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Brief Note

In Vivo Effects of Cadmium Acetate on Rat Complement

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ABSTRACT. Male Wistar rats were injected intraperitoneally or intravenously with various doses of cadmium acetate (i.p.: 1 mg, 2 mg or 4 mg Cd/kg body weight, i.v.: 1 mg, 2 mg or 3 mg Cd/kg body weight). Twenty-four hours following the injection, functional activities of the classical (CH50) and alternative complement pathways (APCH50), and the complement component C3 concentrations in the sera were significantly reduced in the Cd-treated rats.

Histopathological examinations of the livers and kidneys, as well as laboratory tests for serum transaminases (SGOT, SGPT) and serum urea nitrogen (SUN), revealed that cadmium acetate induced moderate to severe hepatic injury depending on the dose administered, whereas kidney lesions were less evident.

Key words : cadmium — rat complement — hepatic injury

The environmental contaminant cadmium (Cd) has recently been demonstrated to enhance the lethality of endotoxin in rats[1] and the susceptibility of rats to bacterial challenge.[2] Although the mechanisms by which Cd alters the host response to bacterial endotoxin or bacterial infection have not been fully clarified, alterations in the reticuloendothelial system, hepatic function[3] and antibody synthesis[4–6] have been reported. It is also well known that complement plays an important role in the nonspecific humoral factor in resistance to various infectious agents. The effect of Cd on the complement system, however, is not fully understood.

Therefore, the present study was initiated to evaluate whether the intraperitoneal or intravenous administration of a single dose of cadmium acetate would alter the complement levels in rats.

MATERIALS AND METHODS

Male Wistar rats of 10 weeks old, average body weight 250 g, were used in this study. The animals were fed Oriental MF pellets and given tap water ad libitum.

In the first experiment, 4 groups of rats were given an intraperitoneal injection of either sterile deionized water or 1, 2 or 4 mg Cd/kg body weight of a freshly made solution of cadmium acetate. In the second experiment, four other groups of rats were injected slowly via the tail vein with either sterile saline or 1, 2 or 3 mg Cd/kg body weight. The 4 mg Cd/kg dose was not employed in the second experiment, since a preliminary study indicated a high mortality rate in this dose group. Twenty-four hours following the injection, rats were lightly anesthetized with ether and blood was drawn from the aorta

望月義夫，菊池和子，植木絢子

with disposable plastic syringes. Blood was allowed to clot at room temperature for 2 hours, after which serum was separated by centrifugation at 4°C.

The serum hemolytic complement titer (CH50 units/ml) was assayed by a modified method of Mayer.[7] In the second experiment, a hemolytic assay of the whole alternative complement pathway (APCH50 units/ml) in serum was carried out using a modified method of Platts-Mills and Ishizaka, with guinea pig erythrocytes.[8] The serum C3 concentration was determined by a single radial immunodiffusion technique (SRID).[9] The total protein concentration in serum was measured by a modified biuret method (Wako). Serum glutamic oxalacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) were assayed by a commercial kit based on the method of Reitman and Frankel (Transaminase B-Test, Wako).[10] The serum urea nitrogen (SUN) level was determined by the urease-indophenol method (Urea NB-Test, Wako).

Immediately following exsanguination, the livers and kidneys were removed, cleaned, weighed, and small portions of each organ were fixed in Bouin's solution, embedded in paraffin, sectioned, and stained with hematoxylin-eosin for histopathological examination.

RESULTS AND DISCUSSION

The in vivo effects of i.p. injected cadmium acetate on serum complement levels and serum transaminases activities in the rats are summarized in Table 1. There was a significant reduction in both the serum hemolytic complement level and the serum C3 component concentration in the rats treated with 2 mg and 4 mg Cd per kg body weight.

SGOT and SGPT levels were elevated in the Cd treated rats. SUN levels were also significantly elevated in the rats receiving 4 mg Cd per kg body weight.

