EFFECT OF PRETREATMENT ON LEAD DISTRIBUTION IN LIVER AND BODY OF RAT IN ACUTE LEAD POISONING

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Male rats were given 5 mg/kg of lead intraperitoneally and after an interval of 3 days were given 50 mg/kg of lead intraperitoneally. The distribution of lead in the body after receiving challenge dose of lead was compared with that of rats non-pretreated with lead. Lead contents of each organ, especially that of liver, of the pretreated rats were found to be reduced. The rate of lead content in nuclei fraction, where lead taken into liver mainly concentrated, to lead content in liver was significantly low in rats pretreated with lead compared with non-pretreated rats after challenging. The rate of lead content in other fractions of liver to lead in liver of pretreated rats rose from that of non-pretreated rats. From these results, it was recognized that the pretreatment remarkably changes the lead distribution in body and in liver.

It has already been reported that acute toxicity of lead is reduced by a prior administration of small doses of lead¹⁾ and the pretreated animals reduced the content of lead in liver and enhanced the excretion of lead in feces compared with non-pretreated animals.²⁾ So the present study investigated the change with time of lead distribution in body and in liver of rats pretreated with and without lead.

EXPERIMENTAL METHODS

Experiment I. Lead distribution in body

Fourteen male rats of Sprague-Dawley strain (13 weeks old) were divided into two groups. The rats of the first group were given intraperitoneally 5 mg Pb/kg of $\text{Pb}(\text{NO}_3)_2$ as pretreatment, and after an interval of 3 days they were injected intraperitoneally 50 mg Pb/kg as challenging (Group Pb-Pb). The rats of the second group were given intraperitoneally 50 mg Pb/kg (Group Pb). At 1st and 7th day after challenging 3 and 4 rats respectively were killed and heart, lungs, liver, spleen, and kidneys were obtained. Lead in these samples was determined by atomic absorption spectrometry after wet-ashing of sample with nitric acid and hydrogen peroxide.

Experiment II. Lead distribution in liver

Twenty-four male rats of Sprague-Dawley strain (7 weeks old) were divided into two groups. The first group was treated as Group Pb-Pb and the second group as Group Pb as described above. The method and dosage of lead administration was the same as in the Experiment I. Six normal male rats were also used as control.

Four rats each were killed at 1st, 3rd and 7th day after challenging of lead and livers were obtained and immediately perfused with cold 0.25 M sucrose, 0.01 M tris-HCl buffer, pH 7.4. These livers were homogenized in a cold buffer with a glass-teflon homogenizer spinning at 800 r.p.m. for 2 min. Then the homogenized solutions were separated into nuclei, mitochondria, microsome and supernatant fractions by the centrifugation method³⁾. Lead in each fraction was determined by atomic absorption spectrometry.

EXPERIMENTAL RESULTS

Experiment I. Lead distribution in body

Lead contents in each organ at 1st and 7th day after challenging of lead are shown in Table 1. The effect of pretreatment is recognized on the contents of lead in various organs, that is, at 1st day after challenging the lead content in liver of Group Pb-Pb was significantly lower and that of lung was significantly higher compared with those of Group Pb; at 7th day the lead content in heart, lung and liver of Group Pb-Pb was significantly lower, and that in spleen and kidney was lower, than that of Group Pb.

Also, lead content from 1st to 7th day showed no change in liver and spleen which contained a high concentration of lead in both the Groups, but the lead content of heart, lung and kidney increased in Group Pb while it did not increase in Group Pb-Pb from 1st to 7th day.

These results showed that pretreatment of lead suppressed the lead accumulation after challenging of lead, and it will be able to presume that pretreatment accelerates the excretion of lead.

