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The Effects of Multi-minerals on Susceptibility to Lead Toxicity in Rats

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ABSTRACT *: Female Wistar rats were randomly divided into 5 groups: Control, received distilled water; Low lead, received 0.5 g/l lead (as acetate) in drinking water; High lead, received 2.0 g/l lead; Low lead + Minerals, received 0.5 g/l lead in drinking water and received minerals (Ca^{2+}, 25 mg/kg/day; Fe^{3+}, 0.47 mg/kg/day; Zn^{2+}, 0.33 mg/kg/day; Se, 0.83 μg/kg/day) by gavage; High lead + Minerals, received 2.0 g/l lead and received the same minerals. Animals exposure to lead was from 10 days before mating till postnatal day 21; and the minerals was administered from the first day of pregnancy and during lactation. No statistical difference was found either in body weights or in blood lead levels between the pups received minerals and those only exposed to lead at the same dose. The developmental and behavioral teratological effects of lead on pups, such as time-lag of eye opening, pinna detachment, fur developing, incisor eruption, ear unfolding, and surface righting were observed in this study; and the minerals decreased the toxicity of lead either in low or in high lead exposure pups. The numbers of step-down were significantly increased in lead exposed animals, and the effect of intervention by the minerals was appeared only in the pups exposed to low lead. The ChAT activity and levels of glutamate and aspartate in hippocampus decreased in treated animals compared to control animals, no effect of intervention by the minerals was found. The results of this study indicate that the applied multi-minerals can alter the outcome of developmental lead poisoning in rats.*

Key Words *: Lead, mineral, Development, Intervention, Neurotoxicity*

I. INTRODUCTION

China is the largest populated country with about 120 million children of 0-6 years old. It is estimated that almost 50% of unexposed children have blood lead levels higher than 100 μg/l (Shen, 1996). However, few environmental and health regulations specially address lead exposure in China. Childhood lead poisoning is even a "new disease" for most of Chinese pediatricians (Shen, 1996). There is a strong need to explore economical, simple and available ways to minimize the environmental lead hazards to children particularly in developing countries. The nutrition minerals-lead interactions have been investigated using models of isolated cells, whole animals, and humans. It is well known that calcium- or iron-deficiency may increase the lead toxicity (Mahaffey, 1990; Wright *et al.*, 1999). Zinc or selenium has been reported protective against the toxicity of lead, even though the role of zinc or selenium on lead toxicity is not very understood. Most of previous studies have not evaluated the combined effects of multi-minerals on lead toxicity. Anyway, it is important. For general health purpose, taking multi-minerals is becoming popular. To determine whether additional multi-minerals may modify susceptibility to lead toxicity, we conducted a study in rats.

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II. MATERIALS AND METHODS

Female Wistar rats (Institute of Laboratory Animals, Chinese Academy of Medicine) were randomly divided into 5 groups: Control, received distilled water; Low lead, received 0.5 g/l lead (as acetate) in drinking water; High lead, received 2.0 g/l lead in drinking water; Low lead + Minerals, received 0.5 g/l lead in drinking water and received minerals [Ca^{2+}(as $CaCo_3$), 25 mg/kg/day; Fe^{3+}(as $FeSO_4$), 0.47 mg/kg/day; Zn^{2+}(as $ZnSO_4$), 0.33 mg/kg/day; Se(as Na_3SeO_3), 0.83 μg/kg/day] by gavage; High lead + Minerals, received 2.0 g/l lead in drinking water and received the same minerals. Animals exposure to lead was covered a period of

10 days before mating till 21 days postnatal; and the minerals was administered from the first day of pregnancy and during lactation.

The pups of groups were examined daily for developmental and behavioral teratological effects (Suter and Schon, 1986). The Step-down test (Tang *et al.*, 1994 (passive avoidance) was performed at postnatal day 21 and day 35, and the levels of blood lead, hippocampal choline acetyltransferase (ChAT), glutamate (Glu) and aspartate (Asp) were determined in the pups. The techniques of atomic absorption spectrometry (for lead; Spect/AA800, Varian), radiochemistry (Fonnunm, 1975) (for ChAT), and capillary electrophoresis (Camilleri, 1998) (for glu and asp; P/ACE5000 with LIF, Beckman) were employed. The main chemicals of chloracetylcholine, acetyl-CoA, glutamate, aspartate, FTIC (Sigma), and ^{3}H-acetyl-CoA (1.835 Ci/mmol, Dupont) were used.

III. RESULTS

The body weights of pups were no different between groups till weaning, and at postnatal day 35 the body weights of pups in low or in high lead group were less than that in control or in the two groups received minerals (Table 1). The blood lead levels obviously increased in pups exposed to lead, and the pups received minerals had lower levels of blood lead than those only exposed to lead at the same dose, particularly in high lead exposure group (Table 2). However, no statistical difference was found either in body weights or in blood lead levels between the pups received minerals and those only exposed to lead at the same dose. The developmental and behavioral teratological effects of lead on pups, such as time-lag of eye opening (low or high lead group), pinna detachment, fur developing, incisor eruption, ear unfolding, and surface righting (high lead group) were observed in this study; and the minerals decreased the toxicity of lead either in low or in high lead exposure pups (Table 3). Table 4 shows the results of step-down test(passive avoidance) performed at postnatal day 21

Table 1. The body weights of pups by group(g, χ±s)*

Group	Postnatal day					
	4	8	12	16	21	35
Control	10±1	15±3	22±4	27±4	38±7	85±9
Low lead	9±1	15±3	21±3	26±6	35±6	79±17
High lead	9±1	6±2	22±4	27±8	33±9	75±13
Low lead+Minerals	9±2	15±2	19±3	24±4	32±7	85±12
High lead+Minerals	8±1	15±1	20±3	25±5	33±5	81±9

*: n=5.

