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Effects of Maternal Pb^{2+} Poisoning during Pregnancy on the Development of the Jaw (Meckel's Cartilage) of Rat Fetus

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ABSTRACT: The purpose of this study was to investigate the effects of maternal lead poisoning during pregnancy on the development of the jaw (Meckel's cartilage) of rat fetuses by histologic and morphometric methods. Pregnant rats received a single intraperitoneal injection of 2.5 mg of lead acetate/100g body weight on the 10th day of pregnancy. Meckel's cartilage of fetuses of the lead-treated group showed smaller volume density and size of the lacunae, as well as modification of the lacunae shape. Moreover, the number density of lacunae and the volume density of the matrix increased significantly in the Meckel's cartilage in treated group fetuses. The results suggest that lead poisoning during the period of organogenesis can induce disturbances in the development and differentiation of the fetal stomatognathic system. Reducing the consumption of alcoholic beberages and smoking cessation by women in childbearing age, along with a strict policy of control of the environmental lead exposure can bring great benefits to the future generations of children. @ JASEM

Lead is toxic to living things. The metal can be free in the environment or in industrialized products such as gasoline, derived from fuel combustion and cement production, cigarettes, mobile batteries, alloys used in fuses, crystals, foods of animal or vegetal origin, plastic stabilizers, biocides used in wood preservation, welding with lead and jewelry (Sanin et al., 1998). During the last decades the reduction of exposure to lead have been attained by decreasing the metal content in house paints, plumbing, insecticides, batteries, milk, whisky, wine and cutlery. Several researches on bioengineering were devoted to develop materials for lead-free solder, with the aim of improving public health conditions. Nowadays, there is great concern in avoiding the presence of pregnant women in endemic areas of lead contamination in order to protect their reproductive health and the development of their children. However, lead from other sources such as tobacco, *Cannabis sp*. and alcohol can also intoxicate user women. Furthermore, the sub clinical intoxication of non-pregnant women can cause fetal alterations in later pregnancies, by releasing of lead stored in the maternal bones. Most of the time, because of misdiagnosis, the problem is not considered (Gulson et al., 1998).

The fetus is susceptible to lead and may be intoxicated because of the ability of the metal to be transferred across the placental barrier, resulting in delayed fetal development, mainly with high doses of lead and during the teratogenic period (Carpenter et al., 1973, Gerber et al ., 1978, McClain and Becker, 1975). Lead has toxic effects on the development of various organs and physiological processes, including hematopoiesis, urogenital and cardiovascular systems, and probably the most serious, central nervous system (Sanin et al., 1998). Since bone tissue is the major depository of lead in the body, the purpose of this study was to evaluate histologically and morphometrically the development of Meckel's cartilage (jaw primordium) in fetuses of rats experimentally intoxicated with lead.

MATERIALS AND METHODS

Nulliparous albino Wistar rats (*Rattus norvegicus*), 180-200 g body weight, were used. After two weeks of acclimation under controlled conditions of light, temperature, and humidity and fed with pelletized rodent chow and water *ad libitum*, the rats were mated overnight with fertile males. The next morning was considered the first day of pregnancy when the sperm was present in the vaginal smear. On the 10th day of pregnancy, five rats (treated group), randomly chosen, received a single intraperitoneal injection of lead acetate solution (Merck KGaA, Germany), reaching a concentration of 2.5 mg/100 g body weight, and another five (control group) received a single intraperitoneal injection of saline. On the morning of the 20^{th} day of pregnancy, the rats were anesthetized with 2.5% tribromoethanol and killed by cervical dislocation. Fetuses and placentas were extracted and fixed for 24h in a solution of 85 ml 80% alcohol, 10 ml formalin, and 5 ml glacial acetic acid. Five fetuses of the treated group and five of the control group, randomly chosen, were used in this study. Heads, separated from the bodies, were cut along the midsagittal plane and embedded in paraffin. Serial 6 μm-thick sections were sliced and stained with hematoxylin and eosin. The morphometric analysis was performed on 50 lacunae of Meckel's cartilage of each fetus. The following parameters were determined: diameters, greatest diameter/smallest diameter ratio, perimeter, area, surface, volume, volume/surface ratio, eccentricity, shape factor, and contour index (Sala et al., 1994). Volume densities of both lacunae and ground substance of the Meckel's cartilage were estimated as well as the number density of lacunae (Sala et al, 1992). Statistical analysis was performed by the Wilcoxon-Mann-Whitney (Sprent and Smeeton, 2001).

RESULTS AND DISCUSSION

Lead is toxic and may induce alterations in fetuses of intoxicated pregnant women. The metal is rapidly transferred to the fetus at different stages of

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pregnancy, reaching equilibrium between fetus and mother serum concentration 24 hours after poisoning (McClain and Becker, 1975). The lead intoxicated embryo shows delayed development, low birth weight, and often malformations (Carpenter et al., 1973). These authors report smaller size and presence of malformations such as exencephaly, sacral vertebrae, and equine tail at different levels, in fetuses of lead-intoxicated hamsters.

Embryos of lead-intoxicated chickens show reduced body size, microcephaly, shortened members and curved neck and beak (Gilani, 1973). In fetuses from lead-intoxicated rats, McClain and Becker (1975) demonstrate absence of the posterior part of the body, absent or reduced tail and defects in the axial skeleton. Fetuses of rats intoxicated by lead and fed a low-calcium diet present cleft palate and malformations of the tail (Carpenter, 1982).

