

## Lipid Peroxidation and Ultrastructural Modifications in Brain after Perinatal Exposure to Lead and/or Cadmium in Rat Pups<sup>1</sup>

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**Objective** To assess lipid peroxidation and ultrastructural modifications in rat brains following perinatal exposure to lead (Pb) and/or cadmium (Cd). **Methods** Female rats were divided into four groups: control group, Pb (300 mg/L) group, Cd group (10 mg/L) and Pb+Cd (300 mg/L, 10 mg/L) group. The compounds were delivered in the drinking water throughout pregnancy and lactation. **Results** The levels of compounds in blood and brain of the Pb+Cd group were similar to those of other groups, but the effects of Pb+Cd on pups' body and brain weights were higher than on other compounds. Electron microscopy revealed that Pb and Cd had effects on mitochondrial swelling, disruption and cristae loss, Nissl body dissolution, degenerated organelles and vacuoles, cytomembrane disappearance, and nuclear chromoplasm concentration. The activity of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), acetylcholinesterase (AChE) was decreased, whereas the activity of maleic dialdehyde (MDA) was increased. **Conclusion** Perinatal exposure to low doses of Pb and Cd can produce alterations in lipid peroxidation and ultrastructural modifications in rat brains, and exposure to both metals can result in greater damages.

**Key words:** Lead; Cadmium; Lipid peroxidation; Ultrastructure; Brain; Rat

### INTRODUCTION

In recent years, the levels of heavy metals, particularly lead (Pb) and cadmium (Cd), have increased in air, water and soil in both urban and petiurban areas<sup>[1]</sup>. Cd and Pb are the elements that cause concerns in terms of their potential adverse effects on animal and human health. Due to such concerns, the scientific community has focused its attention on the toxicological correlation between these trace metals. Today, exposure to high-doses of these elements seldom occurs in most countries, but chronic co-exposure to their low doses remains a major public health concern.

The central nervous system of newborn animals is highly susceptible to metals, and neurotoxicities of Cd and Pb have been well-established<sup>[2]</sup>. It has been demonstrated that Pb and Cd are particularly dangerous to the developing central nervous system (CNS) due to lack of a functional blood-brain barrier (BBB) and intense cellular proliferation, differentiation

and synaptogenesis, which take place during gestation and early postnatal period in mammals<sup>[3-4]</sup>. During gestation and early postnatal period, a significant amount of Pb and Cd can reach the brain upon chronic exposure because immature BBB does not protect mammals against xenobiotics. Both Cd and Pb can cross placenta. Cd, instead of Pb, accumulates in placenta during gestation. Transplacental transport of Cd to fetus appears to be restricted because trophoblasts synthesize metallothionein (MT), which is an important complex protein rich in sulfhydryl groups that binds to heavy metals like Cd<sup>[5]</sup>.

Neurotoxicity associated with Pb and Cd exposure may be the result of a series of small perturbations in brain metabolism, especially due to oxidative stress. Both metals have a high affinity for free sulfhydryl groups in enzymes and proteins, and binding of these metals to enzymes can alter normal enzyme functions. It has been shown that glutathione can induce oxidative stress, which is associated with Pb and Cd poisoning<sup>[6]</sup>. Furthermore, it has been

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reported that Pb and Cd can enhance lipid peroxidation by inhibiting superoxide dismutase and other related enzymes<sup>[7-8]</sup>.

Most of the above studies were carried out following exposure to a single metal. Studies on co-exposure to Pb and Cd showed that these metals had additive effects on acetylcholine release at the neuromuscular junction of frogs<sup>[9]</sup> and inhibited the activity of some enzymes in the brain<sup>[10]</sup>. While some studies showed that Pb and Cd had additive effects<sup>[11]</sup>, Nation *et al.*<sup>[12]</sup> found that combined exposure to Pb and Cd produced smaller effects than exposure to Pb or Cd alone. Because the long-term accumulation of metals in tissues is the major cause of metal toxicity<sup>[13]</sup>, it is of interest to study the toxic effects of Pb and Cd when they are co-administered during the entire perinatal period.

