Morphometric analysis of brain lesions in rat fetuses prenatally exposed to low-level lead acetate: correlation with lipid peroxidation

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Summary. The effect of prenatal lead acetate exposure was studied microscopically together with the concentration of lead and lipid fluorescent products (LFP) in the brain of rat fetuses. Wistar rats were intoxicated with a lead solution containing either 160 or 320 ppm of lead acetate solution during 21 days through drinking water. The control group (ten rats) received deionized water for the same period. The rats were killed on gestation day 21 and fetuses were obtained; the placenta, umbilical cord and parietal cortex (Cx), striatum (St), thalamus (Th) and cerebellum (Ce) were collected for measuring tissue lead concentration, LFP as an index of lipid peroxidation and histopathologic examination. Lead contents were increased in placenta, umbilical cord, St, Th and Cx in both lead-exposed groups. Lead exposure increased (LFP) in placenta and umbilical cord, St, Th and Ce as compared to the control group. Histopathological examination showed severe vascular congestion in placenta, the Cx, St, Th and Ce with hyperchromatic and shrunken cells. Interstitial oedema was found in all regions studied of both lead exposed groups. The morphometric evaluation of the studied brain regions showed an absolute decrease in total cell number and increased number of damaged cells and interstitial oedema. Our results show that morphological changes in rat brain are correlated with increased lipid peroxidation, and the lead levels of the umbilical cord, however it is not clear whether oxidative stress is the cause or the consequence of these neurotoxic effects of lead.

Key words: Lead, Fetuses, Brain, Lipid Peroxidation, Placenta, Umbilical cord

Introduction

Lead (Pb)-induced neurotoxicity in children is still a major public health problem both in environmental and occupational settings. The central nervous system (CNS) is one of the most vulnerable organs to lead toxicity and it is known from numerous studies that this is particularly true in children (Davis et al., 1993; Adonaylo et al., 1999). During pregnancy the maternal blood Pb levels increase about 15-20% (Angle, 1994; Gulson et al., 1997; Antonio et al., 1999). Pb also crosses the placental barrier readily as early as the 12th week and the fetus is thus exposed to maternal circulating Pb levels during neurodevelopment (Enhart, 1992; Antonio et al., 2003).

The findings with respect to prenatal lead exposure and preterm birth are inconsistent; some studies have found an inverse correlation between blood lead levels and gestational age (Burdette and Goldstein, 1986; West et al., 1994) and an increase in the frequency of preterm birth in areas highly contaminated with lead (Flowler et al., 1993). The umbilical cord plays a paramount role in intrauterine existence because it is the only connection between the fetus and mother (Robin et al., 2003). Although umbilical cord Pb levels are lower than in maternal blood, Pb transfer from the mother to the fetus has been well documented (Antonio et al., 2003). Previous findings suggest that there is a correlation between maternal blood lead and umbilical cord lead (Hernandez-Avila et al., 1997), and that there is a significant association between low levels of lead exposure and deficits in neurobehavioral development in infants (Needleman, 1990)

Various biological markers of dose have been used or proposed as umbilical cord blood lead level, maternal blood level at different time during pregnancy, amniotic fluid lead level and the concentration of lead in cord or placenta tissue, the biomarkers of prenatal lead exposure used in previous prospective studies of lead and child development has been the concentration of lead in

whole, blood, sample either from the umbilical cord of the time of delivery or from maternal venous blood at various point during pregnancy (Gomaa et al., 2002).

It is clear that a biomarker of prenatal lead exposure is required to assess the risk of adverse health effects. Other authors have proposed the placenta and umbilical cord as a biomarker that could be taken as an alternative to repeated maternal blood sampling for assessing lead exposure (Falcon et al., 2003).

Perinatal exposure to low levels of Pb is particularly dangerous for the developing central nervous system due to the lack of a functional blood-brain barrier and the intense cellular proliferation, differentiation and synaptogenesis that takes place during gestation (Goyer, 1993). On the other hand, neurotoxicity associated with lead exposure may result from a series of small perturbations in brain metabolism and, in particular, of oxidative stress (Villeda-Hernández, 2001). Among the mechanisms that produce cell damage after lead intoxication, oxidative damage and lipid peroxidation in the brain have been frequently reported (Ding et al., 2000; Ercal et al., 2000).

