Effects of Zinc Coadministration on Lead Toxicities in Rats

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Abstract: In order to investigate the effects of Zn on Pb toxicities. Proportion of abnormal sperm, percentage of micronucleated polychromatic erythrocyte (MPCE), serum thyroid hormones (T₃, T₄) and cortisol were measured. Rats received intraperitoneal injection of 25 mg/kg Pb acetate, 4 mg/kg Zn acetate, both Pb acetate and Zn acetate, or normal saline as controls, once every two days, 7 times in total. No significant differences in whole blood Pb were detected between groups received Pb alone or both Pb and Zn. On the contrary, the concentration of whole blood Zn in the group given Zn alone was significantly higher than that in the group that received both Pb and Zn. In the groups given Pb alone or both Pb and Zn, proportion of abnormal sperm, frequency of MPCE and serum cortisol were significantly higher than those in controls, whereas serum T₃ and T₄ were significantly lower than in controls. In the group given both Pb and Zn, T₄ was decreased most obviously among the four groups. While the proportion of abnormal sperm was less in the group given both Pb and Zn than in the group given Pb alone. These findings suggest that Zn coadministration might alleviate toxic effects of Pb on the male reproductive system, whereas it could enhance the toxicity on thyroid function. Zn did not affect the toxicities of Pb on cytogenetic systems as indicated by MPCE percentage, and on serum cortisol levels under the dose of the present study. Our results suggested the double-edged effects of Zn on Pb toxicities in different organs. Therefore, the effects of Zn on Pb toxicities should be evaluated systematically.

Key words: Lead acetate, Zinc acetate, Male reproductive system, Cytogenetics, Endocrine system

Introduction

Since the recognition of zinc (Zn) as an essential nutrient, many researchers have studied its role in the prevention and treatment of toxic metals such as lead (Pb), cadmium and mercury, as well as organic solvents such as ethanol and carbon tetrachloride^{1–6}. In particular, these studies have mainly focused on the protective effects of Zn on Pb toxicities.

Cerklewski and Forbes²) reported that as dietary Zn increased, severity of Pb toxicity decreased, including decreases of Pb concentration in the blood, liver and kidneys, excretion of urinary delta-aminolevulinic acid, and inhibition of kidney delta-aminolevulinic acid dehydratase (ALAD) activity in rats. Hasan and Seth⁷) reported that erythrocytic ALAD activity was significantly decreased by Pb but not by a combination of Pb and Zn. Flora *et al.*⁸) demonstrated that Zn administration together with Pb decreased hepatic and renal uptake of Pb and reduced Pb-induced inhibition of

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blood ALAD activity. In addition, Batra *et al.*⁹⁾ reported that testicular degeneration was observed in rats exposed to high doses of Pb and that, with a concomitant administration of Pb and Zn, both testes and epididymides presented nearly normal pictures. However, these experimental studies mainly involved in the protective effects of Zn against Pb toxicity to the hematopoietic and male reproductive systems, and there are few reports on the interactions of Zn in Pb toxicities to other organs.

To further explore the interactions of Zn on Pb toxicities to male reproductive system, cytogenetics, thyroid and adrenal cortex, the present study was designed to examine the proportion of abnormal sperm, percentage of micronucleated polychromatic erythrocyte, serum thyroid hormones (T₃, T₄) and cortisol in rats administered Pb, Zn, or both.

Materials and Methods

Chemicals

Pb acetate (purity: 99.6%) and Zn acetate (purity: 98.9%) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Nitric acid, hydrogen peroxide and the other chemicals were obtained from Shenyang Chemical Industries (Shenyang, China).

Animals and treatment

Fifty-six Wistar rats weighing 160–190 g were purchased from the animal center of China Medical University and were caged under a 12-h dark-light cycle. The animals were acclimatized to their new environment for 1 wk before the initiation of treatment. The rats were randomly segregated into four groups (7 males and 7 females in each group). In order to induce obvious toxic effects of Pb, a dose of 25 mg/kg was selected according to literatures^{9–11)}. Pb acetate or Zn acetate was dissolved in sterile double distilled water (DDW). Rats received intraperitoneal injection of 25 mg/kg Pb acetate (Pb group), 4 mg/kg Zn acetate (Zn group)^{12–14)}, both Pb acetate and Zn acetate (Pb+Zn group) or DDW (controls), once every two days, 7 times in total. Approximately 2.6 ml of blood was collected from the heart of each rat for analyses of whole blood Pb, whole blood Zn, serum thyroid hormones and cortisol after the last administration, and then all rats were undergone necropsy. Seven rats were used for analyses of micronucleated polychromatic erythrocytes (MPCE). Testes and epididymides dissected from all seven males in each group, and the proportion of abnormal sperm was assessed. Animal experiment was conducted in accordance with the Guiding Principles in the Use of Animals in Toxicology,

which was adopted by the Chinese Society of Toxicology.

