in the sera (151 %), the livers (267 %), the kidneys (368 %) and the nails (120 %), and decreased in the blood (21 %). The value of Fe increased in the livers (474 %), the kidneys (512 %), the skins (147 %) and the nails (172 %) and decreased in the blood (42 %). And the Zn level increased in the livers (235 %) and in the skins (164 %), and decreased in the blood (33 %) (Table 3).

Table 3. Metal content in the various tissues

		Zn	Fe	Cu
Serum	control	0.96 ± 0.10	5.50 ± 0.66	1.28 ± 0.06
(ppm)	treated	0.75 ± 0.11	7.47 ± 1.00	1.94** ± 0.10
Blood	control	0.34 ± 0.07	7.84 ± 0.67	4.79 ± 0.40
(ppm/Ht)	treated	0.11** ± 0.02	3.22** ± 0.23	1.01** ± 0.17
Kidney	control	0.53 ± 0.14	1.02 ± 0.28	2.05 ± 0.42
(μg/mg)	treated	0.82 ± 0.17	5.24** ± 0.69	7.53** ± 1.78
Liver	control	0.50 ± 0.19	1.25 ± 0.28	0.08 ± 0.02
(μg/mg)	treated	1.17* ± 0.30	5.91** ± 1.43	0.22** ± 0.04
Nails	control	83.4 ± 17.9	88.0 ± 15.6	64.3 ± 4.3
(ng/mg)	treated	117.5 ± 12.9	151.4** ± 32.7	77.3* ± 5.8
Skin	control	324.9 ± 30.8	146.5 ± 15.5	10.2 ± 1.3
(ng/mg)	treated	534.4** ± 95.1	216.0** ± 13.4	12.4 ± 1.4

* $P < 0.05$, ** $P < 0.01$, mean ± S.E.

The CCl_4 treatment is given by three methods, per os, subcutaneous injection and inhalation. But p.o. and s.c. methods are not good for chronic experiments because they weaken the rats. Inhalation treatment does not enfeeble the rats and certainly causes liver damages.

It is known that Zn is decreased in plasma (Halsted et al 1968, Halsted and Smith 1970) and serum (Boyett and Sullivan 1970) by liver diseases. In this study, though feed and water contained metals (Table 1), we saw the serum Zn did not statistically decrease in CCl_4

treated rats. But in the liver, Cu, Fe and Zn were increased and, in the other organs except the blood and serum, these metals were increased, too (Table 3).

As Cikrit et al (1975) found the CCl_4 treated rats did not alter the total excretion of Cu and Zn, these results suggested that the transport of the metals were inhibited by the injury of the liver. Abnormally high levels of Cu in the liver induce various symptoms for man and Cu presents negative relation with Fe in the liver (Underwood 1975). And, in the Zn deficiency rats, serum alkaline phosphatase activity is decreased and alkaline phosphatase and LDH activity in the tissues are decreased, too (Underwood 1975). But these changes are the direct effects of administration or deficiency caused by the metals.

The serum metal levels are secondary effect after the inhibition of protein synthesis and destruction of the liver cells when liver damages caused by the CCl_4 inhalation, though Loyke found that there was a relation between serum Cu, Zn (1984) or blood Pb (1985) and blood pressure in the CCl_4 s.c. treated rats.

We think that a stream of metals in the body is stopped when a chronic liver disease occures, and metals, which were absorbed in the body as feeds and drinks, accumulated in the liver. And as Cu and Zn are reported their toxic effects in the organs, there is a possibility that metal accumulation into the tissues lead to various toxic effects. Therefore, it is significant to assay the organs' metal levels rather than the serum. And as metal levels in the nails increased in the CCl_4 treated group, we suggest that the metal levels in the nails be measured as the index of alteration of these in the organs.

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