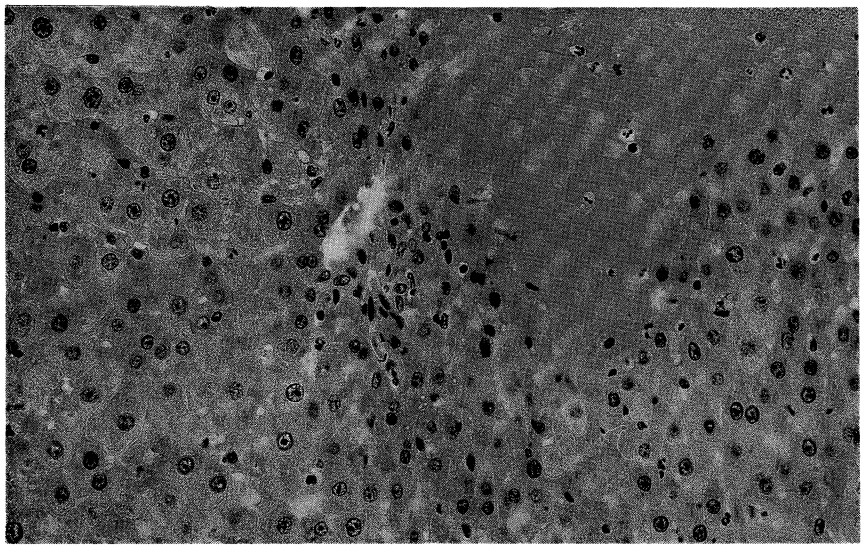

Fig. 1. Liver tissue of a Cd-treated rat sacrificed 24 hr after an i.p. injection of 4 mg Cd/kg body weight. (HE, ×280)

TABLE 1. Levels of serum complement and transaminases in rats following i.p. administration of cadmium acetate

treatment	no. of rats	CH50	C3 mg/dl	total protein g/dl	SGOT Karmen U.	SGPT Karmen U.	SUN mg/dl	liver g/100g b.w.	kidneys g/100g b.w.
Control	7[a] / 7	84.08±6.04[b]	0.99±0.05	5.80±0.08	123.0±14.1	21.4± 1.8	16.61±0.54	2.37±0.07	0.66±0.02
Cd 1 mg/kg b.w.	6 / 6	74.72±9.74	0.89±0.06	5.42±0.18	157.2±22.6	26.3± 3.4	16.88±0.89	2.52±0.09	0.68±0.05
Cd 2 mg/kg b.w.	6 / 6	53.10±6.86**	0.90±0.05	5.28±0.28	172.7±37.4	33.3±10.7	18.73±1.35	2.70±0.19	0.60±0.02
Cd 4 mg/kg b.w.	4 / 7	12.43±1.94**	0.67±0.04*	4.10 (n=2)	292.5±32.1**	172.3±24.5**	42.65±3.42**	3.55±0.03**	0.63±0.02

a) No. of survivors b) Mean±S.E. Value differed significantly from control: * $p<0.05$, ** $p<0.01$

TABLE 2. Levels of serum complement and transaminases in rats following i.v. administration of cadmium acetate

treatment	no. of rats	CH50	APCH50	C3 mg/dl	total protein g/dl	SGOT Karmen U.	SGPT Karmen U.	SUN mg/dl	liver g/100g b.w.	kidneys g/100g b.w.
Control	10[a] / 10	63.68±1.52[b]	26.27±0.40	0.98±0.04	5.69±0.07	85.3± 4.7	26.2± 2.2	23.1±0.91	2.69±0.05	0.68±0.01
Cd 1 mg/kg b.w.	10 / 10	60.51±1.19	25.62±0.43	0.85±0.03**	5.71±0.10	85.6± 4.7	23.0± 3.0	20.0±0.73*	2.81±0.16	0.71±0.03
Cd 2 mg/kg b.w.	9 / 10	56.06±0.83**	22.88±0.38**	0.79±0.03**	5.09±0.07**	117.2± 7.1**	33.6± 5.1	18.9±1.21*	3.29±0.26*	0.67±0.04
Cd 3 mg/kg b.w.	6 / 7	36.81±5.72**	15.87±2.38**	0.54±0.13**	4.25±0.16**	1305.4±603.4*	1419.2±689.2*	30.0±3.92*	3.77±0.17**	0.76±0.03*

a) No. of survivors b) Mean±S.E. Value differed significantly from control: * $p<0.05$, ** $p<0.01$

