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BRIEF NOTE

PROTECTIVE EFFECT OF ZINC CHLORIDE ON CARBON TETRACHLORIDE-INDUCED REDUCTION OF COMPLEMENT ACTIVITY IN RATS

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It has been demonstrated that total hemolytic complement activity in rats was markedly reduced following the administration of a sublethal dose of carbon tetrachloride (CCl_4)[1]. In other experiments, it has also been reported that CCl_4-induced liver damage could be significantly prevented by pretreatment of animals with divalent metals, including zinc[2,3] and cobalt[4]. In this respect, a preliminary study was carried out to see whether zinc chloride ($ZnCl_2$) would protect CCl_4-induced reduction of total hemolytic complement activity in rats.

MATERIALS AND METHODS

Male Wistar rats, 11 weeks old, were used in this study. The animals were fed Oriental MF pellets (zinc content 6.05mg/100g), and given tap water *ad libitum*. They were divided in 4 groups of 6 rats each. Rats were injected intraperitoneally with aqueous solution of $ZnCl_2$ (Kojundo Kagaku Kenkyusho) so that they received 250 μmol. Injection volume was 5 ml/kg and control animals received an equal volume of saline. Twenty-four hours following the pretreatment with $ZnCl_2$ or saline, rats were given intraperitoneally 1ml/kg of olive oil or 0.5ml/kg of CCl_4 in a 50% olive oil solution. Twenty-four hours later, the rats were sacrificed under light ether anesthesia and blood was drawn from abdominal aorta with plastic syringes. It was allowed to clot at room temperature for one hour, then left standing overnight at 4°C in a refrigerator, and serum was separated by centrifugation.

Total hemolytic complement activity in serum was assayed by a modified method of Mayer et al.[5] as the 50 percent hemolytic unit (CH50). Total protein content of serum was determined by a modified biuret method (Wako). Composition of serum proteins was analyzed by the cellulose acetate membrane electrophoresis. Serum alkaline phosphatase (Al-P) was assayed by a modified method of Bessey-Lowry (Alkalinephospha B-Test, Wako). Serum glutamic oxalacetic transaminase (SGOT) was determined by a modifiied method of Reitmann and Frankel (S. TA-Test, Wako). Serum triglyceride level (TG) was assayed by the method of Moteki et al. (Triglyceride-Test, Wako). Concentration of zinc in serum was determined by a flame atomic absorption method (Perkin-Elmer 503)[6].

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RESULTS AND DISCUSSION

The results of the present investigation are summarized in Table 1,2 and Fig. 1.

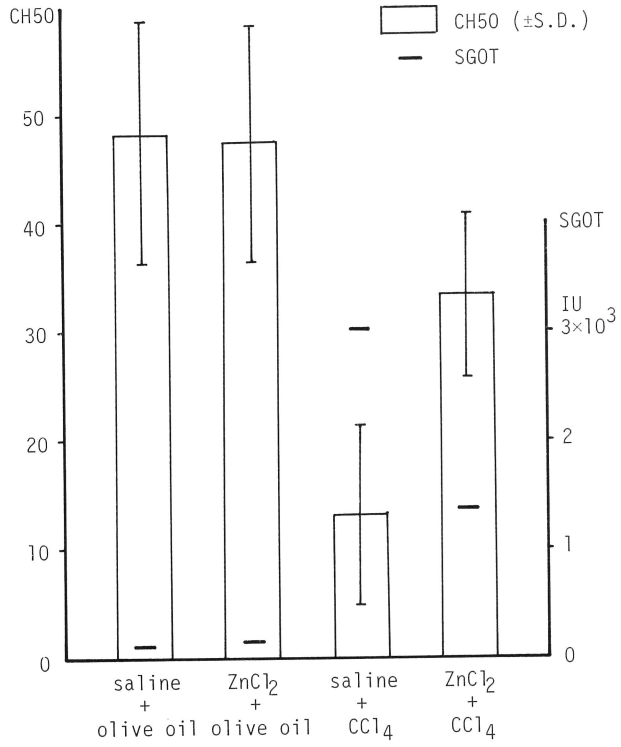

Fig. 1. Effect of $ZnCl_2$ on CCl_4-induced alterations of complement and SGOT levels

Animals pretreated with $ZnCl_2$ had significantly higher CH50 values following CCl_4 than those of CCl_4-treated animals, and the results demonstrated that pretreatment of rats with $ZnCl_2$ prior to CCl_4 resulted in significant protection against CCl_4-induced reduction of total hemolytic complement activity (Table 1, Fig. 1). The results also suggested that the acute hepatic damage following CCl_4 intoxication, as measured by hepatic transaminase release (SGOT), could be partially prevented by pretreatment with $ZnCl_2$, as reported by others (Table 2, Fig. 1). A close inverse correlation was observed between CH50 values and SGOT values, as an index of hepatic damage ($r=-0.691$, $p<0.02$).

