Effect of Prenatal Lead Exposure on Superoxide Dismutase Activity in the Brain and Liver of Rat Fetuses

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Prenatal lead exposure had a damaging effect on Cu/Zn superoxide dismutase activity in the brain and liver of rat fetuses (20 days of gestation). The decrease in Cu/Zn superoxide dismutase activity in the brain and liver of treated fetuses reflects activation of free radical processes and impairment of the antioxidant defense system during prenatal lead exposure.

Key Words: *superoxide dismutase; brain; liver; lead citrate; fetus*

Lead is one of the most common pollutants in nature [7]. Lead exposure during pregnancy produces embryotoxic and teratogenic effects [13].

We hypothesized that free radical processes play an important role in the pathogenetic mechanisms of prenatal brain injury [4].

However, little is known about activity of the antioxidant system in fetal organs during chronic prenatal intoxication with lead.

In the present work, activity of one of the major antioxidant enzymes, Cu/Zn superoxide dismutase (COD), was measured in the brain tissue and liver of rat fetuses after intrauterine exposure to $Pb(NO_3)_2$ in various concentrations.

MATERIALS AND METHODS

Experiments were performed on female Wistar rats. The rats of treatment groups received $Pb(NO_3)_2$ in concentrations of 0.3 (groups 1 and 2) and 3.0 mg/liter (groups 3 and 4) as the sole source of fluid. Groups 1 and 3 received $Pb(NO_3)_2$ over 1 month before pregnancy and during pregnancy. Groups 2 and 4 received $Pb(NO_3)_2$ over 5 months before pregnancy and during pregnancy. Table 1 illustrates daily lead consumption. Control animals received distilled water.

Plasma lead concentration in females was measured by the method of non-flame atomic absorption spectroscopy and electrothermal atomization on an AAS-3 device (Karl Zeiss) equipped with a E TA-3 electrothermal atomizer [11].

Fetuses were removed from pregnant females on day 20 of pregnancy under combined anesthesia (intramuscular injection of 30 mg/kg calipsol and 2 mg/kg relanium). SOD activity was measured in brain and liver samples [3,5]. The enzyme activity assay is based on the ability of SOD to inhibit epinephrine autooxidation in the alkaline medium [10]. The amount of SOD inhibiting epinephrine autooxidation was taken as a unit of enzyme activity. SOD activity was expressed in arb. units per mg protein. Protein content was measured by the method of Lowry.

The significance of differences was evaluated by Student's *t* test.

RESULTS

Blood lead concentration in control rats was 161.6± 6.31 μg/liter, which is consistent with published data [6]. Blood lead concentration in females of treatment groups surpassed the control by 26.5 (groups

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1 and 2) and 51.8% (groups 3 and 4). We conclude that lead accumulation in the blood from pregnant rats of treatment groups is a dose-dependent process, which reflects the amount of lead consumption by these animals. These results are supported by published data [8].

Lead easily crosses the placental barrier and accumulated in target embryonic tissues (primarily in the brain and liver) [13].

SOD activity in the brain of fetuses from groups 1-2 (administration of $Pb(NO_3)_2$ in a concentration of 0.3 mg/liter) and 3-4 (administration of $Pb(NO_3)_2$ in a concentration of 3.0 mg/liter) decreased by 29.5, 32.0, 21.2, and 39.4%, respectively, compared to the control. Hence, the effect of $Pb(NO_3)_2$ did not depend on its dose. No intergroup differences were revealed in enzyme activity.

Various changes in SOD activity were observed in liver tissue from fetuses of treatment groups. SOD activity in liver tissue tended to increase in group 1 fetuses (by 13.2%) and significantly increased in group 2 fetuses (by 31.1%) after administration of $Pb(NO_3)_2$ in a concentration of 0.3 mg/liter.

SOD activity in liver tissue decreased in fetuses of groups 3 and 4 receiving $Pb(NO_3)_2$ in a concentration of 3.0 mg/liter (by 15.7 and 37.5%, respectively, compared to the control).

Hence, SOD activity in the brain significantly decreased in fetuses of treatment groups. The increase in SOD activity in the liver in groups 1 and 2 reflects activation of this enzyme in response to increased production of free radicals. Previous experiments showed that SOD produces a hepatoprotective effect during toxic liver injury, which is related to antioxidant, antiinflammatory, and membrane-protective properties [1].

The decrease in SOD activity in the brain of treated fetuses, as well as in the liver of group 3 and 4 fetuses, suggests that prenatal lead exposure impairs the antioxidant defense system. These changes potentiate the damaging effect of free radicals on tissue structures. The toxic effect of lead is partly due to substitution of polyvalent cations (Zn, Ca, and Mg) in metal-binding proteins and inhibition of their activity [6,7]. The decrease in enzyme activity is probably associated with substitution of Zn for Pb in the binding site of Cu/Zn superoxide dismutase. Our hypothesis is consistent with published data that substitution of Zn for Pb in active site of δ-aminolevulinic acid hydratase inhibits enzyme activity [12]. It cannot be excluded that SOD activity decreases due to the influence of free radicals and peroxides. It should be emphasized that females of treatment groups were subjected to lead treatment before and during pregnancy, i.e. the fetus developed under conditions promoting the formation of free radicals against the background of suppressed antioxidant defense system. Published data show that lead-induced damage to mitochondria contributes to free radical generation [9]. We found that the brain is more sensitive to lead than the liver. This conclusion was derived from the significant decrease in SOD activity in the brain of fetuses from all treatment groups. These changes did not depend on the duration of intoxication and dose of lead (Table 1). Our results are confirmed by published data, which show that lead causes most severe damage to developing central nervous system [6].

There is a theoretical possibility that inorganic nitrate $Pb(NO_3)_2$ can undergo transformation into NO, which produces a prooxidant effect and inhibits SOD. However, the formation of NO from $Pb(NO_3)_2$ is catalyzed by metallic copper, which is absent in mammals. Other reactions of NO formation from inorganic nitrate $Pb(NO_3)_2$ proceed at temperatures, which cannot be observed in the living organism [2]. Moreover, the NO_3^- anion formed from inorganic nitrates possesses no oxidative properties in near-neutral media. Previous studies showed that chronic exposure of mice to lead acetate is followed by a significant decrease in NO concentration in brain tissue during postnatal ontogeny [14].

TABLE 1. Effect of Lead Consumption by Female Rats on SOD Activity in Fetal Brain and Liver ($M \pm m$)

Group	SOD activity, arb. units/mg protein		Mean daily lead consumption, μg/kg
	brain	liver	
Control	34.06±1.03 (8)	46.43±1.60 (8)	
Treatment group 1	24.00±0.97** (8)	52.58±2.56 (8)	26.7
Treatment group 2	26.83±3.13* (6)	39.15±2.21* (8)	270.2
Treatment group 3	23.23±0.47** (11)	60.88±1.92**(11)	26.6
Treatment group 4	20.64±1.75** (8)	29.03±1.50** (8)	250.3

Note. *$p<0.05$ and **$p<0.001$ compared to the control. Number of animals is shown in parentheses.

Our study showed that long-term administration of $Pb(NO_3)_2$ to female rats is followed by impairment of SOD activity. These changes are accompanied by a decrease in glucose-6-phosphate dehydrogenase activity in the placenta, kidneys, and lungs and appearance of morphological signs for damage and inhibition of tissue growth and differentiation. These data confirm the fact that free radical processes play an important role in the mechanisms of prenatal disorders [4].

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