The weight of the liver was significantly increased in the rats treated with 4 mg Cd per kg body weight. Morphologically, the liver was markedly altered following Cd administration. Severe liver injury was evident 24 hours following i.p. injection of a challenge dose of 4 mg Cd per kg body weight (Fig. 1). Changes included hepatocyte necrosis and pyknosis of the nucleus of hepatocytes, focal to massive areas of centrilobular necrosis accompanied by infiltration of neutrophils and severe congestion.

Kidney lesions were less evident, and included insignificant changes in the proximal tubulus, such as occasional vacuolar degeneration and desquamation of epithelial cells, and formation of renal casts. These findings are similar to those described in the literature.[11]

The in vivo effects of intravenously injected cadmium acetate were essentially the same as those for intraperitoneal injection, as shown in Table 2. The functional activities of the classical and alternative complement pathways were significantly reduced in the rats treated with 2 mg and 3 mg Cd per kg body weight. The serum C3 concentration was also decreased in the rats injected with 1 mg, 2 mg and 3 mg Cd per kg body weight.

The possible anticomplementary effect of Cd was tested in vitro. Fresh normal rat serum was diluted with a gelatine-veronal buffer solution (pH 7.5) containing Cd of various concentrations (10^{-3}mol–10^{-6}mol), and the resultant mixture was tested for total hemolytic complement activity (CH50). No anticomplementary effect was observed up to a Cd concentration of 10^{-4}mol, which was much higher than the serum Cd level expected in the Cd-treated rats in the present study.

Heavy metals such as lead, cadmium or mercury are environmental contaminants for which immunosuppressive effects have been demonstrated in a variety of animals.[12] However, only a limited number of reports concerning the in vivo effects of heavy metals on complement system have been found in the literature.[13,14]

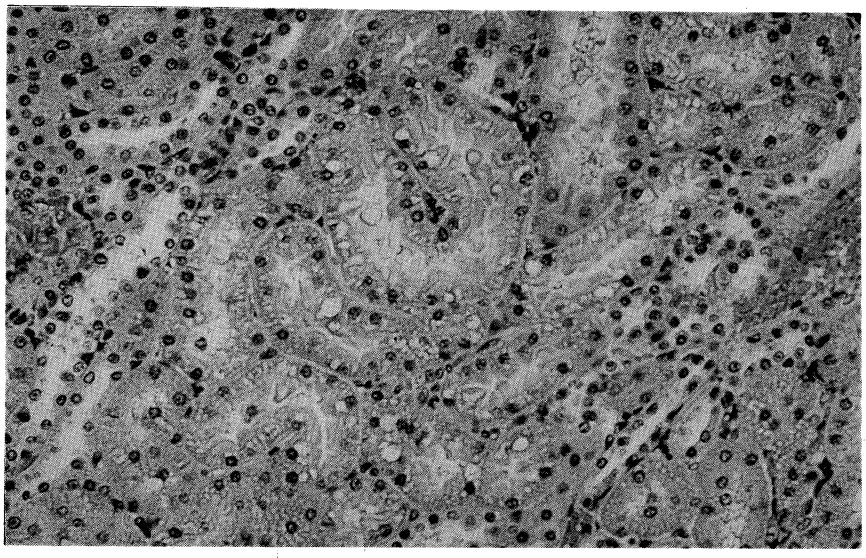

Fig. 2. Kidney tissue of a Cd-treated rat sacrificed 24 hr after an i.p. injection of 4 mg Cd/kg body weight. (HE, ×280)

It seems likely from the results of the present study that the increased susceptibility to infectious agents caused by Cd could be due in part to alteration of the complement system following Cd exposure.

Yoshio MOCHIZUKI, Kazuko KIKUCHI and Ayako UEKI

Department of Hygiene,
Kawasaki Medical School,
Kurashiki 701-01, Japan

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