The neuropathological effects of lead by light microscopy showed gliosis and demyelination of nerve fibers, increase of oligodendrocytes around the neuron of several brain regions, but lead exposure also retards transition of young postmitotic neurons into adult neurons, the alteration in neurogenesis are accompanied by an enhanced gliogenesis since a higher proportion of astroglial cells. (Soltaninejad et al, 2003; Jaako-Movits et al., 2005), the reduction in the number of mature neuron might result in the decreased level of the neuronal plasticity to the mature brain (Hirano and Iwata, 1989; Jaako-Movits et al, 2005). Studies in cerebellar explants exposed to lead through the host organism showed histopathological and physiopathological damage, and evidence for abnormal developmental and significant delays in maturation of Purkinje cells (Hirano and Iwata, 1989; Needleman, 1990). Also, somatic processes are delayed up to 10 days and longer in lead-treated rats (Wells et al., 1976; Hirano et al., 1989. Morphologic changes are increased in size and numerical density of mossy fibers and cells, and cell density decreased (Hirano and Iwata, 1989; Robin et al., 2003).

In this study, the effects of prenatal lead administration were studied microscopically and biochemically in the brain regions, placenta and umbilical cord of rat fetuses. The aim of this the work was to search for possible correlations between the lesions observed in the placenta and umbilical cord with lesions already present in the brain regions that allowed us to propose the use of placenta and umbilical cord tissues as surrogate indicators of possible brain damage.

Materials and methods

Chemicals

Lead acetate, methanol, chloroform and formaldehyde were obtained from Mallinckrodt, J.T. Baker (Mexico). Some staining reagents were obtained from Sigma Chemical Co. (St Louis, MO.). All other chemical were obtained from E. Merck (Mexico). Deionized water (Milli R/Q System, Millipore) was used for preparation of all reagents and solutions.

Animal treatment

Thirty pregnant Wistar rats, NIH bred in-house strain, weighing 200-250 g and their fetuses (45 controls and 90 treated) were used on gestation day 21. The rats were fed a standard chow diet (Purina Chow) ad libitum and had free access to water. Room darkness was maintained between 19:00 and 07:00 h, room temperature was kept at 25°C, and relative humidity was 40%. Rats were housed individually and were mated during the night. The morning on which a sperm plug was found was designated day 0 of gestation. Twenty pregnant female Wistar rats were treated with a solution containing either 160 or 320 ppm (ten rats each group) of lead acetate during the 21 days of gestation through their drinking water. The control group (ten rats) was treated with deionized water for the same period. On day 21 of gestation, rats from both groups were killed by decapitation, the placentas and the umbilical cords were removed from membranes, and fetuses were obtained to extract the following brain regions: cortex, striatum, thalamus and cerebellum were dissected out according to the described technique (Glowinski and Iversen., 1966).

Lead analyses

Animals were euthanized by decapitation for analyses. Lead in the blood, as well as in the placenta, umbilical cord, parietal cortex (Cx), corpus striatum (St), thalamus (Th) and cerebellum (Ce) were analyzed by graphite furnace atomic absorption spectrophotometry (GFAAS), according to previously reported methods (Miller, 1987). All laboratory glassware, polypropylene tubes, and disposable micropipette tips were immersed for several hours in 1:1 v/v concentrated HNO_3/H_2O, thoroughly rinsed in deionized water, and nitrogen gas dried before use, to avoid any possible contamination. Blood samples (200 ml) were added to 800 ml of HNO_3 Suprapur (E. Merck), centrifuged at 15000 rpm for 15 min, and 100-ml aliquots were taken from the clear solution and diluted (1:5 v/v) with deionized water.

