Bull Environ Contam Toxicol (2012) 89:240–244
DOI 10.1007/s00128-012-0683-y

Detrimental Effects of Maternal Lead Exposure during Pregnancy and Lactation on Molar Development in the Young Rat

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Received: 16 December 2011 / Accepted: 8 May 2012 / Published online: 22 May 2012

Abstract The purpose of this animal study was to investigate the influence of maternal lead exposure during pregnancy and lactation on molar development in the offspring. Scanning electron microscopy revealed no significant differences in the molar morphology among the groups. However, in all the experimental groups, deep, wide cracks were found in the occlusal enamel. Further, the experimental groups had smaller molar diameters than the control group, lead exposure during lactation had a greater influence on the molar size in the offspring, and the groups with the higher dose of lead exposure during pregnancy and lactation had significantly smaller molar sizes than the groups that received the lower dose. The mesiodistal and buccolingual diameters of molars were measured as 3.10 ± 0.07 and 1.95 ± 0.04 mm for control group, 2.97 ± 0.08 and 1.94 ± 0.01 mm for lactation group of low dose, 2.96 ± 0.05 and 1.84 ± 0.02 mm for lactation group of high dose, 3.09 ± 0.06 and 1.94 ± 0.04 mm for pregnancy group of low dose, and 3.02 ± 0.06 and 1.85 ± 0.06 mm for pregnancy group of high dose, respectively.

Keywords Rat · Molar · Lead exposure · Morphology · Pregnancy · Lactation

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Numerous studies have confirmed the impact of lead exposure on human health (Fletcher et al. 1999; Osman et al. 2000; Weizsaecker 2003). For example, Lead exposure could damage the physiological system via the blood circulation and also directly affect the oral environment (Gomes et al. 2004). In the oral cavity, lead exposure has a proven negative effect on tooth development and eruption (Appleton 1991; Gerlach et al. 2000a, 2002; Youravong et al. 2008). Youravong et al. (2008) noted that lead damages odontoblast function and affects tooth calcification, which further affects the formation of dentin.

The lead concentration in fetal and infant blood is reportedly influenced by that in maternal blood during pregnancy and lactation, respectively (Korpela et al. 1986; Osman et al. 2000; Téllez-Rojo et al. 2004). Téllez-Rojo et al. (2004) reported that fetuses are mainly exposed to lead via the lead-polluted environment in which the mother lived during pregnancy as well as the long-term lead deposits in bones.

Previous studies on the effect of maternal lead exposure on fetal tooth development mostly emphasized

measurement of the lead content of teeth (Gomes et al. 2004; Youravong et al. 2008). Fewer studies have focused on the effect of maternal lead exposure during pregnancy and lactation on the tooth morphology of the offspring. In this study, we used a rat model to examine whether maternal lead exposure during pregnancy and lactation affects tooth development of the offspring. We hypothesized that lead exposure during pregnancy or lactation would have a significant impact on the tooth morphology and size of the young.

Materials and Methods

Twenty-five 2-day-pregnant Sprague–Dawley rats (average body weight ≈ 175 g) were used in this study. The rats were purchased from BioLASCO Taiwan Co., Ltd. and bred at the Kaohsiung Medical University Laboratory Animal Center. Their duration of pregnancy was 20–22 days; they bore 6–12 young from a single pregnancy; and weaning occurred 21 days after birth on average.

The pregnant rats were randomly divided into 5 groups (n = 5/group), including a control group and 4 experimental groups, and exposed to lead in drinking water on the basis of the Geng et al. method (2005). The control group received deionized drinking water during pregnancy and lactation. Among the experimental groups, the Preg50 and Lact50 groups received 50 ppm (low dose) of lead acetate in drinking water during pregnancy and lactation, respectively, whereas the Preg200 and Lact200 groups received 200 ppm (high dose) of lead acetate in drinking water during pregnancy and lactation, respectively.

To obtain leaded drinking water, an electronic analytical balance (Model Precisa 205A, Swiss) was used to measure 50 and 200 mg of lead acetate powder (99.5 % purity). Each quantity was then added to 1 L of double-deionized water (>17.2 Ω). The drinking water for the control group was also double deionized above 17.2 Ω.

Young rats were sacrificed with carbon dioxide 35 days after birth, and their left and right mandibular first molars were extracted. Ultrasonic vibration was used to clean the tooth surfaces for 5 min; then, the teeth were fixed onto the sample platform with the cementoenamel junction parallel to the ground and the long axis perpendicular to the ground. An ion-sputtering device (JFC-1100E, JEOL, Tokyo, Japan) was used to treat the tooth surfaces to enhance the contrast between the observed samples. Finally, a scanning electron microscope (JSM-5300, JEOL) and dynamic mathematics software (GeoGebra, USA) were used to measure the mesiodistal and buccolingual diameters.

