Kawasaki Med. J. Vol. 7: No. 1, 71-76, 1981

INFLUENCE OF LEAD ACETATE AND MERCURIC CHLORIDE ON HEMOLYTIC COMPLEMENT ACTIVITY IN RATS

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Accepted for Publication on February 5, 1981

Abstract

***In vivo* effect of a single intravenous administration of lead acetate or mercuric chloride on total hemolytic complement activity (CH 50) in male Wistar rats was investigated, in order to assess the influence of heavy metals to host defense mechanisms against infectious agents. A significant reduction of the CH 50 values was observed in both groups of lead and mercury treated animals.**

INTRODUCTION

Several environmental contaminants, including heavy metals, have been demonstrated to enhance the susceptibility to a variety of infectious agents[1,2]. Some of the heavy metals are apparently immunosuppressive[3,4], since circulating antibody titers to the infectious agents in animals exposed to lead, cadmium or mercury were significantly lower than those from the control animals. It is also well known that complement plays an important role in nonspecific humoral factor in resistance against various infectious agents, however, the effect of heavy metals on the complement system has not been fully understood.

In the present study, a preliminary study was undertaken to evaluate whether the intravenous administration of a single dose of lead acetate or mercuric chloride would alter the complement activity in male Wistar rats, as a part of host defense mechanisms against infection.

MATERIALS AND METHODS

Male Wistar rats, 10–11 weeks old, were used in this study. The animals were fed Oriental MF pellet and given tap water *ad libitum*. Two separate experiments were carried out in two groups of 20 rats, and each group of rats was administered freshly made solution of lead acetate (Merck) or mercuric chloride (Wako) via tail vain, so that they received 2 mg, 1 mg or 0.5 mg of

Part of this study was presented at the 48th Annual Meeting of the Japanese Society for Hygiene in Maehashi, May 29, 1978.

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each metal for every 1 kg of body weight and the injection volume was 1 ml /kg body weight. A group of 5 rats in each experiment was injected intravenously with an equal volume of saline solution and served as a control. Twenty-four hours following the injection, animals were sacrificed under light ether anesthesia, and blood was drawn from abdominal aorta with disposable plastic syringes. A portion of blood was placed in a heparinized test tube and the rest of blood 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 of serum was assayed by a modified method of Mayer et al.[5] as the 50 percent hemolytic unit (CH 50). Total protein concentration of serum was determined by a modified biuret method (Wako). Compositions of serum proteins were analyzed by the cellulose acetate membrane electrophoresis[6].

Concentration of lead in the blood was determined with an atomic absorption spectrometer (Perkin-Elmer 503), equipped with a deuterium background corrector and a graphite furnace (Perkin-Elmer HGA-2000)[7]. Content of mercury in the blood was assayed by the flameless atomic absorption method with the Perkin-Elmer mercury analysis system (Type 303-0833)[8]. Concentration of zinc in serum was assayed by the flame atomic absorption method[9].

Erythrocyte δ-aminolevulinic acid dehydratase (ALA-D) activity was assayed by the method of Tomokuni[10]. Hemoglobin (Hb) content was measured as cyan-methemoglobin and hematocrit (Ht) value was determined by the microcapillary method. Blood urea-nitrogen level (BUN) was measured by the urease-indophenol method (Urea NB-Test, Wako).

RESULTS

Effect of lead acetate on rat complement :

The *in vivo* effect of a single intravenous injection of lead acetate on serum hemolytic complement activity (CH 50) is shown in Table 1. CH 50 levels in the rats treated with various doses of lead acetate were found to be lower than

TABLE 1

Effect of lead acetate on rat complement and serum protein fractions

Treatment	No. of animals	CH 50	Total protein g/dl	Alb. %	α_1-glob. %	α_2-glob. %	α_3-glob. %	β-glob. %	γ-glob. %	A/G
Control	5	54.8±14.0[(a)]	6.75±0.07	50.32±1.38	18.93±0.91	5.81±0.99	3.22±0.57	12.82±1.11	8.89±1.08	1.01±0.05
0.5 mg Pb/kg	4	49.7± 6.0	6.46±0.31	51.56±1.91	17.27±0.36**	5.97±0.85	3.34±0.77	12.60±1.03	9.27±0.55	1.07±0.08
1.0 mg Pb/kg	5	38.7±14.1	6.84±0.49	49.05±1.01	18.42±0.92	5.83±0.90	3.59±0.75	13.82±0.77	9.30±0.82	0.96±0.12
2.0 mg Pb/kg	5	39.2± 7.5*	6.48±0.16*	45.87±1.48**	19.77±0.85	7.27±0.97*	4.51±0.97*	14.59±0.70*	7.99±0.87	0.85±0.05**