Whole Blood Pb (BPb) and Zn (BZn) analyses

Blood samples (0.5 ml) were digested with nitric acid and 3% of hydrogen peroxide. Concentrations of Pb (283.3 nm) and Zn (307.6 nm) were measured on an atomic absorption spectrophotometer (Varian AA-40) using appropriate standards of the two metals. Duplicate tests were carried out for each sample. Quality control was performed by determination of the reference samples from the Institute of Environmental Monitoring, Chinese Academy of Preventive Medicine (Calf blood serum GBW091039 and Calf blood GBW09131 as the reference for determination of Pb and Zn, respectively). The reference values (Pb: $109 \pm 23 \mu\text{g/l}$; Zn: $698 \pm 0.2 \mu\text{g/l}$) measured by the present laboratory were almost the same as the authentic standard (Pb: $110 \pm 20 \mu\text{g/l}$; Zn: $710 \pm 0.1 \mu\text{g/l}$). The accuracy of this method was checked by the analytical recoveries of added Pb or Zn, and found to be satisfactory. The blood Pb recoveries ($M \pm \text{SD}$) were $106 \pm 8\%$ (RSD = 1.61%) and $105 \pm 8\%$ (RSD = 3.7%) when the concentrations of standard Pb solution were 50 $\mu\text{g/l}$ and 100 $\mu\text{g/l}$, respectively. Detection limit of Pb was 1.78 $\mu\text{g/l}$. The blood Zn recoveries ($M \pm \text{SD}$) were $98 \pm 8\%$ (RSD = 2.35%) and $99 \pm 8\%$ (RSD = 1.89%) when the concentrations of standard Zn solution were 50 $\mu\text{g/l}$ and 100 $\mu\text{g/l}$, respectively. Detection limit of Zn was 1.8 $\mu\text{g/l}$ ^{15, 16)}.

Abnormal sperm examination

The epididymides of 7 male rats were removed within 2 min of death, finely minced, and immediately placed in 3 ml Tyrode's solution [0.8 g NaCl, 0.02 g KCl, 0.02 g CaCl₂ (anhydrous), 0.01 g MgCl₂ (anhydrous), 0.005 g NaH₂PO₄ (anhydrous) and 0.01 g glucose; volume adjusted to 100 ml with distilled water] at 37°C for 20 min to ensure an even distribution of sperm¹⁷⁾. The suspension was filtered through a nylon mesh. To examine the sperm morphology, a drop of the sperm suspension was placed on a glass slide, the smear was fixed in ethanol for 1 h, stained with Giemsa stain for 15–20 min, washed, dried, and examined with a light microscope at 100 × magnification. One thousand sperms were counted and the percentage of abnormal sperm was determined. Morphologic abnormalities included enlarged, undersized, deformed, or double-headed sperm and sperms with coiled, short, or double tails¹⁷⁾.

Percentage of micronucleated polychromatic erythrocytes (MPCE)

Sterna were dissected from the sacrificed rats and bone

Table 1. Concentrations of whole blood lead (BPb) and zinc (BZn) in four groups of rats

Groups	Treatment (mg/kg)		BPb (mean $\pm$ SD) $\mu\text{g/l}$	BZn (mean $\pm$ SD) $\mu\text{g/l}$
	Pb acetate	Zn acetate		
Controls	0	0	18 $\pm$ 8	690 $\pm$ 225
Zn	0	4	15 $\pm$ 2	728 $\pm$ 162
Pb	25	0	67 $\pm$ 28 ^{a,c}	643 $\pm$ 217
Pb+Zn	25	4	71 $\pm$ 36 ^{a,c}	571 $\pm$ 125 ^b

^a $p < 0.01$, compared with control group; ^b $p < 0.05$ and ^c $p < 0.01$ compared with Zn group. Statistical analysis was conducted using the Scheffe's test.