Protective effect of $ZnCl_2$ on CCl_4-toxicity, as measured by serum TG, Al-P levels, composition of serum protein fractions, as well as relative weights of the livers and the kidneys, was not clearly observed (Table 2).

TABLE 1. Effect of $ZnCl_2$ on CCl_4-induced reduction of complement activity

treatment	no. of animals	body weight g	CH 50	TP g/dl	A/G	serum Zn μg/dl
saline+olive oil	6	324±17[a)]	47.6±11.3	6.36±0.18	0.94±0.16	117± 22
$ZnCl_2$+olive oil	6	308±28	47.4±10.9	5.60±0.19**	0.80±0.07	302±105**
saline + CCl_4	6	322± 9	13.0± 8.2**	5.55±0.27**	0.80±0.09	154± 32
$ZnCl_2$ + CCl_4	6	326±39	33.3± 7.6**	4.91±0.23**	0.79±0.11	349± 92**

a) mean±s. d. statistically significant *$p<0.05$ **$p<0.01$

TABLE 2. Effect of $ZnCl_2$ on CCl_4-induced release of GOT into serum

treatment	no. of animals	GOT IU/l b)	TG mg/dl	Al-P BL c)	liver g/100g b. w.	kidneys g/100g b. w.
saline+olive oil	6	83± 22	79.4±44.3	12.1±5.0	3.62±0.32	0.68±0.04
$ZnCl_2$+olive oil	6	124± 117	94.3±32.3	8.6±3.5	3.81±0.17	0.67±0.09
saline+CCl_4	6	3023± 965**	51.0±31.0	11.4±3.8	3.74±0.48	0.68±0.04
$ZnCl_2$+CCl_4	6	1382±1307*	47.4±35.7	12.8±6.6	3.84±0.29	0.64±0.04

b) International Unit c) Bessey-Lowry Unit

Carbon tetrachloride is well known to cause significant liver damage, and this damage is due to peroxidation of membrane lipids by reactive free radicals produced by the hepatic microsomal drug oxidizing system[7]. It has been demonstrated that CCl_4-induced liver injury could be prevented by pretreatment of animals with $ZnCl_2$. The possible mechanism of the protective effect of $ZnCl_2$ has not been fully understood, however, divalent metal zinc was shown to be a stabilizer of biological membrane possibly because of its interference with lipid peroxidation[8]. It has also been suggested that zinc-induced metallothionein could protect the CCl_4-induced liver damage by sequestering reactive metabolites of CCl_4[3]. However, the mechanism of CCl_4-induced reduction of total hemolytic complement activity, as well as that of protective effect of zinc against the alteration of complement system have not been understood, and further studies should be needed to clarify these mechanisms.

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REFERENCES

1) Asahara, H. and Mochizuki, Y. : Effect of carbon tetrachloride on rat complement, a preliminary report. Jap. J. Hyg. **34** : 176, 1979 (Abstract) (in Japanese)
2) Chavapil, M., Ryan, J. N., Elias, S. L. and Peny, Y. M. : Protective effect of zinc on carbon tetrachloride-induced liver injury in rats. Exp. Mol. Pathol. **19** : 186-196, 1973
3) Cagen, S. Z. and Klaassen, C. D. : Protection of carbon tetrachloride-induced hepatotoxicity by zinc : Role of metallothionein. Toxicol. Appl. Pharmacol. **51** : 107-116, 1979
4) Suarez, K. A. and Bhonsle,, P. : The relationship of cobaltous chloride-induced alterations of hepatic microsomal enzymes to altered carbon tetrachloride hepatotoxicity. Toxicol. Appl. Pharmacol. **37** : 23-27, 1976
5) Imai, S. and Yasuda, R. : Hotai. Rinsho-kenkyu **23** : 1137-1144, 1979 (in Japanese)
6) The Perkin-Elmer Co. Ed. : Analysis of serum-determination of copper and zinc. In Analytical Methods for Atomic Absorption Spectrometry. Norwalk, Connecticut, The Perkin-Elmer Co., 1973, BC-5
7) Recknagel, R. O. : Carbon tetrachloride hepatotoxicity. Pharmacol. Rev. **19** : 145-208, 1967
8) Chvapil, M., Ryan, J. N. and Zukoski, C. F. : Effect of zinc on lipid peroxidation in liver microsomes and mitochndria. Proc. Soc. Exp. Biol. Med. **141** : 150-153, 1972