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Lipid Peroxidation Induced by Maternal Cadmium Exposure in Mouse Pups

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Cadmium as an environmental pollutant has received considerable attention and its toxic effects have been studied extensively in human and adult animals (Shulka & Singhal. 1984). Moreover, an International Task Group on Metal Accumulation (1973) has established that although it is in a limited quantity cadmium can be transported across placenta and excreted through milk in animals. Likewise, it can pass through placenta in humans. Furthermore, the fact is that women in the cadmium-polluted areas are continuously exposed to cadmium during gestation and lactation. Even if they are removed from the exposure, the body burden of cadmium probably remains high because of the very long biological half-time of cadmium which is estimated to be between 17.6 and 33 years. Thus, it is possible that fetuses and pups may be exposed to cadmium during maternal gestation and lactation. Although placenta affords some protection from cadmium exposure, cadmium exposure prior to day 10-11 when placenta forms may be deleterious. Cadmium exposure during pregnancy and its effects on offsprings, which were mainly focused on litter size, pup survival, pup growth and cadmium contents in pups following maternal cadmium exposure (Schroedre & Mitchener. 1971; Pond & Walker. 1975; Webster. 1978; Welton *et al.* 1988; Payan *et al.* 1990). have been reported. Lipid peroxide has been considered as a sensitive toxicological index for environmental pollutants (Sagai. 1986). The inhibited antioxidant enzymes and enhanced lipid peroxidation due to cadmium exposure have been demonstrated both in humans and animals (Jamall *et al.* 1989; Hussain *et al.* 1987; Xu et al. 1991). Therefore, the present study was designed to evaluate the toxic effects of maternal cadmium exposure on mouse pups using both the indices used in the previous studies and determinations of lipid peroxide concentrations in various pup organs.

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MATERIALS AND METHODS

Adult healthy Wistar mice (both males and females) weighing 18-20 g, obtained from the Experimental Animal Center of China Medical University, were used in the experiments. Both male and female mice were equally divided into three groups and were kept in separate polypropylene cages with a thick layer of husk at the bottom. Considering the fact that both men and women are exposed to cadmium in cadmium-polluted areas, in this study both male and female mice were exposed to cadmium by daily drinking the tap water with 0 ppm cadmium (control), 30 ppm cadmium added or 75 ppm cadmium added for two months. Then, the mice in the same group were mated (two females with one male) for six days while continuing to receive their respective cadmium exposure. Pregnancy was evaluated by vaginal smear test and pregnant mice were removed to other cages. When they delivered the litter sizes were recorded and the pups remained with the mother until they were sacrificed for the experiments. During gestation and lactation, cadmium was continuously administered to maternal mice. Pup survival rates in the three groups were observed on birth and postnatal days 4 and 10. To examine the cadmium exposure in the pups following the maternal cadmium exposure, cadmium concentrations in the whole body of the pups at birth and cadmium concentrations in the liver, heart, kidney and brain (pooled tissue per individual pup) of postnatal day 20 pups were determined by atomic absorption spectrometry following the digestion by nitric acid and heating. The pups used for the determinations of cadmium contents were from different litters (one pup per litter). Finally, on day 20 postpartum cadmium concentrations in the kidneys of female mice were also detected using the same method.

On postnatal day 7, the pups (one pup per litter) were sacrificed by decapitation. Liver, heart, kidney and brain were removed and weighed quickly, and the percentages of each organ weight to body weight were calculated. Then. 10% tissue homogenates were prepared in 0.9% cold saline solution. Lipid peroxide (LPO) concentrations in the unfractionated homogenates of these tissues were determined using TBA (thiobarbituric acid) method as described by Shimasaki(1985). The results were expressed as nmoles MDA (malondialdyhyde)/ ml homogenate.

All data in the present study are presented as mean $\pm$ standard deviation. ANOVA (analysis of variance) was used for statistical analysis. If a significant F value was obtained, Duncan's t-test was further employed to determine differences between the groups. $p < 0.05$ was considered to be significantly different.

