Short Communication

**DISSOCIATION OF THE ENHANCING EFFECTS OF LEAD
ACETATE ON LETHAL AND BODY-WEIGHT-
DECREASING ACTIVITIES OF ENDOTOXIN
BY THE ADMINISTRATION ROUTE**

HIROSHI FUJIWARA and KAZUO KURATSUKA

*Department of General Biologics Control, National Institute
of Health, Kamiosaki, Shinagawa-ku, Tokyo 141*

(Received June 25, 1982. Accepted July 30, 1982)

SUMMARY: The enhancing effects of lead acetate on the lethal and the body-weight-decreasing activities of endotoxin in mice with different combinations of the administration routes. The effect of lead acetate on the lethality was shown by iv administration, but not by ip administration. The body-weight-decreasing activity of endotoxin was enhanced by ip administration of lead acetate but not by iv administration. In any case, the route of administration of endotoxin was not concerned with the enhancement by lead acetate.

Selye et al. (1966) found the enhancing effect of lead acetate on the lethal activity of endotoxin by iv administrations of both in the rat. Truscott (1969) reported that the enhancing effect of lead acetate on the lethal activity of endotoxin in chicks was shown by iv administrations of lead acetate, but not by the ip administration. Similar results were also obtained in mice (Terao and Katsuno, 1978). Thus, it is suggested that it may be important to give lead acetate intravenously for the enhancement of endotoxin lethality. While, we have demonstrated in mice that lead acetate administered intraperitoneally enhanced the body-weight-decreasing activity of endotoxin given by the same route (Kuratsuka and Fujiwara, 1972, 1974; Fujiwara and Kuratsuka, 1978, 1980, 1982). In mice, then, it is of interest to compare the enhancement of the lethal and the body-weight-decreasing activities of endotoxin by the combinations of the ip and iv administration routes of both substances.

A lipopolysaccharide (LPS) preparation (Control No. 613122, Difco, Detroit, MI) prepared by Westphal's method from *Escherichia coli* 0 111 B4 cells was used throughout as endotoxin. For the experiments of the lethal activity, 5 mg (ip) or 1 mg (iv) per mouse of lead acetate (Wako Junyaku, Osaka) was used. These doses were led from the data of LD₅₀ of lead acetate, 11.6 mg per mouse

(ip) and 2.61 mg per mouse (iv), respectively (Fujiwara and Kuratsuka, 1976). For the body-weight-decreasing activity, 83 μ g per mouse of lead acetate, which had no effect by itself (Kuratsuka and Fujiwara, 1974) was used. Other materials including mice, diluent and equipments were prepared and used as described in the preceding paper (Fujiwara and Kuratsuka, 1982). The effects of lead acetate on the lethal and body-weight-decreasing activities were studied by possible combinations of the two routes (ip and iv) and sequences of administrations. Lethality of mice was calculated from the number of deaths within 24 hr after administration of endotoxin. As a metamer of body-weight-decreasing activity of endotoxin, body-weight ratio on the first day (BWR_1) was adopted and calculated in each mouse by the following formula: $BWR_1 = B_1/A \times 100$, where A and B_1 mean the body weight (gram) before and on the first day after endotoxin administration, respectively. Ip and iv administrations of endotoxin and lead acetate were carried out in volumes of 0.25 ml and 0.2 ml per mouse, respectively.

The experiment for the lethal activity was carried out simultaneously. Immediately after lead acetate, 500 μ g per mouse of endotoxin was administered. As shown in Table I, iv administration of lead acetate enhanced significantly both the lethalities of endotoxin given intraperitoneally or intravenously. On the contrary, lead acetate administered intraperitoneally showed no effect on the lethality of endotoxin regardless of the administration routes of endotoxin. This phenomenon was confirmed also with a smaller dose such as 200 μ g per mouse of endotoxin.

As for the body-weight-decreasing activity, the lead acetate was given prior to, simultaneously with or after endotoxin administration. The interval between administrations of lead acetate and 0.5 μ g per mouse of endotoxin was an hour. The experiments were carried out separately, depending on the administration method of lead acetate. The results are summarized in Table II. As we have already reported, the body-weight-decreasing activity of endotoxin was signifi-

TABLE I

Combinations of administration routes of lead acetate and endotoxin and the lethal activity of endotoxin

Administration route of		Lethality of mice(%)	
Lead acetate	Endotoxin (500 μ g/mouse)		
ip (5 mg/mouse)	ip	0/10†	(0)
	iv	1/10	(10)
iv (1 mg/mouse)	ip	8/10	(80)
	iv	9/10	(90)
—	ip	1/10	(10)
	iv	1/10	(10)

† The numerators are numbers of mice died within 24 hr after administration of endotoxin given immediately after lead acetate and the denominators are numbers of mice tested.

TABLE II

Enhancing effect of lead acetate on the body-weight-decreasing activity of endotoxin in several methods of administrations

Endotoxin (0.5 µg/ mouse)	Time of administration of lead acetate	Ip administration of		Iv administration of	
		Lead acetate (83 µg/ mouse)	Water (Control)	Lead acetate (83 µg/ mouse)	Water (Control)
ip (0.25 ml/mouse)	Pre-	93.1¶*	95.7¶	96.9¶	95.3¶
	Simultaneous	95.0*	99.3	97.9	97.6
	Post-	95.4*	98.3	96.3	97.5
iv (0.2 ml/mouse)	Pre-	92.4*	96.1	95.6	95.9
	Simultaneous	92.5*	97.6	97.0	96.8
	Post-	94.9*	100.4	96.6	96.2

¶ Mean value of body weight ratios on the first day (BWR₁).

* Significantly different ($\alpha < 0.05$) from control in the *t*-test using a common variance (3.70). The common variance was calculated as the homoscedasticity of variances in the experiments summarized in the table was not denied by the Hartley's method.

As the experiments were not always performed simultaneously except those in the same row of this table, the *t*-tests were carried out only about the results in the same row.

The number of mice was 10 a group.

A 1-hr interval was adopted between administrations of lead acetate and endotoxin.

cantly enhanced by the ip administration of lead acetate. Now it was confirmed that the lead acetate administered intravenously showed no enhancing effect.

No report other than ours (Fujiwara and Kuratsuka, 1978, 1980, 1982; Kuratsuka and Fujiwara, 1972, 1974) about the enhancing effect of lead acetate on the body-weight-decreasing activity of endotoxin in mice has appeared. In respect of the lethality, our results in this report agree well to those in the reports by other workers (Truscott, 1969; Terao and Katsumo, 1978), though they did not make such many combinations of the administration routes as we did.

The relation between the enhancing effect and the route of administration of lead acetate on the lethal activity of endotoxin was opposite to that on the body-weight-decreasing activity. Such dissociation of the enhancing effects on the activities of endotoxin was demonstrated at least in mice. The fact must depend on the difference in the mode of action of the lethal and the body-weight-decreasing activities of endotoxin. Schaedler et al. (1961) suggested different mechanisms for the two activities and reported that the loss of body weight occurred even in mice which are remarkably resistant to the lethal effects of endotoxin. These findings suggest that lead acetate may serve as a useful tool for elucidating the two distinct activities of endotoxin.

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