As shown in Fig. 1, the contour of the mandibular first molar is oval, with the distal margin being wider than the mesial margin. According to Hunt et al. (1970), the occlusal surface shows 6 tubercles (T) and 2 supplementary tubercles (ST). T1, T3, and T5 are located on the lingual side, and T2, T4, and T6 are located on the buccal side; the STs are located on the buccal and distal walls of T6. The reference point for measuring the molar size was based on the method of Chowdhury and Bromage (2000). In brief, the salient points of T2 and T4 were joined; parallel to this line, a tangential line was drawn from the lingual enamel projection to create the lingual tangential line. Parallel to the lingual tangential line, another tangential line was drawn from the buccal enamel projection to obtain the buccal tangential line. The distance between these tangential lines was considered the buccolingual diameter. Further, a line was drawn to join the midpoints of the buccal and lingual tangential lines. Perpendicular to this line, two parallel tangential lines were drawn from the salient points of the mesial and distal margins. The distance between these lines was considered the mesiodistal diameter. The accuracy of the linear measurement was ±1 μm.

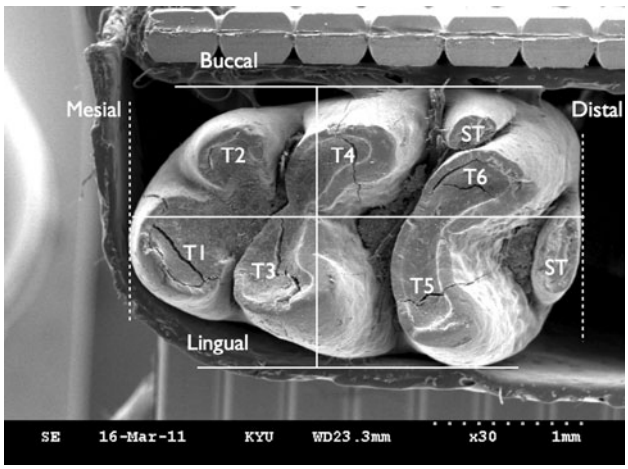

Fig. 1 Scanning electron micrograph showing the occlusal surface of a mandibular first molar from the control group (×30 magnification). Dynamic geometry software (GeoGebra, USA) was used to measure the distances between the buccal and the lingual tangential lines and between the mesial and the distal tangential lines as the buccolingual and mesiodistal diameters, respectively. *T* tubercle *ST* supplementary tubercle

A statistical analysis software package (JMP 8.0, SAS Institute, Inc., Cary, NC) was used for all analyses. Differences in the physiological features of the pregnant rats and their offspring among the groups were analyzed with the Wilcoxon/Kruskal–Wallis test (rank sums). Differences in the mesiodistal and buccolingual molar diameters among the groups were analyzed by one-way ANOVA. A p value of less <0.05 indicates a statistically significant difference, and data represent means (standard deviation).

Results and Discussion

The groups did not show significant differences in the average amounts of daily drinking water and average weight gain during pregnancy and lactation (Table 1). They also did not show significant differences in the number of offspring per rat, birth weight, and weight gain during lactation (Table 2).

In terms of the molar morphology, no significant differences were noted among the groups (Fig. 2). However, the occlusal surface of the molars from the offspring in the experimental groups displayed deep, wide cracks near the dentinoenamel junction. Comparatively, the occlusal surface of molars from the control offspring had many small and shallow cracks in the enamel.

As shown in Table 3, the control group had the longest mesiodistal diameter (3.10 [0.07] mm), whereas the Lact200 group had the shortest mesiodistal diameter (2.96 [0.05] mm). One-way ANOVA showed significant differences ($p < 0.0001$) among the different groups. The mesiodistal diameter of the control group was significantly larger than those of all the experiment groups. Moreover, the Preg50 and Lact50 groups had significantly larger mesiodistal diameters than the Preg200 and Lact200 groups, respectively. However, the Preg50 and Preg200 groups had significantly larger mesiodistal diameters than the Lact50 and Lact200 groups. Regarding the buccolingual diameter, the control group had the largest diameter of 1.95 (0.04) mm, and the Lact200 group had the smallest diameter of 1.84 (0.02) mm. Again, there were significant differences ($p < 0.0001$) among the different groups.