The placenta, umbilical cord, and brain regions were dissected out according to the described technique (Glowinski et al., 1966) Tissue samples were carefully weighed, of placenta (mean 0.176 w/w), umbilical cord (mean = 0.04 w/w), brain regions Cx (means= 0.0290 w/w), S (mean=0.0156 w/w), Th (mean= 0.187 w/w) and Ce (mean=0.245 w/w), placed in the polypropylene tubes, and digested in 0.5 ml of concentrated HNO_3 Suprapur (E. Merck) in a shaking water bath at 60°C for 30 min. This treatment ensures complete destruction of organic matter without lead loss (Christian, 1969). After digestion, a 100 µl aliquot was taken from the clear

solution and diluted (1:5 v/v) with 400 µl of deionized water. Calibration curves were constructed by adding known amounts of lead standard (E. Merck). Analysis of diluted samples of blood and the brain regions were injected into the atomic absorption spectrophotometer (Perkin-Elmer model 3110) with furnace (HGA-600) and autosampler (AS-60) adjusted to a wavelength of 283.3 nm (Perkin-Elmer, Norwalk, CT, USA). Blood lead (Pb). Results were expressed as mg of Pb/dl blood and content of (Pb) in brain regions was expressed as mg of lead/g tissue weight.

Assay of lipid fluorescent products (LFP)

The formation of lipid-soluble fluorescence (an index of lipid peroxidation) was monitored using the described technique (Press, 1977; Santamaría and Rios, 1993). The placenta (0.316 w/w), umbilical cord (0.122 w/w and brain regions (Cx X= 0.244 w/w, St X= 0.199 w/w, Th X=0.226 w/w, Ce X= 0.403 w/w were homogenized in 2.5 ml of ice-cold saline solution (0.9% NaCl). One ml aliquots of the homogenate were placed into glass tubes with 4.0 ml of chloroform-methanol (2:1 mixture). The tubes were capped, gently mixed and placed on ice for 30 min to permit phase separation. The aqueous phase was discarded and 900 µl of the chloroformic layer was transferred into a quartz cuvette, and 100 µl of methanol added. Fluorescence was measured in a Perkin-Elmer LS 50B fluorescence spectrophotometer at 370 nm of excitation and 430 nm of emission. Sensitivity was adjusted to 140 fluorescence units with 0.1 mg/l quinine standard prepared in 0.05 M aqueous sulfuric acid solution prior to the measurement of the samples. Final results were expressed as fluorescence units per milligram of protein.

Protein measurement

The protein concentration was assayed in 1 ml aliquot of the homogenate used for LFP and was estimated by the method described (Lowry, 1951) using bovine serum albumin as a standard. Results of lipid peroxidation were corrected by protein content in each sample.

Histopathological study

On day 21 of gestation the rats from both groups were sacrificed by i.p. injection of 0.3 ml of 3.5% chloral hydrate and then perfused through the heart with cold saline solution, followed by 4% w/v paraformaldehyde solution at 4°C. The placenta, umbilical cord and the brain were dissected out and kept in the same fixative for 15 days. The tissue samples were processed separately in Histokinette 2000 (Reichert Jung) with a program specific for brain, placenta and umbilical cord. The tissues were embedded in paraffin, and sectioned to 5µm thin slices. We applied hematoxilin-eosin, Masson's trichrome, and

Bielschowsky (Luna, 1995) for quantitative assessment of lesions. The placenta, umbilical cord and brain regions were then examined with a Zeiss light photomicroscope.

Morphometric evaluation

Morphometric measurements were applied in all brain regions. The morphometric parameters were measured following the "random systematic sampler". The details of both sampling strategies are described elsewhere (Weis, 1991). Five sections per brain region and per animal were evaluated in both treatment groups, each section was 5 µm thick, was applied to coronal sections stained with hematoxilin-eosin and Bielschowsky to measure the number of cells, either preserved, damaged or the total and interstitial edema was quantified. The general scoring criteria were damaged neurons with included nuclei that were rather intensely stained, cytoplasmic vacuolation scarcely separated from surrounding cytoplasm, shrunken perykaria and neuronal atrophy. The sections were examined with a light microscope, Leica DMLS (Leica, Germany). A halogen light source connected to a stabilized, adjustable power supply (12 V, 100 W) provided illumination. A tree-channel system (RGB) color Sony video camera (SSC-DC14, Japan) was connected to the microscope through an adapter with color neutral optics. A true-color image analyzer, IM1000 (Leica, Cambridge, UK) was used.