In this study, the effects of simultaneous Pb and Cd treatment during the perinatal period on brain ultrastructure and activity of critical antioxidative enzymes in the brain were detected in order to study the effects of long-term, combined exposure of rats to Pb and Cd during gestation and lactation, which is the critical period of the central nervous system development.

## MATERIALS AND METHODS

### *Chemical Reagents*

Lead acetate, cadmium acetate and all other chemicals used in our experiments were purchased from Sigma-Aldrich (St. Louis, USA).

### *Animals*

The animals used in this study were approved by the Animal Care Committee of Yangzhou University. Sprague-Dawley rats were purchased from the Yangzhou University Laboratory Animal Center (Yangzhou, China).

### *Experimental procedures*

All experiments were performed in 60-days old female Sprague-Dawley rats weighing 200-220 g. After a week adaptation in a room with controlled temperature ( $21 \pm 1$  °C) in a 12 h light/12 h dark cycle, the rats were mated overnight with males (1 male and 2 females). A sperm-positive vaginal smear was taken as an indication of the first day pregnancy. Then, the rats were housed individually in plastic cages and maintained under the supervision of a licensed veterinarian, in accordance with the principles set forth in the NIH guide for the care and use of laboratory animals.

The animals were divided into four groups ( $n=6$ ) on day 1 of pregnancy: control group with access to distilled water as drinking water, Pb group with access to a solution of lead acetate (300 mg/L) as drinking water, Cd group with access to a solution of cadmium acetate (10 mg/L) as drinking water, and Pb+Cd group with access to a solution of lead acetate (300 mg/L) and cadmium acetate (10 mg/L) as drinking water.

Metal solutions were prepared in distilled water and provided as drinking water *ad libitum* to pregnant rats throughout the entire experimental period (42 days). Maternal water consumption and weight gain were measured during the experiment, and neonate weight gain was assessed during the postnatal period. On postnatal day 21, the pups were weighed. Litters that stayed with their mother were culled to 10-12 pups in each group on the day of birth. Experiments were performed at two time points: on the day of parturition (postnatal day 0), and at weaning (postnatal day 21). On either day 0 or day 21, the pups were weighed and anesthetized with ether vapor, and blood samples were collected from the heart with a syringe. After the pups were sacrificed by decapitation, their brains were quickly removed and weighed.

### *Biochemical Assays*

Brain tissue samples were homogenized (1:10, w/v) in a cold (4 °C) buffer containing Tris base (20 mmol/L), EDTA (1 mmol/L), dithiothreitol (1 mmol/L), sucrose (0.5 mmol/L), KCl (150 mmol/L) and phenylmethylsulfonyl fluoride (1 mmol/L), with the pH adjusted to 7.6. Homogenates were centrifuged at 4000 g for 20 min at 4 °C, and enzyme and protein levels in supernatants were measured as previously described<sup>[14]</sup>, using serum bovine albumin as the standard.

*Measurement of Thiobarbituric Acid-reactive Substance (TBARS) Levels:* Brain TBARS levels were measured as previously described<sup>[15]</sup> based on reaction with thiobarbituric acid at 90-100 °C. In the thiobarbituric (TBA) test, malondialdehyde (MDA) or MDA-like substances and TBA reacted to produce a pink pigment with an absorption maximum at 532 nm. Results were expressed as nmol per gram of wet tissue, according to the standard graphic prepared from measurements with a standard solution (1, 1, 3, 3-tetramethoxypropane).

*Determination of Superoxide Dismutase (SOD) Activity:* The total (Cu-Zn and Mn) superoxide dismutase (t-SOD) enzyme activity was determined based on the inhibition of nitroblue tetrazolium (NBT) reduction by  $O_2^-$ , generated by the xanthine/XO system<sup>[16]</sup>. One unit of SOD was defined as the

amount of enzyme causing 50% inhibition of the NBT reduction rate. Tissue SOD activity was expressed as units per milligram protein (U mg prot<sup>-1</sup>).