RESULTS AND DISCUSSION

Table 1 shows that the cadmium concentrations in the maternal kidneys of both exposed groups were significantly higher than that in the control group, indicating significant maternal cadmium exposure in both cadmium-exposed groups. As presented in Table 2, although the mean cadmium concentrations of the whole body of pups at birth in both exposed groups were higher than that in the control group, there were no statistical differences because of the observed large standard deviation. Table 2 shows also that on postnatal day 20 the cadmium concentrations in the pooled pup organs in both exposed groups were significantly higher than that in the control group ($p<0.01$). The cadmium concentration of the pooled organs in the pups whose mothers were exposed to 75 ppm cadmium was also higher than that in the pups whose mothers were exposed to 30 ppm cadmium ($p<0.01$). Clearly, the present study indicates that the pups can be significantly exposed to cadmium following the maternal exposure from pre-gestation through lactation and that the extent of the exposure in the pups was associated with the degree of maternal exposure. Thus, our results agree with the previous studies (International Task Group on Metal Accumulation 1973; Flis *et al.* 1978; Payan *et al.* 1990). An International Task Group (1973) documented that cadmium could be transported through placenta and could also be excreted by milk in animals. Flis *et al* (1978) detected cadmium concentrations ranging from 1.7-4.2 ng/g in maternal milk on the second day of lactation following maternal exposure to 5.1 ppm cadmium (5.0 ppm in drinking water and 0.1 ppm in food) from conception through lactation in rats. They (Flis *et al*, 1978) reported also that although the placenta was an efficient barrier to cadmium transfer to the fetuses, 0.4-0.6 ppm, 0.4-1.2 ppm and 0.4-0.5 ppm cadmium were detected in the pup liver, kidney and brain, respectively, following maternal exposure to 5.1 ppm cadmium. More recently, Payan *et al*(1990) further demonstrated that administration of cadmium (in the form of metallothionein) to pregnant mice resulted in a significant increase in the cadmium contents of the fetuses, pointing out the possibility that fetuses can be exposed to cadmium through placental transport. Therefore, we suppose that under our experimental conditions placental transport and milk may be important routes of cadmium exposure for the fetuses and pups. In addition, fetuses may also be exposed to cadmium before placental formation.

Litter size and pup survival rates at birth and postnatal days 4 and 10 of birth were not statistically different among the three groups (data not shown). We

Table 1. Cadmium concentrations of the maternal kidneys on postpartum day 20

Group	No. of mice	Cadmium concentration(μg/g)
Control	11	0.069 ± 0.013
30 ppm Cd	9	1.482 ± 0.072**
75 ppm Cd	10	1.961 ± 0.062**,a

Duncan's t-test, **p<0.01 compared with control group, [a]p<0.01 compared with 30 ppm Cd group.

Table 2. Comparison of the cadmium concentrations of the pup organs and whole body in control and cadmium treated groups

Group	No. of pups	Cadmium concentration(μg/g)	
		Body (At birth)	Organ (On postnatal day 20)
Control	11	0.010 ± 0.003	0.012 ± 0.007
30 ppm Cd	9	0.024 ± 0.023	0.075 ± 0.044**
75 ppm Cd	10	0.035 ± 0.040	0.418 ± 0.183**,b

Duncan's t-test, **p<0.01 compared with control group, [b]p<0.01 compared with 30 ppm cadmium-exposed group.

found that the brain weight of the pups following the exposure of maternal mice to 30 ppm cadmium and the weights of liver, kidney, heart and brain of the pups following the exposure of the maternal mice to 75 ppm cadmium were significantly lower compared with the control group (data not shown), but there was no significant changes in body weights among the three groups (Table 3). However, when the percentages of organ weights to body weights were calculated, it was found that although the percentages of the liver weight/body weight and brain weight/body weight in the pups following the exposure of maternal mice to 75 ppm cadmium tended to be lower compared with the control group, there were no statistical difference (Table 3). Concerning the effects of maternal cadmium exposure on the fetus and pups, Pond and Walker (1975) reported that 200 ppm dietary cadmium significantly decreased the mean rat pup weight by 16% at birth. Webster (1978) also demonstrated significant reductions in mean fetal weights for three cadmium-exposed groups (10, 20 and 40 ppm cadmium) of dams in mice compared with the control group (0 ppm cadmium). More recently, Whelton *et al* (1988) observed that female mice fed 50 ppm cadmium

Table 3. Percentages of the pup organ weights/body weights in the control and cadmium treated groups on postnatal day 7

Organ	Control(11)	30 ppm Cd(9)	75 ppm Cd(10)
BW(g)*	6.36 ± 0.53	6.16 ± 0.64	5.98 ± 0.58
Liver	3.03 ± 0.23	3.33 ± 0.41	2.84 ± 0.20
Kidney	1.29 ± 0.13	1.21 ± 0.13	1.22 ± 0.14
Heart	0.48 ± 0.05	0.47 ± 0.07	0.45 ± 0.04
Brain	5.35 ± 0.41	5.23 ± 0.61	4.34 ± 0.47

Data in parentheses represent the cases of mouse pups examined. *body weight.

