Endotoxin Susceptibility of Mice Treated with Lead Acetate

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Endotoxin Susceptibility of Mice Treated with Lead Acetate

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Summary

Effects of lead acetate on susceptibility of ddY mice to lethal activity of endotoxin from *E. coli* were studied. Mice which received the lead were more sensitive to the endotoxin than normal ones. This effect of lead acetate depended on the injection doses and routes, as well as on the intervals between injections of the lead and endotoxin. The most effective lead-treatment was simultaneous, intravenous injection of maximal sublethal dose, 0.5 mg per 10 grams of mouse, of lead acetate with the endotoxin, though pretreatments with lead acetate were more effective than the post-treatments. In mice injected simultaneously with the lead and endotoxin, the susceptibility to the lethal activity of endotoxin increased about 1,400 times over normal, and a linear relationship was obtained between the logarithm of the doses of the endotoxin and probits of mortality of mice. This relation was highly significant in the range of 0.2 to 6.25 μg per 25 grams of mouse, thus this system is useable for quantitative detection of small amounts of endotoxin.

It has been known that various treatments involving an inoculation of BCG (1) and injections of carbon tetrachloride (2), saccharrated ion (3), actinomycin D(4) or lead acetate (5) promote the lethal activity of endotoxins in laboratory animals. The promotion by lead acetate has been reported in rats (6), mice (7), chicks (8) and baboons (9). And several papers have been published on the mechanisms of an enhanced susceptibility to endotoxins in lead-treated animals (10, 11, 12). In addition, a bioassay of the microquantity of endotoxin using lead-treated rats was established (13). However, in mice such information is comparatively little, and no standardized method is available for microanalysis of endotoxins.

In this paper, altered susceptibilities of mice to an endotoxin by lead-treatments and a practicable quantitative method for the endotoxin are investigated.

Materials and Methods

Endotoxin: Lypopolysacchardie from *E. coli* 029; B6 (Difco) was dissolved and diluted with sterilized pyrogen-free physiological saline solution.

Lead Acetate: Diluted lead acetate solutions were prepared with 5% dextrose solution and sterilized in boiling water for 10 minutes.

Mice: Male mice of ddY strain weighing 25 to 30 grams, 6 to 7 weeks old, were maintained in a room kept at constant temperature (25±2°C) and humidity (55±5%) through out the experiment and from 10 days before. They were injected intravenously or intraperitoneally with 0.05 ml of lead acetate solution and 0.1 ml of the endotoxin solution per 10 grams of their body weight. Mortality of the mice within 72 hours after injections was determined and LD_{50} values of the lead acetate and the endotoxin were calculated by means of Behrence. The injected or lethal doses were usually expressed per 10 grams of body weight of mouse.

Results

Toxicity of Lead Acetate: Serially, 1.2-fold, diluted lead acetate solution was injected into the caudal vein of mice to determine the toxicity. The results are shown in Table 1. LD_{50} of lead acetate was 0.86 mg and MDL and 0.7 mg.

TABLE 1. *Toxicity of Lead Acetate in Mice.*

Dose of lead aceate[1]		Death/Tested
Dilution	mg/10 g BW	
1.2^{-0}	1.00	11/12
1.2^{-1}	0.83	3/12
1.2^{-2}	0.69	1/12
1.2^{-3}	0.58	0/12
1.2^{-4}	0.48	0/12

1) Injected intravenously. LD_{50}=0.86 mg/10 g BW

Lethal Dose of Endotoxin: To determine the toxicity of the endotoxin used, 2-fold serial dilutions of the endotoxin were intravenously injected into the mice. From the results, it was known that LD_{100} and MLD were 0.8 mg and 0.1 mg respectively, as shown in Table 2. LD_{50} calculated was 0.179 mg.

TABLE 2. *Lethal Activity of Endotoxin from E. coli in Mice.*

Dose of Endotoxin[1]		Death/Tested
Dilution	μg/10 g BW	
2^{-0}	800	16/16
2^{-1}	400	10/11
2^{-2}	200	5/ 9
2^{-3}	100	2/10
2^{-4}	50	0/14

1) Injected intravenously. LD_{50}=179.3 μg/10 g BW

Effects of Lead Acetate on Lethal Activity of Endotoxin: To determine what dose of lead acetate enhanced the susceptibility to endotoxin, mice were injected with sublethal doses, 5 μg and 50 μg, of endotoxin immediately after treatments with varying sublethal doses of lead acetate, 8 μg to 500 μg. The results are shown in Table 3. No mouse died in the case of a single injection of endotoxin or of lead acetate, however some or all mice injected with both materials died and the mortality rate increased with the increment of lead acetate. This indicates that the maximal sublethal dose, 0.5 mg, of lead acetate was the most effective on the enhancement of the susceptibility to endotoxin in mice.