**Determination of Glutathione Peroxidase (GSH-Px) Activity:** GSH-Px activity was measured as previously described<sup>[17]</sup>. The enzyme reaction in a tube containing NADPH reduced the level of glutathione (GSH), sodium azide, and glutathione reductase. The reaction was initiated upon addition of H<sub>2</sub>O<sub>2</sub>, and the change in absorbance at 340 nm was monitored using a spectrophotometer. Results were expressed as U mg prot<sup>-1</sup>.

**Determination of Catalase (CAT) Activity:** CAT activity was assayed with a spectrophotometer by measuring the decrease in absorbance of H<sub>2</sub>O<sub>2</sub> at 240 nm, as previously described<sup>[18]</sup>. The activity was expressed as U mg prot<sup>-1</sup>.

**Determination of AChE activity:** The method created by Ellman and his colleagues<sup>[19]</sup> was used to estimate the acetylcholinesterase (AChE) activity in tissues, which was expressed as U mg prot<sup>-1</sup>.

#### Metal Analysis

The Pb and Cd concentrations in blood and brain samples were measured on postnatal day 21. The samples were digested in a reagent grade of nitric acid-perchloric acid (2:1) mixture until the samples became colorless. Then, the acid mixture was evaporated and the remaining precipitate was dissolved in a few drops of concentrated HCl. The samples were diluted in 10 mL of distilled water, and then the Pb and Cd concentrations were measured by inductively coupled plasma-mass spectrometry (Elan DRC-e, USA). The sensitivity of the assays was 0.1 µg/mL for Pb and 0.01 µg/mL for Cd, respectively.

#### Transmission Electron Microscopy

The cerebral cortex samples were prefixed in a 2.5% glutaraldehyde solution, diced into 1 mm<sup>3</sup> cubes, and then rinsed three times for 15 min with 0.1 mol/L phosphate buffer (pH 7.4). The tissue was post-fixed in cold 1% aqueous osmium tetroxide for 1 h. The specimens were then rinsed with phosphate buffer, dehydrated in a graded ethanol series of 50%-100%,

and embedded in Epon 812. The tissue was cut into ultrathin sections with glass knives on a LKB-V ultramicrotome (Nova, Sweden), stained with uranyl acetate and lead citrate, and examined under a TecNai 12 transmission electron microscope (Philips Company, Holland).

#### Statistical Analysis

Results were expressed as  $\bar{x} \pm s$ . All data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons. Comparisons were made between control group, Pb+Cd group, Pb group and Cd group.  $P < 0.05$  was considered statistically significant.

## RESULTS

#### *Effects of Pb and/or Cd on the Growth and Development of Offsprings*

The amounts of metals ingested during the experimental periods (21 and 42 days, respectively) are shown in Table 1. These values were calculated following the formula: (mL water consumed/day × metal dose)/rat weight (kg). There were no specific signs attributable to the Pb and/or Cd treatment. No effects were observed on body weight gain from gestation to lactation (data not shown). Metal exposure either alone or in combination had no significant effect on dam reproductive performance, including period of gestation, litter size, sex ratio, live births, still births, and deaths after birth (data not shown). No gross malformations were observed in any pups born from the dams treated with Pb and/or Cd. Exposure to either Pb or Cd produced a statistically significant decrease in weight of the pups at postnatal days 0 and 21 and in their brain weights on postnatal day 21. The Pb+Cd group also showed a significant decrease in weights of the pups, their brains and relative brain weights in comparison to the Pb, Cd and control groups (Table 2). However, co-exposure to Pb and Cd produced more severe effects on the weight of pups and their brains than exposure to either element alone.

TABLE 1

Lead and Cadmium Consumption in the Treated Groups ( $\bar{x} \pm s$ )

	Lead	Cadmium	Pb+Cd	
			Lead	Cadmium
Metal Consumption During Pregnancy <sup>a</sup>	65.75 ± 8.97	1.65 ± 0.06	57.93 ± 6.83	1.54 ± 0.18
Metal Consumption During Lactation <sup>a</sup>	94.68 ± 6.34	2.56 ± 0.25	76.25 ± 5.76	2.11 ± 0.09

Note. <sup>a</sup>mg metal/kg body weight/day.