Table 2. Effect of Zn on the MPCE

Groups	Treatment (mg/kg)		No. of PCEs ^a	No. of MPCEs ^b	Percentage (%)
	Pb acetate	Zn acetate			
Controls	0	0	7,000	23	0.33
Zn	0	4	7,000	25	0.35
Pb	25	0	7,000	40	0.57 ^c
Pb+Zn	25	4	7,000	44	0.63 ^c

^aPCEs: Polychromatic erythrocytes; ^bMPCEs: Micronucleated polychromatic erythrocytes; ^c $p < 0.05$, compared with control group by the χ^2 test.

marrow was flushed out with 0.5 ml of bovine serum albumin into a 10 ml test tube. Bone marrow smears were then prepared from each rat. The number of MPCE in 1,000 polychromatic erythrocytes (PCE), which provides an index of chromosomal damage, was counted¹⁸⁾.

Endocrine assays

Two ml of blood was centrifuged and serum was isolated and stored at -80°C until use. Levels of thyroid hormones (T_3 and T_4), and cortisol (adrenocortical hormone) were measured using radioimmunoassay (RIA) kits purchased from Shanghai Institute of Chemical Agents (Shanghai, China)¹⁰⁾.

Statistical analysis

The analysis of variance (ANOVA), the Scheffe's test and Chi-square test were performed on analyses of the data obtained in this study using StatView Ver. 5.0 (SAS Institute Inc., USA, 1998). The probability less than 0.05 was considered to be significant.

Results

Concentrations of BPb and BZn in each group of rats were shown in Table 1. BPb in Pb group and Pb+Zn group was significantly higher than that in controls or Zn group. In the Pb+Zn group, BZn was significantly lower than in Zn group.

Percentages of MPCE were shown in Table 2. In Pb group and Pb+Zn group, MPCE was significantly higher than that in controls, whereas there was no change in Zn group. Proportion of abnormal sperm in four groups of rats was shown in Table 3. In Pb group and Pb+Zn group, Proportion of abnormal sperm was significantly higher than that in controls, however proportion of abnormal sperm in Pb+Zn group was significantly lower than that in Pb group. There was no significant difference in proportion of abnormal sperm between Zn group and controls.

The levels of serum T_3 , T_4 and cortisol in four groups of rats were shown in Table 4. In Pb group or Pb+Zn group, serum T_3 and T_4 were significantly lower, and on the contrary, serum cortisol were significantly higher than those in controls. In addition, level of serum T_4 in Pb+Zn group was the lowest among the four groups. In Zn group, serum T_3 was significantly lower than that in controls and whereas there were no significant differences in the serum T_4 and cortisol compared with controls.

Discussion

Flora *et al.*⁸⁾ reported a decrease in BPb level of rats during Zn co-administration. In the present study, there were no significant differences in concentrations of BPb between the groups that received Pb alone and that received both Pb and Zn. No effects of Zn on BPb may be associated with

Table 3. Effect of Zn on the proportion of abnormal sperm

Groups	Treatment (mg/kg)		No. of sperm	No. of abnormal sperm	Proportion of abnormal sperm (%)
	Pb acetate	Zn acetate			
Controls	0	0	7,000	54	7.7
Zn	0	4	7,000	42	6.0
Pb	25	0	7,000	106	15.1 ^{a, b}
Pb+Zn	25	4	7,000	78	11.1 ^a

^a $p < 0.05$, compared with control group; ^b $p < 0.05$, compared with Pb+Zn group by the χ^2 test.