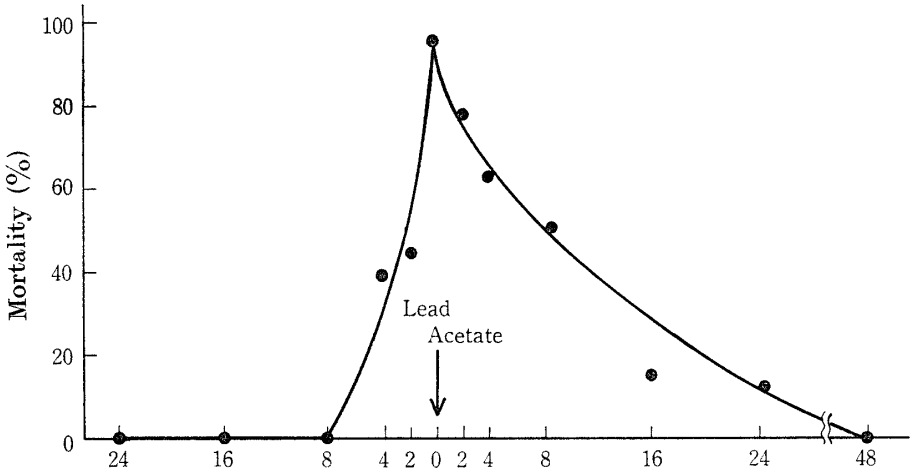
TABLE 3. *Effect of Injection Dose of Lead and Endotoxin on Mortality in Mice.*

Lead Acetate[1] mg/10 g BW	Endtoxin[1] 50 μg/10 g BW	Endotoxin[1] 5 μg/10 g BW	Without Endotoxin
0.5	12/12[2]	11/12[2]	0/10[2]
0.25	10/12	6/12	0/10
0.12	9/12	3/12	0/10
0.06	3/12	1/12	0/10
0.03	2/12	0/12	—
0.015	0/12	0/12	—
0.008	0/12	0/12	—
None	0/10	0/10	—

1) Lead acetate was administerted intravenously, simultaneously with endotoxin.
2) Death/Tested

Critical Period for Interaction between Lead Acetate and Endotoxin: As stated above, mice injected with lead acetate became more sensitive to the endotoxin, but a suitable interval between the treatments of the two materials to give the highest sensibility is unknown. So, a sublethal dose, 5 μg, of the endotoxin was injected intravenousely at various times from 0 to 48 hours before or after the injection of lead acetate. The results are shown in Fig. 1. The highest mortality was given in the case of simultaneous injections, though the death of mice was obtained by pretreatments with lead acetate within 24 hours and by post-treatments within 4 hours. When comparing the same intervals, the pretreatment always showed a higher mortality rate than the post-treatment with lead acetate.

Effect of Injection Routes of Lead Acetate on Lethal Activity of Endotoxin: The next experiment was conducted to compare the efficiencies of lead acetate given through different routes. As shown in Table 4, no effect was observed in the case of simultaneous, intraperitoneal injection of endotoxin and lead acetate. While, the susceptibility to the endotoxin was increased by intravenous injection of lead acetate in mice injected intraperitoneally with the toxin, in which the increment was 395-fold in MLD and 26-fold in LD_{50}. However, the efficiency was lower than in the case of intravenous injection with the two materials, in which the susceptibility was 1,400-fold or 256-fold increment in MLD or LD_{50} respectively.



Hours of Endotoxin-injection after or before Lead-injection

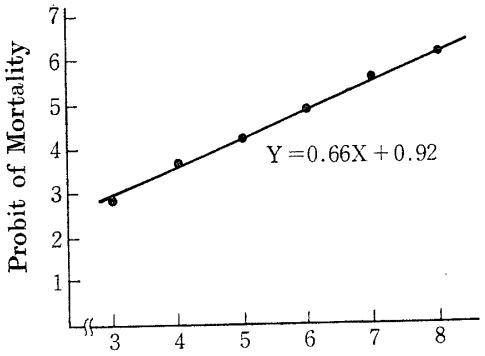
Injection Dose ; Endotoxin : 5μg/10g BW, Lead Acetate : 0.5mg/10gBW.

FIG. 1. Influence of Intervals between Injections of Lead Acetate and Endotoxin on Mortality in Mice.

TABLE 4. *Influence of Injection Routes on Lethality of Endotoxin.*

Lead Acetate		Routh of endotoxin	Toxicity of endotoxin[1]		
Route	Dose[1]		LD_{50}	LD_{100}	MLD
—	—	i.p.	356 μg	800 μg	237 μg
i.p.	1.0 mg	i.p.	379	800	237
i.v.	0.5 mg	i.p.	13.5	100	0.6
—	—	i.v.	179	800	100
i.v.	0.5 mg	i.v.	0.70	5	0.07

1) Per 10 grams of body weight.



Logarithmic Dose of Endotoxin
($2^X$$\mu$g/kg BW)

Dose of lead acete : 0.5mg/10g BW

FIG. 2. Dose Response of Lead-Treated Mice to Endotoxin.