Table 1. Lead content in organs ($\mu\text{g/g}$ wet wt)

	n	Heart $\bar{x} \pm \text{SD}$	Lungs $\bar{x} \pm \text{SD}$	Liver $\bar{x} \pm \text{SD}$	Spleen $\bar{x} \pm \text{SD}$	Kidneys $\bar{x} \pm \text{SD}$
Pb 4	0.84 $\pm$ 0.16	2.35 $\pm$ 0.64	202.57 $\pm$ 41.29	785.45 $\pm$ 196.73	41.46 $\pm$ 13.03	
After 1 day Pb-Pb 4	0.87 $\pm$ 0.07	5.14 $\pm$ 1.41	75.58 $\pm$ 59.03	526.30 $\pm$ 433.95	39.46 $\pm$ 6.79	
		p<0.05	p<0.05			
Pb 3	1.71 $\pm$ 0.47	26.58 $\pm$ 7.57	195.14 $\pm$ 93.66	407.08 $\pm$ 200.64	62.63 $\pm$ 31.96	
After 7 days Pb-Pb 3	0.74 $\pm$ 0.16	5.03 $\pm$ 2.09	48.24 $\pm$ 31.57	224.17 $\pm$ 152.43	31.65 $\pm$ 15.40	
	p<0.05	p<0.01	p<0.05			

Experiment II. Lead distribution in liver

Lead contents of liver are shown in Table 2. Lead content of liver in Group Pb

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increased till the 3rd day, thereafter decreased. But lead content of liver in Group Pb-Pb reached maximum at the 1st day and decreased from 1st to 3rd day, after that maintained its value until 7th day. From this result, it was recognized that pretreatment remarkably diminished the lead content and changed the lapse of lead content in liver.

Table 2. Lead distribution in liver of rats pretreated with and without lead

		After 1 day		After 3 days		After 7 days	
Group		$\mu\text{g Pb/g}$	% ^b	$\mu\text{g Pb/g}$	%	$\mu\text{g Pb/g}$	%
Liver homogenate	Pb	64.1± 9.2		68.8±11.7		47.8± 6.4	
	Pb-Pb ^a	45.8±18.5		21.2± 8.6		22.9±10.6	
				p<0.01		p<0.05	
Nuclei fraction	Pb	41.3± 8.8	63.9± 4.8	55.3± 9.1	80.3± 0.7	34.6± 5.1	72.3±3.2
	Pb-Pb	29.2±16.7	58.9±14.7	13.9± 6.9	63.0±10.2	14.2± 8.3	59.1±6.9
				p<0.01		p<0.05	
Mitochondria fraction	Pb	5.8± 0.6	9.2± 1.8	2.7± 0.7	3.8± 0.4	2.8± 0.4	5.9±0.7
	Pb-Pb	4.9± 0.5	12.8± 5.8	1.7± 0.5	9.1± 3.1	2.4± 0.9	8.4±2.4
						p<0.05	
Microsome fraction	Pb	2.4± 0.2	3.9± 0.6	1.9± 0.5	2.7± 0.5	2.2± 0.6	4.5±0.7
	Pb-Pb	2.2± 0.5	5.5± 1.7	1.0± 0.3	5.4± 1.8	1.6± 0.3	8.1±2.8
				p<0.05		p<0.05	
Supernatant fraction	Pb	14.6± 1.8	23.1± 3.0	9.0± 1.1	13.2± 0.7	8.2± 1.2	17.3±2.4
	Pb-Pb	9.5± 3.2	22.9± 7.9	4.6± 1.6	22.6± 5.7	4.7± 2.7	24.4±2.3
				p<0.05		p<0.05	
						p<0.01	

^a Rats were given 5 mg Pb/kg intraperitoneally, after an interval of 3 days they were injected 50 mg Pb/kg intraperitoneally.