Table 4. LPO concentrations in the various organs of postnatal day 7 mouse pups following maternal cadmium exposure

Organ	LPO concentration(nmoles MDA/ml homogenates)		
	Control(9)	30 ppm Cd(9)	75 ppm Cd(12)
Liver	92.1 ± 30.6	92.7 ± 17.2	121.1 ± 17.2*,a
Kidney	75.2 ± 17.8	70.3 ± 12.1	66.8 ± 27.4
Heart	44.8 ± 30.2	42.8 ± 24.6	69.9 ± 37.6*,a
Brain	11.5 ± 7.8	20.7 ± 16.4	27.3 ± 10.6**

Data in parentheses represented the cases of the mice pups. Dunncan's t-test, *$p<0.05$, **$p<0.01$ compared with the control group. [a]$p<0.05$ compared with 30 ppm cadmium-exposed group.

containing purified diet through six consecutive rounds of gestation and lactation caused a 25% decrease in pup growth. However, under our experimental conditions we did not detect a significant toxic effect of maternal cadmium exposure on mouse pup weight.

To further document the influences of maternal cadmium exposure on mouse pups, LPO concentrations in various pup organs were determined in the present study. Although TBA test is nonspecific compared with the method using high pressure liquid chromatography (Knight *et al*, 1988) TBA test is still commonly used. Because the latter method has not been established in our laboratory and the materials used for the detection of LPO concentration in the present study were limited,

only TBA test was performed. As presented in Table 4, although LPO concentration in the brain tissue of the pups following exposure of maternal mice to 30 ppm cadmium was higher compared with control group it did not reach statistical significance. In contrast, pups from mice consuming the tap water with 75 ppm cadmium added resulted in significantly higher LPO concentrations in all organs observed, except that in kidney. The LPO concentrations in the pups liver, heart and brain were 131.5%, 156.0% and 237.4%, respectively, of the control group. Thus, our results indicate that during gestation and lactation maternal cadmium exposure can lead to increased LPO concentrations in various pup organs, especially in the brain. We further suppose that the elevated LPO concentrations in the various organs of the pups may reflect the toxic effect of maternal cadmium exposure on the mouse pup. Recently, lipid peroxidation induced by cadmium has been well documented in adult animals. For example, Hussain *et al*(1987) observed that intraperitoneal administration of 0.4 mg Cd/Kg (cadmium acetate)to growing rats daily for 30 days significantly depressed SOD (superoxide dismutase) activities to 46% for the liver tissue and to 44% for the kidney tissue, respectively, as compared with the control group, and LPO concentrations in both tissues increased to 127% of the control group. Shukla *et al* (1987) reported also that under the same cadmium exposure conditions, the SOD activities in cerebellum, cerebral cortex, corpus striatum, hypothalamus, midbrain and pons-medulla were significantly inhibited by cadmium, but significant increases in LPO concentrations were detected only in cerebellum, cerebral cortex, corpus striatum and midbrain. Manca *et al*(1991) confirmed that in rats with intraperitoneal injection of 25 micrograms Cd/Kg as $CdCl_2$, LPO concentrations (nmoles TBARS/g tissue) in lung and brain tissues were elevated by 138% and 170%, respectively, as compared with the control group, whereas significant increases in LPO concentrations of liver and kidney tissues were observed after intraperitoneal administration of 500 micrograms Cd/Kg as $CdCl_2$. With regard to the high susceptibility of the pup brain to lipid peroxidation induced by cadmium we assume that it may be associated with the several factors. First, brain is not rich in antioxidant enzymes. As reported by Hussain *et al* (1987) and Shukla *et al* (1987) the SOD activity (less than 25 micrograms/mg protein in most brain regions) in the brain was significantly lower than that (40.6 micrograms/mg protein) in the liver in growing rats. Second, brain has high amounts of polyunsaturated fatty acids which are susceptible to peroxidation. Third, brain consumes oxygens much more than other organs which accordingly releases more free radicals responsible for lipid peroxidation.

In conclusion , data from the present study indicate that the detection of LPO concentration in selected pup tissues is a sensitive index for evaluating the effects of maternal cadmium exposure on mouse pups.

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