Table 2. Blood lead levels of pups by group (μg/l, χ±s)

Group	Postnatal day	
	21	35
Control	16±4	17±4
Low lead	204±89*	232±29*
High lead	387±101*	331±84*
Low lead+Minerals	189±58*	172±61*
High lead+Minerals	252±127*	203±133*

*: p<0.01; vs Control, n=5.

Tab 4. Step-down test by group (numbers of step-down/5 min, χ±s)

Group	Postnatal day			
	21		35	
	Training	Test	Training	Test
Control	1.4±0.5	0.2±0.5	1.7±0.7	0.4±0.5
Low lead	3.4±1.8*	1.6±1.1*	3.2±1.6*	1.2±0.8*
High lead	3.0±1.5*	1.7±1.2*	2.8±0.9*	1.1±0.7*
Low lead+Minerals	3.3±1.6*	0.5±0.8	1.9±0.8	0.5±0.5
High lead+Minerals	2.8±1.8*	1.5±1.7*	2.7±1.0*	1.1±0.7*

*: p<0.05; vs Control, n=5.

Table 5. ChAT activities in pup hippocampus by group (nmol/mg protein/min, χ±s)

Group	Postnatal day	
	21	35
Control	0.92±0.15	1.16±0.22
Low lead	0.69±0.07*	0.89±0.09*
High lead	0.56±0.06*	0.79±0.08*
Low lead+Minerals	0.62±0.09*	0.83±0.14*
High lead+Minerals	0.62±0.07*	0.87±0.06*

*: p<0.05, vs Control; n=5.

Table 3. Behavioral teratological effects of lead on pups by group (day, χ±s)

Group	Pinna detachment	Fur development	Ear eruption	Ear unfolding	Eye opening	Surface righting	Cliff avoidance
Control	2.7±0.9	10.1±2.5	10.5±1.3	14.4±1.4	14.8±1.5	9.1±1.8	8.1±2.1
Low lead	3.5±0.6	12.2±0.8	11.5±1.1	14.7±1.9	16.8±1.6*	10.2±1.0	10.0±2.6
High lead	4.0±0.8*	14.0±2.9*	13.8±1.0*	17.3±1.7*	18.0±3.9*	11.7±1.7*	11.0±2.6
Low lead+Minerals	3.2±1.2	11.0±1.4	10.7±1.0	14.3±1.6	15.8±1.0	8.8±3.1	8.0±3.0
High lead+Minerals	3.4±1.2	12.3±2.3	11.8±0.9*	16.3±0.7**	15.5±1.6	9.6±3.2	9.5±2.6

*:p<0.05; **: p<0.01; vs Control, n=5.

Table 6. Glutamate and aspartate levels in pup hippocampus by group(μmol/g wet tissue, χ±s)

Group	Postnatal day			
	21		35	
	Glu	Asp	Glu	Asp
Control	732±39	455±38	818±11*	366±44
Low lead	595±59*	381±24*	634±37*	217±25*
High lead	623±44*	303±20*	624±27*	237±39*
Low lead+Mineral	576±71*	371±22*	639±87*	232±33*
High lead+Mineral	601±47*	308±16*	661±32*	232±43*

*: $p<0.05$, vs Control; n=5.

and day 35. The numbers of step-down were significantly increased in lead exposed animals, and the effect of intervention by the minerals was appeared only in the pups exposed to low lead. The ChAT activity and levels of glutamate and aspartate in hippocampus decreased in treated animals compared to control animals, no effect of intervention by the minerals was observed (Table 5 and Table 6).

IV. DISCUSSION

Developmental lead exposure resulted in reduction of hippocampal ChAT activity, ChAT mRNA, and loss of neurons in rats (Tian *et al.*, 1995; Sun *et al.*, 1997; Bourjeily and Suszkiw, 1997). The cholinergic system changes may related with behavioral changes in lead exposed rats (Widmer *et al.*, 1992). Various investigators (Cory-Slechta, 1997; Lasley *et al.*, 1999; Yi and Lim, 1998; Winter and Kitchen, 1984) have demonstrated that lead will decrease depolarization-evoked neurotransmitter release and stimulate spontaneous neurotransmitter release with an inverse dose-response relationship between the basal levels of neurotransmitter and increasing doses of lead. It is appeared that glutamatergic system disturbances play a key role in the learning impairments induced by lead (Cory-Slechta, 1997). To our understanding, this indicated that lead may have direct effects on neurons. Surviving neurons released neurotransmitters more rapidly than those of normal neurons. However, with more severe lesions, either depolarization-evoked or spontaneous neurotransmitter release should be attenuated because of neurotransmitter system function deficits. Our study showed correlation between behavioral and neurochemical effects of lead, but no association between the reversed behavioral and neurochemical effects of minerals on lead toxicity. However, the results of this study indicate that the applied multi-minerals can alter the outcome of developmental lead poisoning in rats particularly in low lead exposed rats, and the mechanism involved and the dose-response relationship are not yet clear. The multi-minerals may have multi-effects on altering the toxicological process induced by lead.

Prenatal exposure to lead has adverse effects on cognitive, neuro-behavioral, and neurophysiological development. Childhood lead poisoning has become a most common public health problem. Chelation therapy is recommended for the treatment of lead poisoned children with blood lead higher than 250 μg/l (CDC, 1991). However, in view of the epidemiological data, most of "unexposed" children have blood lead lower than 250 μg/l. And in developing countries it is hardly to test blood lead regularly for childhood lead poisoning survey. Taking additional multi-minerals is acceptable and available for pregnant women and children. Further human-based research on effects of multi-minerals on susceptibility to lead toxicity is needed.

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