Correlation between Doses of and Response for Endotoxin in Lead-treated Mice: To know the quantitative sensitivity of the lead-treated mice to endotoxin, mice were injected intravenously with lead acetate at a dosage of 0.5 mg per 10 grams of body weight and with 2-fold serially diluted endotoxin ranging from 5.0 μg to 0.04 μg. Fig. 2 shows the results, in which it is shown that a highly significant linear relationship (t=49.4) exists between the logarithm of the doses of endotoxin (X) and the probits of mortalities of the mice (Y). The regression line is given by $Y=0.66X+0.92$, hence the dose of endotoxin is expressed by 2^X μg per Kg of body weight of mouse.

Discussion

Since Selye *et al.* (5) reported that the lethal activity of endotoxin to rats was remarkably promoted by injection of lead acetate, this synergism has been used as a convenient bioassay for minute amounts of endotoxin (13). In this paper, we researched some conditions for the assaying of endotoxin from *E. coli* by use of mice treated with lead acetate.

The results indicate that the enhanced susceptibility to endotoxin in lead-treated mice depends on the injected doses and routes of lead acetate and on the intervals between injections of the endotoxin and lead, and that the most effective sensitization is obtained by simultaneous, intravenous injections of the toxin and 0.5 mg of lead acetate. Under these conditions, the minimal lethal dose of endotoxin is 0.07 μg per 10 grams of lead-treated mouse, of which value means that the lead-treatment increases the susceptibility to the endotoxin about 1,400 times above normal mice. Also, a highly significant linear relationship was obtained between the logarithmic increments in dose of endotoxin and probits of mortality of mice. These findings show that the lead-treated mice are available for the detection of submicrograms of endotoxins. The lower limit of detection of the employed endotoxin was about 0.2 μg per 25 grams of mouse body weight. This value is approximately the same as that obtained in lead-treated rats (13), in which the susceptibility to endotoxin, however, increased about 100 thousands times above the normal.

Milner *et al.* (14) showed data indicating that the minimal lethal dose of endotoxins from *Salmonella* in lead-treated mice was 0.04 μg per 20 grams of body weight and they indicated that the enhancements of susceptibility by lead were over 1,000-fold in one endotoxin and 10,000-fold in another as compared with MLD in normal mouse. An importance of strains of mice for bioassay of endotoxin was mentioned by Pieroni *et al.* (4). Therefore, if a more sensitive strain of mice were searched to be used or the other endotoxins were used, the susceptibility in lead-treated mice might be more enhanced.

Fujiwara and Kuratsuka (15) reported that effects of lead acetate on an endotoxic action which decreased the body weight of ddY mice were detected in those

injected intraperitoneally with lead acetate, though in our experiment, no effect was observed on the intraperitoneal injection with lead acetate. These facts seem to suggest that the lethal action and the decreasing action in body weight of endotoxin are caused by different mechanisms.

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References

1) Suter, E., *J. Immunol.*, **92**, 49 (1964)
2) Farrer, W.E. Jr. and Magnani, R.J., *Proc. Soc. Exp. Biol. Med.*, **115**, 396 (1964)
3) Beeson, P.B., *J. Exp. Med.*, **86**, 29 (1947)
4) Pieroni, R.E., Broderick, E.J., Bundeally, A. and Levine, L., *Proc. Soc. Exp. Biol. Med.*, **133**, 790 (1970)
5) Selye, H., Tuchweber, B. and Bertok, L., *J. Bact.*, **91**, 884 (1966)
6) Trejo, R.A. and DiLuzio, N.R., *Proc. Soc. Exp. Biol. Med.*, **136**, 889 (1971)
7) Rippe, D.F. and Berry, L.J., *J. Reticuloendothel. Soc.*, **13**, 527 (1973)
8) Truscott, R.B., *Can. J. Comp. Med.*, **34**, 134 (1970)
9) Hoffman, E.O., DiLuzio, N.R., Holper, K., Brettschneider, L. and Coover, J., *Lab. Invest.*, **30**, 311 (1974)
10) Bertok, L., *J. Bact.*, **95**, 1974 (1968)
11) Trejo, R.A., DiLuzio, N.R., Loose, L.D. and Hoffman, E., *Exp. Mol. Path.*, **17**, 145 (1972)
12) Filkins, J.P. and Buchanan, B.J., *Proc. Soc. Exp. Biol. Med.*, **142**, 471 (1973)
13) Buchanan, B.J. and Filkins, J.P., *Amer. J. Physiol.*, **231**, 258 (1976)
14) Milner, K.C., Rudbach, J.A. and Ribi, E., "*Microbial Toxins. Vol. IV. Bacterial endotoxins*," ed. by G. Weinbaum, S. Kadis, and S. Ajl, Academic Press, New York and London, pp. 34
15) Fujiwara, H. and Kuratsuka, K., *Medicine and Biol.*, **93**, 461 (1976) (in Japanese)