(a) Mean±S.D. Statistically significant * $p<0.05$ ** $p<0.01$

those observed in the controls. Total protein concentration, electrophoretic distribution of serum protein fractions (in percent) and A/G ratio are also summarized in the same table. In the lead treated group (2.0 mg Pb/kg body weight), total protein concentration, albumin fraction and A/G ratio were significantly less than those of the controls, whereas α_2-globulin, α_3-globulin and β-globulin fractions were significantly higher than those of the control group ($p<0.05$). As the indices of lead body burden and its intoxication of rats, blood lead levels (Pb-B), serum zinc levels (Zn-S), hematocrit values (Ht), hemoglobin contents (Hb), erythrocyte ALA-D activities and BUN levels were determined and summarized in Table 2. As shown in the table, Pb-B levels in the lead treated animals were evidently higher than those of the controls, according to the injected dose of lead acetate. Mean Zn-S level in the lead treated group (2.0 mg/kg body weight) was significantly less than that of the control group. Blood ALA-D activities were significantly reduced by the administration of lead acetate.

TABLE 2

Effect of lead acetate on hematological findings and weights of organs

Treatment	No. of animals	Blood Pb μg/dl	Serum Zn μg/dl	Ht %	Hb g/dl	ALA-D (b)	BUN mg/dl	Body weight g	Liver (c)	Kidneys (c)
Control	5	n.d.	214±16	44.4±1.5	15.4±0.5	0.12±0.02	22.2±1.2	306±40	3.78±0.25	0.65±0.03
0.5 mg Pb/kg	4	16.3± 8.8	233±24	44.5±2.2	15.3±0.9	0.03±0.04**	22.6±1.6	295±10	3.70±0.13	0.65±0.04
1.0 mg Pb/kg	5	36.4±11.2	215±25	46.3±2.2	16.3±0.5	0.01±0.005**	21.6±1.9	310±20	3.47±0.38	0.68±0.04
2.0 mg Pb/kg	5	88.5±15.0	178±11**	43.6±1.0	15.5±0.2	n.d.	20.5±1.8	328±16	3.35±0.14*	0.67±0.03

(b) μ mol PBG /hr/ml RBC (c) organ weight g/100 g body weight

Effect of lead treatment on the weights of the liver and the kidneys are also shown in Table 2. Weights of organs are expressed as g per 100 g of body weight. Among them, only the liver weight in the lead treated group (2.0 mg/kg body weight) was significantly lower than that in the control group.

Possible anticomplementary activity of lead acetate was determined *in vitro*. Normal rat serum was diluted with gelatine-veronal buffer solutions (pH 7.5) containing lead acetate (10^{-3}M-10^{-6}M) of various concentrations, and tested the resultant mixture for total hemolytic complement activity. Results of the tests revealed that no anticomplementary activity was observed up to lead concentration of 10^{-4}M, and this level was much higher than the lead levels found in the blood of the lead treated animals.

Effect of mercuric chloride on rat complement :

The *in vivo* effect of a single intravenous administration of mercuric chloride on the CH50 levels is shown in Table 3. Mean CH 50 levels in the mercury treated groups (1.0 mg Hg/kg and 2.0 mg Hg/kg body weight) were significantly less than that of the control group. Total protein concentrations, percentages

of serum protein fractions analyzed by cellulose acetate electrophoresis and A/G ratios are also listed in the same table. In the mercury injected rats, albumin fractions, A/G ratios decreased, whereas α_3-globulin fractions significantly increased.