TABLE 2

Body Weight and Absolute and Relative Brain Weight ( $\bar{x} \pm s$ )				
Group		Weight (g) of Pups	Weight (g) of Brain	Relative Weight ( $\times 10^3$ ) of Brain
Day 0	Control	7.00 $\pm$ 0.43 <sup>a</sup>	0.2131 $\pm$ 0.0051 <sup>a</sup>	30.44 $\pm$ 1.20 <sup>a</sup>
	Pb	6.23 $\pm$ 0.30 <sup>b</sup>	0.2086 $\pm$ 0.0049 <sup>a</sup>	33.48 $\pm$ 1.45 <sup>b</sup>
	Cd	6.25 $\pm$ 0.34 <sup>b</sup>	0.2042 $\pm$ 0.0097 <sup>a</sup>	32.67 $\pm$ 1.87 <sup>b</sup>
	Pb+Cd	6.10 $\pm$ 0.32 <sup>b</sup>	0.2034 $\pm$ 0.0112 <sup>a</sup>	38.36 $\pm$ 2.15 <sup>b</sup>
Day 21	Control	53.78 $\pm$ 2.07 <sup>a</sup>	1.2710 $\pm$ 0.0456 <sup>a</sup>	23.63 $\pm$ 1.15 <sup>a</sup>
	Pb	48.41 $\pm$ 2.49 <sup>b</sup>	1.2421 $\pm$ 0.0309 <sup>b</sup>	25.66 $\pm$ 2.03 <sup>b</sup>
	Cd	45.00 $\pm$ 2.72 <sup>b</sup>	1.2435 $\pm$ 0.0826 <sup>b</sup>	27.64 $\pm$ 1.89 <sup>b</sup>
	Pb+Cd	41.80 $\pm$ 2.35 <sup>b</sup>	1.2234 $\pm$ 0.0610 <sup>b</sup>	29.27 $\pm$ 2.38 <sup>b</sup>

Note. Relative weight of brain = brain weight/body weight. Body weight and absolute and relative brain weights of pups on days 0 and 21 ( $n=12$ ). <sup>a, b</sup>Mean that do not show a common letter in the same column are significantly different from one another.

#### Effects of Pb and/or Cd on Brain MDA Levels and Activity of SOD, GSH-Px, CAT, and AChE in Offsprings

The effects of Pb and/or Cd on brain MDA levels and the activities of SOD, GSH-Px, CAT, AChE in pups are shown in Table 3. On postnatal day 0, the activity of GSH-Px was decreased in all experimental groups, but it was statistically significant only in the Pb+Cd group when compared to the control, Pb and Cd groups. A statistically significant decrease in GSH-Px activity was detected in brain samples from all experimental groups on postnatal day 21. The activity of SOD in brain was significantly inhibited after Pb, Cd, and Pb+Cd exposure on postnatal days 0 and 21. The Pb+Cd group showed a significant decrease in activity of CAT and AChE, whereas exposure

to any single metal did not significantly alter the CAT and AChE activity. The activity of CAT and AChE enzymes was the lowest in the Pb+Cd group. The concentration of MDA was significantly increased in the experimental groups in comparison to the control group on postnatal days 0 and 21. However, the Pb+Cd group had a significantly higher level of MDA than the Pb or Cd group.

#### Pb and Cd Levels in Blood and Brain of Offsprings

The Pb and Cd concentrations in blood of the experimental and control animals on postnatal day 21 are listed in Table 4. The analyses of metal concentrations showed that the Pb+Cd group had a higher level of Pb than the Cd group and a higher level of Cd than the Pb group.