Table 4. Effects of Zn on the thyroid and adrenocortical functions

Groups	Treatment (mg/kg)		Levels of serum thyroid hormones		Level of serum cortisol (nmol/l)
	Pb acetate	Zn acetate	T ₃ (nmol/l)	T ₄ (nmol/l)	
Controls	0	0	1.54 ± 0.59	29.72 ± 7.79	7.54 ± 3.7
Zn	0	4	0.91 ± 0.40 ^a	25.80 ± 6.22	10.82 ± 4.9
Pb	25	0	0.47 ± 0.12 ^b	21.20 ± 10.02 ^a	14.92 ± 5.7 ^a
Pb+Zn	25	4	0.42 ± 0.12 ^b	18.43 ± 7.14 ^b	13.34 ± 2.3 ^a

^a $p < 0.05$ and ^b $p < 0.01$, compared with control group by the Scheffe's test.

the facts that the dose of Zn was considerably lower than that of Pb. On the contrary, the concentration of BZn in the group given Zn alone was significantly higher than that in the Pb+Zn group. It suggests that Pb decreased the concentration of BZn when both Pb and Zn were administered intraperitoneally. It has been recognized that Zn cannot cross biological membranes by simple diffusion and need a trafficking system for its cellular uptake and release¹⁹. Putative mammalian zinc transporters have now been cloned²⁰. Because divalent Pb and Zn potentially have similar chemical characteristics, it may be possible that Pb influences absorption or distribution of Zn in blood or other tissues through interfering the trafficking system of Zn.

Tachi *et al.*²¹ reported that Pb caused a significant increase in the frequency of chromosomal aberrations and micronuclei in rat bone marrow cells. In the present study, an increased frequency of MPCEs was observed in the groups given Pb alone and both Pb and Zn. It suggests that Pb increased frequency of MPCEs and that Zn did not prevent the genotoxic effect of Pb.

The proportion of abnormal sperm was significantly higher in the groups given Pb alone or Pb+Zn group than that in controls. An increase in the proportion of abnormal sperm in the group given both Pb and Zn was less than that in the group given Pb alone. These data suggested that Pb increased abnormal sperm and Zn could decrease it. This agrees with the observation by Batra *et al.*^{19, 22}. That the toxic effects of Pb on male reproductive system could be ameliorated by

Zn supplementation. As evidence has shown that Zn exists in spermatozoa within the seminiferous tubules and helps spermatogenesis^{19, 23}, Pb may result in disruption of the metabolic functions of enzymes containing Zn, inducing testicular damage. Batra *et al.*⁹ reported that there was a 30% reduction in Pb deposition in the testes when Zn was coadministered. The protective effect of Zn on reproductive toxicity of Pb may be attributed to competition between Pb and Zn, or reduction of available Pb-binding sites in the testicular tissue.

Many researchers have studied roles of Zn in the prevention and treatment of toxic effects of Pb. Thyroid gland is a toxic target organ of Pb, however, reports on the interactions of Zn in Pb toxicities in thyroid gland are rare. In this study, serum T₃ and T₄ were significantly lower in the groups given Pb alone or both Pb and Zn than those in control. Especially, T₄ in the group given both Pb and Zn was decreased most obviously among the 4 groups. Alterations in circulating levels of T₃ and T₄ in Pb-exposed workers, experimental animals, and fishes have been reported^{24–26}, as confirmed by decrease in serum T₃ and T₄ in the present study. This may be associated with the fact that Pb inhibits synthesis and release of thyroid hormone at the glandular level through affecting thyroid iodine uptake or TSH release from pituitary or TRH release from hypothalamus^{25, 27, 28}. As excessive amount of Zn decreases thyroid hormone levels^{29, 30}, a greater decrease in serum T₄ in the group received both Pb and Zn seems to be a consequence of the joint toxic action of Pb

and Zn. Hereafter, it is necessary to investigate effect of Zn with low dosage on Pb toxicity to thyroid function. Vyskocil *et al.*³¹⁾ found that Pb increases serum corticosterone level in rats. In the present study, the increased serum cortisol as another adrenal corticoid hormone was observed in groups that received Pb alone or both Pb and Zn, and Zn did not prevent such an increase in serum cortisol. Mechanisms underlying the effects of Pb on corticosterone and cortisol levels should be investigated further.

The present study suggested that Zn exerted a protection against toxic effects of Pb on male reproductive system, while it could enhance the toxic effects of Pb on thyroid function. On the other hand, Zn might have not affect the toxicities of Pb on cytogenetic systems as indicated by MPCE frequencies or serum cortisol levels under the dosing of the present study. It indicates that the effects of Zn on Pb toxicities should be evaluated systematically and Zn should not be used for treatment of the patients exposed to Pb unconditionally. Meanwhile, it is also necessary to explore further the effects of Zn on toxicities of Pb with low dose exposure.

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