Statistical analyses

After checking for the assumption of normality and homogeneity of variances, Statistically analysis are expressed as the mean ± S.E.M and analyzed with one-way ANOVA to determine differences between control and experimental groups, followed by Tukey's multiple comparison test were using Graph Pad Prism (version 4.00) soft-ware (San Diego; CA, USA). Values of $p < 0.05$ were chosen for the level of significance. We also examined the correlation between the lead levels of brain regions with LFP of the umbilical cord and LFP of the placenta. Also was correlated LFP of the brain regions with lead levels of the umbilical cord and of the placenta.
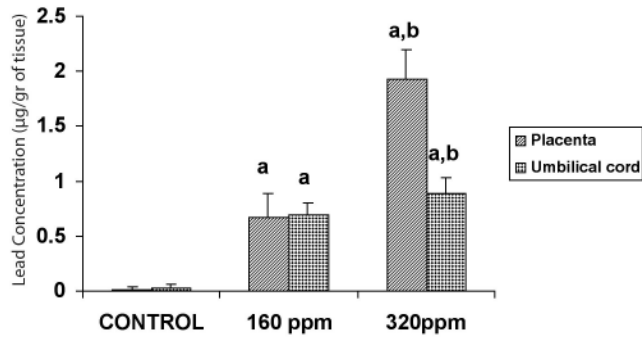
Results

Tissue lead concentration

The content of lead in placenta and umbilical cord of animals exposed to 160 ppm was increased in placenta=4-fold and in umbilical cord=2-fold, respectively, while those treated with 320 ppm showed in placenta=5-fold and in umbilical cord=2-fold increases compared with control group values (Fig. 2). The content of Pb in brain regions of the groups of fetuses exposed to 160 ppm showed an enhance of
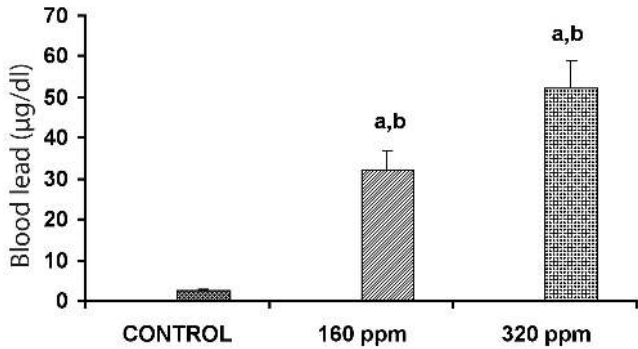
Fig. 1. Lead levels in the blood of rat dams prenatally exposed to either 160 or 320 ppm of lead acetate. Each bar represents the mean ± SEM of 10 independent experiments. [a]p< 0.05 differences vs. control, [b]p<0.05 160 ppm different vs. 320 ppm with respect to the control group analysis of ANOVA followed by Tukey's test.

Fig. 2. The lead concentration in placenta and umbilical cord of rats exposed to 160 and 320 ppm of Pb in drinking water during 21 days of gestation. Results are means±S.E.M. of n=10 independent experiments. [a]p < 0.05 differences vs. control; [b]p<0.05 160 ppm different vs. 320 ppm. ANOVA followed by Tukey's test.
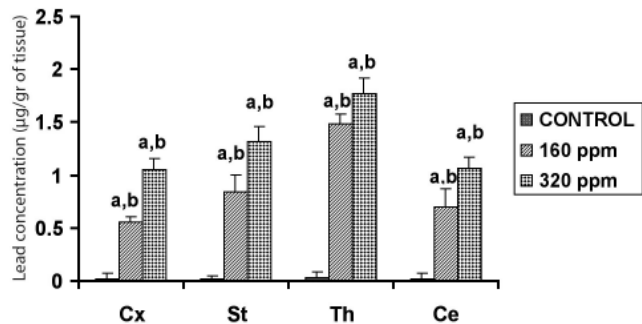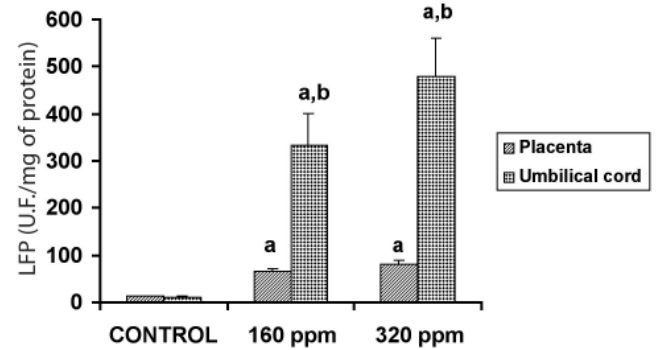