TABLE 3

Effects of Lead and/or Cadmium on Brain MDA Levels and Activities of SOD, GSH-Px, CAT, and AChE in Pups ( $\bar{x} \pm s$ )						
Group		GSH-Px (U/mg prot)	SOD (U/mg prot)	CAT (U/mg prot)	AChE (U/mg prot)	MDA (nmol/mg prot)
Day 0	Control	30.62 $\pm$ 1.71 <sup>a</sup>	11.57 $\pm$ 0.82 <sup>a</sup>	2.26 $\pm$ 0.08 <sup>a</sup>	0.38 $\pm$ 0.02 <sup>a</sup>	1.12 $\pm$ 0.14 <sup>a</sup>
	Pb	29.86 $\pm$ 1.32 <sup>a</sup>	8.36 $\pm$ 0.52 <sup>b</sup>	2.01 $\pm$ 0.11 <sup>a</sup>	0.24 $\pm$ 0.03 <sup>a</sup>	2.51 $\pm$ 0.25 <sup>b</sup>
	Cd	28.29 $\pm$ 1.96 <sup>a</sup>	9.95 $\pm$ 0.41 <sup>c</sup>	1.88 $\pm$ 0.21 <sup>a</sup>	0.35 $\pm$ 0.03 <sup>a</sup>	5.38 $\pm$ 0.49 <sup>c</sup>
	Pb+Cd	23.03 $\pm$ 1.99 <sup>b</sup>	7.95 $\pm$ 0.35 <sup>b</sup>	1.36 $\pm$ 0.13 <sup>b</sup>	0.18 $\pm$ 0.01 <sup>b</sup>	7.13 $\pm$ 0.83 <sup>d</sup>
Day 21	Control	31.10 $\pm$ 3.32 <sup>a</sup>	32.24 $\pm$ 4.48 <sup>a</sup>	2.76 $\pm$ 0.25 <sup>a</sup>	0.352 $\pm$ 0.03 <sup>a</sup>	2.25 $\pm$ 0.58 <sup>a</sup>
	Pb	26.73 $\pm$ 4.49 <sup>b</sup>	27.52 $\pm$ 4.14 <sup>b</sup>	2.26 $\pm$ 0.34 <sup>a</sup>	0.293 $\pm$ 0.07 <sup>a</sup>	3.40 $\pm$ 0.29 <sup>b</sup>
	Cd	23.19 $\pm$ 1.28 <sup>b</sup>	25.25 $\pm$ 1.45 <sup>b</sup>	2.00 $\pm$ 0.16 <sup>a</sup>	0.306 $\pm$ 0.02 <sup>a</sup>	5.36 $\pm$ 0.14 <sup>c</sup>
	Pb+Cd	19.85 $\pm$ 1.93 <sup>c</sup>	21.24 $\pm$ 1.26 <sup>c</sup>	1.81 $\pm$ 0.09 <sup>b</sup>	0.205 $\pm$ 0.04 <sup>b</sup>	6.25 $\pm$ 0.67 <sup>d</sup>

Note. Results are expressed as the mean  $\pm$  standard deviation ( $n=16$ ). <sup>a, b, c, d</sup>Mean that do not show a common letter in the same column are significantly different from one another.

TABLE 4

Lead and Cadmium Levels in Blood and Brains of Control and Experimental Pups on Postnatal Day 21 ( $\bar{x} \pm s$ )

Group	Blood ( $\mu\text{g/L}$ )		Brain ( $\mu\text{g/g}$ )	
	Lead	Cadmium	Lead	Cadmium
Control	$68.5 \pm 0.24^a$	$0.695 \pm 0.031^a$	$16.5 \pm 1.75^a$	$0.565 \pm 0.045^a$
Pb	$384.5 \pm 13.2^b$	$0.721 \pm 0.042^a$	$392.5 \pm 11.8^b$	$0.587 \pm 0.036^a$
Cd	$57.5 \pm 6.5^a$	$8.43 \pm 0.86^b$	$17.0 \pm 1.55^a$	$2.94 \pm 0.25^b$
Pb+Cd	$361.5 \pm 8.3^b$	$7.02 \pm 0.78^b$	$361.0 \pm 9.1^b$	$2.13 \pm 0.43^b$

Note. <sup>a,b</sup>Mean that do not show a common letter in the same column are significantly different from one another.