Table 1: Comparison of mean values for the morphometric parameters of the Meckel's cartilage lacunae in fetuses from control and lead-intoxicated rat fetuses

Parameter	Control	Treated	Ucalc	P
Greatest diameter (μm)	29.6	19.7	2	< 0.05
Smallest diameter (μm)	22.4	13.9	1	< 0.01
Mean diameter (μm)	25.6	16.4	1	< 0.01
D/d ratio	1.35	1.74	0	< 0.01
Perimeter (μm)	82.2	53.2	1	< 0.01
Area (μm^2)	536.8	190.3	0	< 0.01
Surface (μm^2)	2147.2	761.2	0	< 0.01
Volume (μm^3)	9880.4	2009.8	0	< 0.01
Volume/surface ratio (μm)	4.60	2.64	1	< 0.01
Eccentricity	0.61	0.78	0	< 0.01
Shape factor	0.96	1.13	0	< 0.01
Contour index	3.62	4.39	0	< 0.01

Wilcoxon-Mann-Whitney test: Ucalc = U calculated; P = probability; D/d: Greatest diameter to smallest diameter ratio.

Moreover, fetuses of monkeys poisoned with lead are normal, without apparent skeletal abnormalities (Tachon et al., 1983). Furthermore, these authors demonstrate the transfer of lead throughout the placental barrier and its storage in the blood and bone tissue of the fetus. The administration of high doses of lead to pregnant rats and mice causes delayed fetal skeletal development (Kennedy et al., 1975). However, the authors conclude that there are no specific skeletal abnormalities associated to lead exposure. Any center of mandible ossification may be teratogenically induced, causing probably a congenital jaw abnormality. Because of its relationship with the morphogenesis of the jaw, any developmental alteration of the Meckel's cartilage could result in the absence, deformity, or reduction of the mandible size. Frommer and Mergolies (1971) verify that an effective dose of teratogen before the 13th day of pregnancy could inhibit differentiation of Meckel's cartilage in mice by blocking the initial development of the center of intramembranous ossification. Meanwhile, no reports were found about the effects of maternal lead poisoning on the development of the fetal stomatognathic system. In the present study, Meckel's cartilage in the treated-group fetuses was poorly differentiated and composed of small lacunae and abundant ground substance, poorly calcified. Chondrocytes were smaller, irregularly arranged and shrunken into lacunae, with small, central, or slightly eccentric and hyperchromatic nuclei. Variable-sized lacunae were more numerous and smaller. Meanwhile, the cartilage matrix was abundant and poorly calcified in the treated-group fetuses. The morphometric evaluation of lacunae showed smaller diameters, perimeter, area, surface, volume, and volume to surface ratio in the treated group fetuses. Similarly, the shape of lacunae was modified in treated fetuses, appearing significantly elongated (Table 1).

Meckel's cartilage with normal development up to the 13th day of pregnancy shows intense cell proliferation and differentiation. On the 19th day, it is possible to observe calcification of the perichondral collar and extensive calcification of the matrix, whereas on the 20th day there are zones of hypertrophic cartilage, calcification, and erosion (Frommer and Mergolies, 1971). The stereologic analysis showed a significant reduction in the volume density of lacunae and a significant increase in volume density of the cartilage matrix, as well as an increased number density of lacunae in the Meckel's cartilage of intoxicated-rat fetuses (Table 2). This is a typical feature of Meckel's cartilage in the initial phase of growth, reflecting immaturity of this tissue in treated animals. The increase in the number density of lacunae in fetuses of the treated group suggests that Meckel's cartilage chondroblasts did not grow and were not resorbed, as normally occur. Lacunae of the Meckel's cartilage in the treated-group fetuses were smaller than in the control group, allocating an increased concentration of chondrocytes. In the

present experimental conditions, Meckel's cartilage and the mandible bone appeared weakly differentiated, composed by small lacunae and abundant poorly calcified matrix. These alterations could be due to the competition between iron and calcium (Quarterman and Morisson, 1975, Barton et al., 1978; Goyer, 1996), or to the blockage of bone calcification due to alterations of both osteocytes activity and osteoclasis (Goyer, 1996).

Table 2: Comparison of mean values of the stereologic parameters in the Meckel's cartilage from control and lead-intoxicated rat fetuses

Parameter	Control	Treated	Ucalc	P
Lacunae				
Volume density (%)	63.1	34.1	0	< 0.01
Number density (n/mm^3)	67.4	242.7	0	< 0.01
Cartilage matrix				
Volume density (%)	36.9	65.9	0	< 0.01

Wilcoxon-Mann-Whitney test: Ucalc = U calculated; P = probability

The skeleton is the major reservoir of lead in the organism and sub toxic amounts of lead can be mobilized during bone remodeling. In pregnancy, when there are low levels of calcium in the interstitial medium, lead stored in bones can be released into the blood and redistributed by the blood and soft tissues (Gulson et al., 1998; Nashashibi et al. 1999). The inverse relationship between calcium and lead could explain the competition of these elements for the same site, since the presence of lead also increases the levels of calcium in the blood and soft tissues (Kumagai and Sakai, 1966).

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