Fig. 3. The lead concentration in Cortex (Cx), Striatum (St), Thalamus (Th) and Cerebellum (Ce) of rats exposed to 160 and 320 ppm of Pb in drinking water during 21 days of gestation. Results are means ± S.E.M. of n=10 independents experiment [a]p<0.05 differences vs control; [b]p<0.05 160 ppm different vs 320 ppm. ANOVA followed by Tukey's test.

Cx=22-fold, St=32-fold, Th=20-fold and Ce=24-fold the lead levels respectively compared with control group values, while at 320 ppm, the increase was Cx=41-fold, St=41-fold, Th=31-fold, and Ce=35-fold respectively in the Pb treated rats as compared with the control group (Fig. 3). There is a direct correlation between the lead levels of the brain regions and the LFP of the umbilical cord and the placenta.

Effects of lead on lipid peroxidation

Lipid fluorescence products (LFP) of the rats exposed to 160 ppm of lead, was increased in the placenta=1-fold and the umbilical cord=3-fold respectively, while at 320 ppm of lead exposure, the increase was in placenta 2-fold and in umbilical cord 4-

Fig. 4. The formation of lipid soluble fluorescence products (LFP) in placenta and umbilical cord of rats exposed to 160 and 320 ppm of Pb in drinking water during 21 days of gestation. Results are means ± S.E.M. of n=10 independent experiment, [a]p<0.05 differences vs. control; [b]p<0.05 160 ppm different vs. 320 ppm. ANOVA followed by Tukey's test.
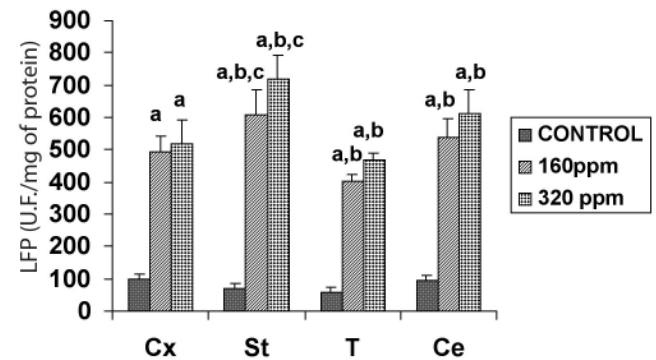
Fig. 5. The formation of lipid soluble fluorescence products (LFP) in Cx, St, Th, and Ce of rats exposed to 160 and 320 ppm of Pb in drinking water during 21 days of gestation. Results are means ± S.E.M. of n=10 independent experiments. [a]p< 0.05 differences vs control; [b]p< 0.05 160 ppm different vs 320 ppm; [c]p < 0.05 different vs regions. ANOVA followed by Tukey's test.

fold (Fig. 4) vs. control group values. The brain regions in the animals treated with the 160-ppm dose were increased Cx=3-fold, St=2-fold, Th=1-fold, and Ce=3-fold, while at 320 ppm in the same regions, the increase was Cx=3-fold, St=3-fold, Th=2-fold and Ce=3-fold in the Pb-treated rats respectively, as compared with the control group values (Fig. 5). There is a direct correlation between the LFP of the brain regions and the lead levels of the umbilical cord and the placenta.