### Effects of Pb and/or Cd on Brain Ultrastructural Changes in Offsprings

Under transmission electron microscope, the control rat brain samples showed intact cytomembranes and numerous spherical or elongated mitochondria with tubular cristae, which are sometimes referred to as “tubular crests” or “transverse cristae” (Fig. 1A). Pb exposure resulted in changes in neuronal mitochondria, including swelling and partial loss of cristae. In addition, partial chromatin dissolution and dispersed arrangement of the rough

endoplasmic reticulum were observed in the animals exposed to Pb (Fig. 1B). In contrast, the animals exposed to Cd had severely damaged mitochondria with loss of nearly all cristae, as well as plasma membrane disorganization and Nissl body dissolution (Fig. 1C). More severe lesions were observed in the animals exposed to Pb+Cd than in those exposed to Pb or Cd alone. Mitochondrial swelling, disruption and total cristae loss, vague texture, severe cytoplasm dissolution, cytomembrane disappearance and neuronal nucleus concentration were observed in the Pb+Cd group (Fig. 1D).

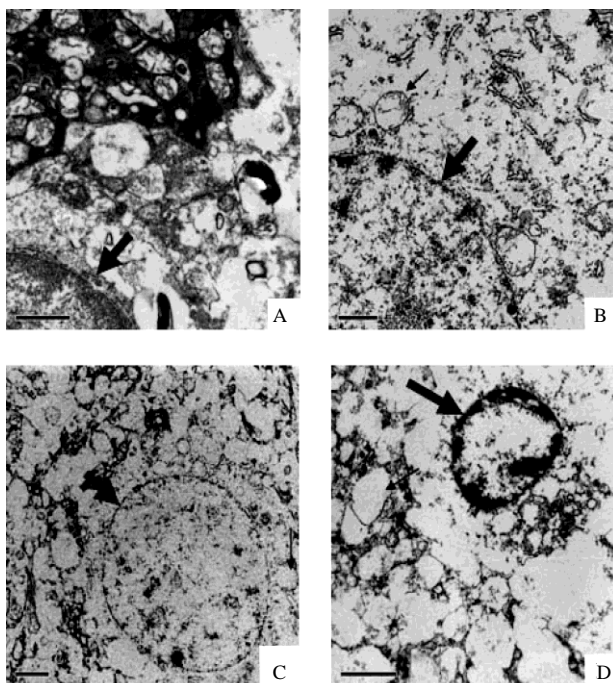


FIG. 1. Electron micrographs of brain tissue from the control, Pb, Cd, and Pb + Cd groups. A: The control group showing normal neuronal ultrastructure (18500 $\times$ , Scale bars=1  $\mu\text{m}$ ); B: the Pb group showing partial chromatin dissolution with rough endoplasmic reticulums dispersed in the extracellular space and some mitochondrial cristae swollen or lost (13500 $\times$ , Scale bars=1  $\mu\text{m}$ ); C: the Cd group showing disruption of plasma membrane, Nissl body dissolution, fragmentation and degeneration of organelles and vacuoles, and loss of mitochondrial cristae (5800 $\times$ , Scale bars=2  $\mu\text{m}$ ); D: the Pb+Cd group showing complete disruption of plasma membrane, neuronal nucleus concentration, severe cytoplasm dissolution, and mitochondrial swelling, disruption and total loss of cristae (9700 $\times$ , Scale bars=2  $\mu\text{m}$ ).

## DISCUSSION

Our results indicate that perinatal exposure to low doses of Pb and Cd can produce alterations in lipid peroxidation and ultrastructure of rat brain. Co-exposure to Pb and Cd produced more severe effects on the weights of pups and their brains than exposure to Pb or Cd alone. Pb and/or Cd exposure induced ultrastructural lesions in brain observed under electron microscope, including mitochondrial swelling, disruption and partial or total loss of cristae, Nissl body dissolution, degenerated organelles and vacuoles, cytomembrane disappearance, and nuclear chromoplasm concentration. A general decrease in the activity of SOD, CAT, GSH-Px, and AChE and an increase in MDA levels following heavy metal treatment were observed, demonstrating that Pb and Cd were able to impair the function and ultrastructure of the central nervous system by producing oxidative stress.