^b Pb μg in fraction/Pb μg in liver $\times 100$

All values in the table represent mean $\pm$ SD (N=4)

The change of lead content with time in each fraction of liver after challenging was shown in Table 2. Lead taken into liver concentrated in the nuclei fraction and the lapse of lead content in the nuclei fraction after challenging was the same as in liver with both the rats pretreated with and without lead, though lead content in the nuclei fraction of Group Pb-Pb was lower that of Group Pb. The lead contents in the mitochondria and microsome fractions were almost the same level with both the groups and decreased rapidly from 1st to 3rd day. Lead content in supernatant fraction in Group Pb-Pb was significantly lower than that in Group Pb, though the lapse content showed the same tendency.

The ratio of lead content in each fraction to lead content in liver of rats admin-

Fig. 1. Lapse of rate of lead contents in each fraction to that in liver of rats pretreated with and without lead.

Table 3. Lead distribution in liver of normal rats^a

	Liver	Nuclei Fraction	Mitochondria Fraction	Microsome Fraction	Supernatant Fraction
$\mu\text{g/g}$	0.82 ± 0.22	0.25 ± 0.05	0.14 ± 0.09	0.09 ± 0.03	0.34 ± 0.15
% ^b		30.8 ± 3.2	17.1 ± 2.2	12.0 ± 3.5	40.1 ± 2.7

^a These rats are 7 weeks old.

^b $\text{Pb } \mu\text{g in fraction} / \text{Pb } \mu\text{g in liver} \times 100$

All values in the table represent the mean $\pm$ S.D. (N=6)

istered with lead and that of normal rats was shown in Tables 2 and 3, and in Fig. 1. The rate of lead in each fraction to lead in liver of normal rats was highest in supernatant fraction (about 40%), followed by nuclei fraction (about 31%), mitochondria fraction (17%) and microsome fraction (12%). These rates of rats administered with lead were fairly changed from the above; 60 to 80% of lead in liver was concentrated in nuclei fraction, on the contrary the rate in other fractions was lower as compared with the value in normal rats, though lead concentration of each fraction in liver of rats injected with lead was remarkably higher compared with that of normal rats.

The rate of lead content in nuclei fraction to lead in liver of the rat of Group Pb-Pb was lower than that of the rat of Group Pb, and the rate of lead content in mitochondria, microsome and supernatant fractions of Group Pb-Pb rose from that of Group Pb. From these results, it will be thought that the suppression of lead intake in liver of the pretreated rats is put under the control of lead intake in nuclei fraction. However, the mechanism of this suppression is still unknown.

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DISCUSSION

It has been reported that pretreatment with small doses of lead reduced the acute toxicity of lead^{1) 4)} and this pretreatment accelerated the excretion of lead from feces²⁾. The present study evidenced that pretreatment suppressed the accumulation of lead in liver and in body.

Lead was injected intraperitoneally in this study, so lead was taken into liver through portal vessel, after when it was dispersed in whole body through blood stream. Accordingly, the fact that lead content in liver of rat pretreated with lead was at low level will be estimated either the increase of lead excretion in bile duct from liver or the increase of lead efflux from liver to blood stream. The increase of lead excretion in feces of the pretreated rat²⁾ suggests that the lead excretion from liver to bile duct is increased by pretreatment. Also, lead concentration in blood after challenging of lead on the pretreated rat increased to about twice the non-pretreated rat⁵⁾, which shows that lead in liver of pretreated rats is more easily effluent in blood stream than that of the non-pretreated rats. From these results, it will be able to suppose that the lead in liver of rat pretreated with lead exists in more mobile form than that of rat non-pretreated with lead.

The rate of lead content in each fraction to lead in liver was changed by an excess administration of lead. That is, lead in liver of rats injected with lead is concentrated in nuclei fraction and the rate of lead content in mitochondria, microsome and supernatant fractions is lower compared with normal rats. But the increase of rate of lead content in nuclei fraction to lead in liver was suppressed and the decrease of its rate in mitochondria, microsome and supernatant fractions was prevented by the pretreatment. That is, the lead distribution in liver of pretreated rats is similar to that of normal rats, though lead concentration increases.

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