By examining the effects of leaded drinking water consumed during pregnancy and lactation on the molar morphology and size of the offspring, we found that lead exposure resulted in a significantly smaller molar size and affected the integrity of the occlusal enamel. Specifically, exposure during lactation and exposure to the high dose had severer effects on the molar size. Further, in all the lead-exposed groups, the occlusal surface showed deep, wide cracks near the dentinoenamel junction.

Numerous previous studies (Appleton 1991; Brook et al. 1997; Gerlach et al. 2000a, 2002; Osman et al. 2000; Téllez-Rojo et al. 2004; Youravong et al. 2008) support the finding that lead exposure during pregnancy or lactation results in significantly smaller molars in the offspring. Osman et al. (2000) showed that lead easily crosses the placental barrier and its presence in cord blood is a negative predictor of growth in children. Gerlach et al. (2002) found that lead exposure via drinking water causes delayed enamel mineralization of the incisors in rats. Therefore, lead could enter the offspring via the mothers, interfere with tooth germ development, and thus cause the development of smaller crowns.

In addition, lead exposure during lactation had the greatest impact on the molar size. In a rat study, Watson et al. (1997) suggested that breast milk contains 10 times more lead than blood in the same period. This finding probably explains why lead exposure during lactation had a greater effect on the molar size.

In this study, although no significant differences were found in the molar morphology and number of tubercles among the groups, the experimental groups had deep, wide cracks on the occlusal surface near the dentinoenamel junction. This result is supported by several other studies (Appleton 1991; Brook et al. 1997; Gerlach et al. 2000a, b, 2002; Kato et al. 1977). In the Brook et al. study (1997), 77 % of the children with lower birth weight likely had defective enamel development and significantly higher bone and tooth lead levels. In 2002, Gerlach et al. (2002) showed that structural damage is more likely to occur in enamel structures infiltrated by lead. Gerlach et al. (2000b) also found a decrease in the microhardness of mature dentin and reduced incisor eruption rate after lead exposure.

In the present study, the experimental rats consumed lead acetate water, and there was no significant difference

Table 1 Comparison of the average amount of daily drinking water and maternal weight gain during pregnancy and lactation in the different groups of rats

Group	Amount of daily drinking water (mL)		Maternal weight gain (g)	
	Pregnancy	Lactation	Pregnancy	Lactation
Control	182.8 (50.6)	345.2 (81.2)	110.7 (6.5)	43.6 (14.2)
Lact50	185.1 (53.9)	321.9 (49.3)	113.8 (9.5)	30.3 (5.4)
Lact200	190.3 (39.5)	314.4 (43.5)	118.1 (4.5)	26.2 (6.6)
Preg50	189.7 (67.5)	347.7 (56.9)	106.5 (18.4)	29.5 (3.6)
Preg200	187.3 (59.9)	330.6 (71.0)	103.9 (7.9)	24.6 (1.0)
p*	0.9953	0.8109	0.6045	0.2160

Data represent means (SD); n = 5/group

* p value calculated by using the Wilcoxon/Kruskal–Wallis test; $p < 0.05$ indicates a statistically significant difference

Table 2 Comparison of the number, birth weight, and weight gain of offspring during lactation in the different groups of rats

Group	Number of offspring	Birth weight (g)	Weight gain (g)
Control	8.00 (3.39)	7.40 (0.21)	63.60 (0.60)
Lact50	8.80 (2.17)	7.34 (0.04)	60.86 (0.27)
Lact200	9.00 (3.00)	7.40 (0.08)	59.05 (0.20)
Preg50	9.80 (2.05)	6.91 (0.15)	58.08 (4.59)
Preg200	9.60 (2.30)	6.67 (0.16)	54.88 (1.98)
p*	0.5080	0.1312	0.2745

Data represent means (SD); n = 5/group

* p value calculated by using the Wilcoxon/Kruskal–Wallis test; $p < 0.05$ indicates a statistically significant difference