Pb and Cd concentrations in blood and brain were measured by inductively coupled plasma-mass spectrometry. The concentration of Pb and Cd was significantly higher in the Pb+Cd group than in the Cd and Pb groups, but not different from that in the Pb and Cd groups, indicating that one metal did not block or antagonize the intestinal absorption of the other.

Heavy metal ions are toxic to the central nervous system because BBB is immature and protein complexes sequestering metals in mature tissues are not present. Both metals are likely transferred from dams to pups in the first three weeks after birth<sup>[5]</sup>. Cd and Pb intoxication during pregnancy and lactation has critical effects on the body and brain weights of pups. Our results showed that co-exposure to Pb and Cd produced more severe effects on the weights of pups and their brains than exposure to Pb or Cd alone.

Oxidative stress is a molecular mechanism that may explain Pb and Cd toxicity. In this study, the activity of SOD, GSH-Px, and CAT was decreased in brain samples from the Pb and Cd groups, indicating that oxygen-derived free radicals may have increased. Our CAT results agree with studies demonstrating that exposure to Cd or Pb alone decreases the CAT activity, which is correlated with the reduced absorption of iron and inhibition of heme biosynthesis<sup>[20-21]</sup>. However, it has been shown that exposure to both Cd and Pb significantly increases the CAT activity in brain on postnatal day 21<sup>[10]</sup>. Free radicals lead to lipid peroxidation and damage to unsaturated fatty acids. The production of MDA parallels lipid peroxidation, which may induce MDA generation. Measurement of MDA is the most widely used test for oxidative stress. In our study, the brain MDA content was

significantly increased in the experimental groups in comparison with the control group.

Impairments of cholinergic function of Cd- and Pb-treated animals have been described, including alterations in choline and acetylcholine levels, acetylcholine turnover rate, AChE and choline acetyltransferase activity<sup>[22]</sup>, but the mechanism of metals underlying the inhibition of cholinesterase (ChE) is not clear. It has been generally accepted that metals deactivate ChEs by binding to their anionic site<sup>[23-24]</sup>, thus preventing acetylcholine from binding to ChE and degradation, suggesting that different metals can inhibit AChE distinctly because of their unique properties, such as ionic size, capacity of forming complex, electro-negativity, and reduction potential<sup>[24-25]</sup>. In the present study, a significant decrease in AChE activity was observed in the experimental groups on postnatal days 0 and 21, and the activity of AChE was significantly lower in the Pb+Cd group than in the Pb or Cd groups. No change was found in the AChE activity of the Pb group, which is consistent with previous findings<sup>[10]</sup>. Further studies are needed to identify other mechanisms underlying metal neurotoxicity and oxidative stress.

Li *et al.*<sup>[26]</sup> have reported ultrastructural lesions, such as widespread swelling and vacuolization of the hepatocytic endomembrane system, mainly in the endoplasmic reticulum (ER), mitochondria and Golgi body after exposure to microcystin in common carp. Mitochondrial changes including cristae loss and swelling induced by Cd or Pb exposure have been described elsewhere<sup>[27]</sup>. In this study, the damaging effect of Pb+Cd on mitochondria in brain tissue was greater than that of Pb or Cd alone. Co-exposure to Pb+Cd produced striking alterations and distortion in mitochondrial cristae and arrangement of cellular organelles.

In conclusion, co-exposure to Pb and Cd produces more severe effects on the weight of pups and their brains than exposure to Pb or Cd alone. Moreover, the general decrease in activity of SOD, CAT, GSH-Px, and AChE and the increase in MDA level observed after the treatment with heavy metals suggest that Pb and Cd are able to impair the function and ultrastructure of the central nervous system by producing oxidative stress.

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