TABLE 3

Effect of mercuric chloride on rat complement and serum protein fractions

Treatment	No. of animals	CH 50	Total protein g/dl	Alb. %	α_1-glob. %	α_2-glob. %	α_3-glob. %	β-glob. %	γ-glob. %	A/G
Control	5	44.6±11.7	6.08±0.11	48.45±0.82	6.29±0.71	14.65±0.45	9.88±1.36	14.68±1.18	6.05±0.47	0.94±0.03
0.5 mg Hg/kg	5	32.5±17.7	5.96±0.13	46.53±0.82**	7.12±0.81	14.83±0.42	11.55±1.14	14.89±0.91	5.09±0.86	0.87±0.03
1.0 mg Hg/kg	5	24.6±11.5*	6.04±0.23	41.19±1.67**	6.71±0.20	15.83±0.93	14.60±1.16**	16.29±0.58*	5.39±0.47	0.70±0.05**
2.0 mg Hg/kg	5	35.2± 2.7*	5.86±0.34	43.40±2.25**	7.09±1.12	14.81±1.70	14.43±0.94**	15.11±1.13	5.16±1.26	0.77±0.07**

Blood mercury concentrations (Hg-B), Zn-S, Ht values, Hb contents, erythrocyte ALA-D activities and serum BUN levels were measured as the indices of mercury body burden and its intoxication. The results are summarized in Table 4. Among them, Hg-B levels and BUN levels were markedly increased by the administration of mercuric chloride, while Zn-S, Ht values, Hb contents and erythrocyte ALA-D activities were unchanged.

TABLE 4

Effect of mercuric chloride on hematological findings and weights of organs

Treatment	No. of animals	Blood Hg μg/dl	Serum Zn μg/dl	Ht %	Hb g/dl	ALA-D (b)	BUN mg/dl	Body weight g	Liver (c)	Kidneys (c)
Control	5	0.02±0.004	278±46	42.3±1.3	13.9±0.4	0.17±0.04	16.2± 3.0	234± 9	3.73±0.24	0.78±0.03
0.5 mg Hg/kg	5	0.35±0.06**	262±33	42.2±2.6	13.5±0.8	0.20±0.04	58.7±16.6**	234± 6	3.36±0.33	1.00±0.03**
1.0 mg Hg/kg	5	0.68 (d)	233±34	41.3±1.2	13.0±0.5	0.17±0.03	94.6±10.6**	238±11	3.63±0.24	1.01±0.05**
2.0 mg Hg/kg	5	0.81±0.22**	248±47	40.8±4.2	13.0±1.2	0.16±0.03	138.2±11.7**	240± 5	3.42±0.18	1.00±0.10**

(d) n=2

Effect of mercuric chloride on the weights of the liver and the kidneys per 100 g of body weight are listed in the table. Among the organs, weights of kidneys in the mercury treated groups (0.5 mg Hg/kg, 1.0 mg Hg/kg and 2.0 mg Hg/kg body weight) was found to be significatly higher than that of the control group ($p<0.01$).

Anticomplementary effect of mercuric chloride in concentrations of 10^{-6}M to 10^{-3}M was also determined *in vitro*, however, no anticomplementary effect was observed up to 10^{-5}M, and this mercury level was much higher than that observed in the blood of mercury treated rats.

DISCUSSION

Heavy metals such as lead, cadmium or mercury are environmental contaminants for which immunosuppressive effects have been demonstrated[3,4].

Interferon synthesis was also suppressed by lead acetate[11]. It is also well known that complement plays an important role in nonspecific humoral factor in resistance against various infectious agents, however, only a limited number of reports for the *in vivo* effect of heavy metals on complement system are available in the literature, in which Fonzi et al.[12] demonstrated that the total hemolytic complement activity in rabbits was reduced by the intravenous administration of lead acetate.

The present findings indicate that the CH 50 levels in the rats were significantly lowered by a single intravenous administration of lead acetate. It is also demonstrated that total protein concentrations of sera, albumin fractions and A/G ratios were significantly reduced in the lead acetate treated rats. These findings are essentially in agreement with the previous observations of Fonzi et al.[12]

In the mercuric chloride treated rats, CH 50 levels, serum albumin fractions and A/G ratios were significantly reduced. It seems likely from the data of the present study that the increased susceptibility to infectious agents caused by lead or mercury could be due in part to alterations in complement system, together with decrease in antibody production or interferon synthesis as reported previously by others[3,4,11,13].

It must be emphasized that the present investigation is of a preliminary nature and that further studies should be carried out to acertain which components of the complement system are being affected.

Acknowledgment

The authors are indebted to Miss Y. Iwasaki for her helpful technical assistance.

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