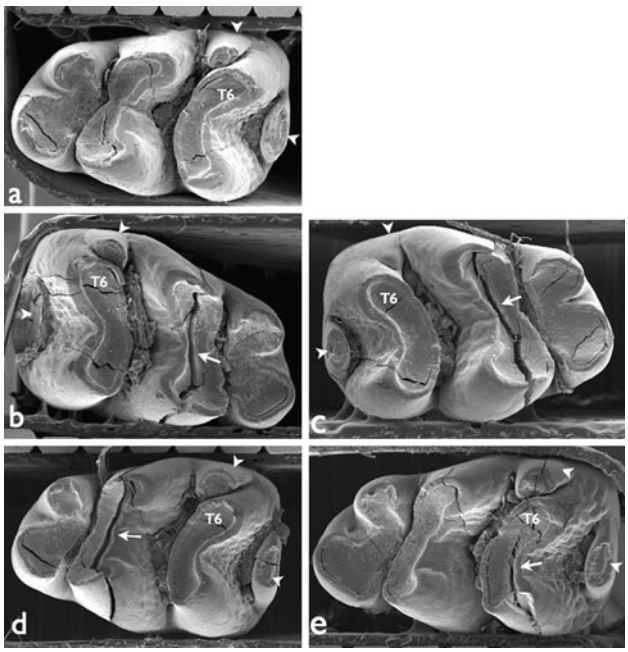

Fig. 2 Scanning electron micrographs showing the occlusal surfaces of mandibular first molars from the **a** control, **b** Lact50, **c** Lact200, **d** Preg50, **e** Preg200 groups (×30 magnification). The occlusal outlines in all the groups were oval shaped, with the distal margin being the widest. Both the buccal margin and the distal region of *T6* showed supplementary tubercles (*arrowheads*). Deep, wide cracks were seen near the dentinoenamel junction in the experimental molars (*arrows*). *T* tubercle

between the control and the experimental groups in the total amount of water consumption. In the experiment by Kimmel et al. (1980), rats were given lead acetate water at the concentrations of 25, 50, or 250 ppm for 7 weeks during pregnancy and lactation. No difference between the control and the experimental groups was found in the amount of food and drinking water consumption. This is consistent with the present result, and therefore, administration of lead acetate water seems to be a practical method of creating lead exposure.

Table 3 Comparison of the molar mesiodistal and buccolingual diameters from the offspring in the different groups of rats

Group	Mesiodistal diameter	Buccolingual diameter
(1) Control	3.10 (0.07)	1.95 (0.04)
(2) Lact50	2.97 (0.08)	1.94 (0.01)
(3) Lact200	2.96 (0.05)	1.84 (0.02)
(4) Preg50	3.09 (0.06)	1.94 (0.04)
(5) Preg200	3.02 (0.06)	1.85 (0.06)
p*	<0.0001	<0.0001
Tukey's pairwise comparison	(1) > (4) > (2) > (3); (1) > (4) > (5) > (3)	(1) > (4) > (2) > (5) > (3)

Data represent means (SD); n = 8/group

* p value calculated by using the Wilcoxon/Kruskal–Wallis test; $p < 0.05$ indicates a statistically significant difference

The different chemical states of lead affect the absorption rate. In the present study, lead acetate water was used to achieve lead exposure, and different concentrations were prepared for consumption. In a study on the levels of lead blood and tissue, Dieter et al. (1993) noted that lead acetate and lead oxide have higher absorption rates than lead ore concentrate and lead sulfide. Another animal experiment with different forms of lead in rat food revealed that the group fed with lead acetate had a higher rate of biological uptake than the group fed with lead in mining waste soil (Freeman et al. 1992). Therefore, lead acetate is often used to simulate conditions of lead exposure in natural or industrial environments.

Andrzejewska et al. (1994) noted that lead exposure at 50 ppm in animal experiments is equivalent to the lead pollution in an urban environment; concentrations of 500 ppm and even 1,000 ppm are equivalent to lead exposure in an industrial environment. In the present study, lead exposure was achieved with 50 and 200 ppm of lead acetate water. Many related studies have utilized similar methods (Andrzejewska et al. 1994; Geng et al. 2005; Kimmel et al. 1980). In 2005, Geng et al. (2005) used 50 and 200 ppm of lead acetate water to observe the effects of maternal lead exposure during pregnancy on the eruption rate of the incisors in the offspring. They discovered that such exposure had a negative effect on the normal eruption of the incisors. Further, Kimmel et al. (1980) administered 50 and 250 ppm of lead acetate water to female rats during pregnancy and lactation to investigate the impact of lead exposure on rats. They found growth retardation after 1–3 weeks of lead exposure.

This study is limited by its experimental design. Although we noted the effects of lead exposure of pregnant rats on the molar development of the offspring, this result cannot be directly applied to humans. Further inference is suitable only after consulting public health survey reports.

In conclusion, lead exposure of pregnant rats has negative effects on the crown size and integrity of occlusal enamel in the offspring. Exposure to high doses of lead and exposure during lactation seem to have severer effects on molar size in the young. However, irrespective of the period of exposure and dose, tooth development is affected to varying degrees. Therefore, lead pollution and residue in the environment may have detrimental effects on tooth development and should be prevented.

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