Histopathological analyses

Histopathological examination of the brain regions (parietal cortex, striatum, thalamus, as well as in
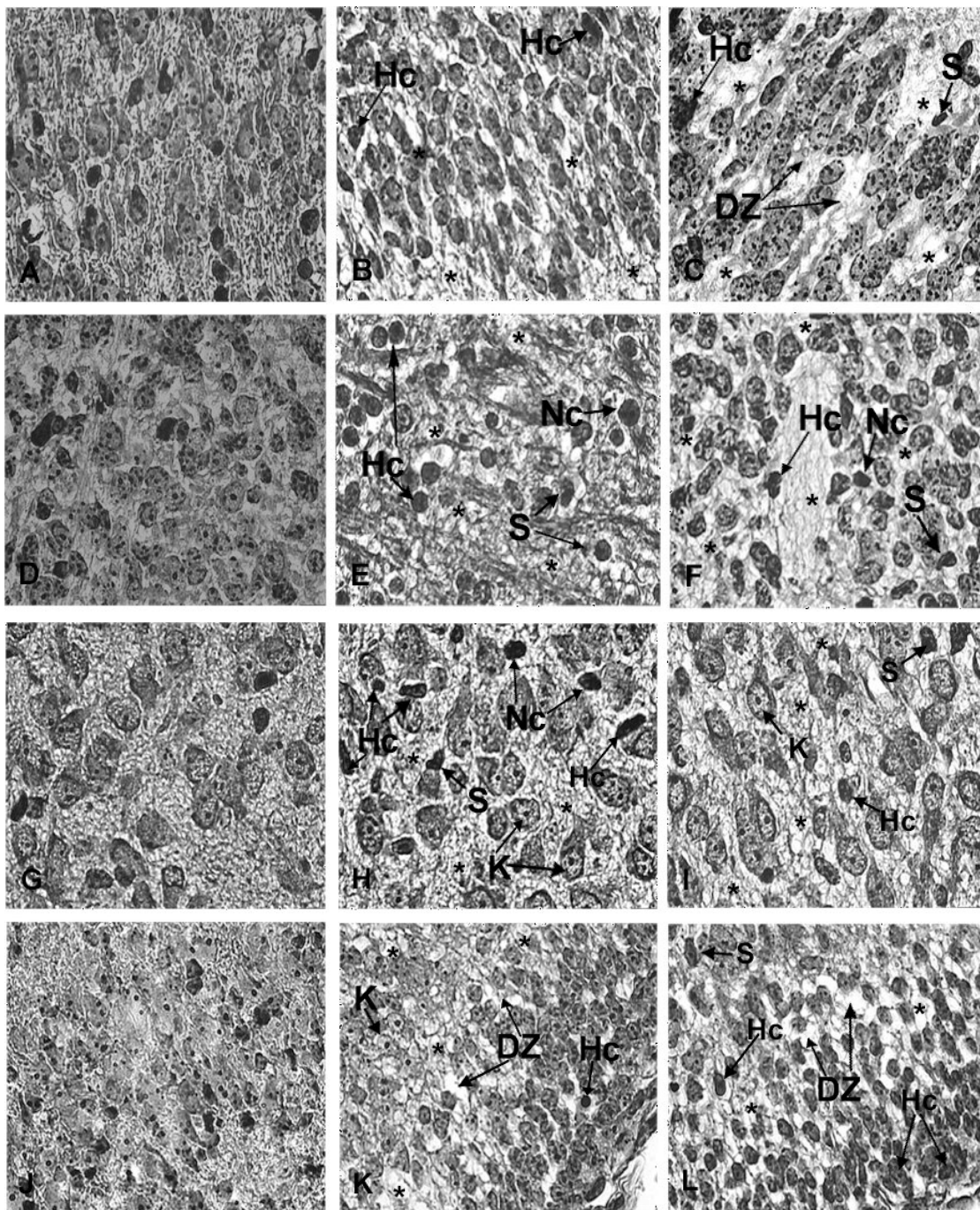
Fig. 6-I Photomicrographs showing the normal appearance of cell bodies and neuropil of **A)** Cx, **D)** St in fetuses of the control group. **B)** Cx, **E)** St represents the group exposed to 160 ppm of Pb, and **C)** Cx, **F)** S correspond to the group exposed to 320 ppm of Pb. Hyperchromatic (Hc) and shrunken (S) cells condensed nuclei (Nc) and devastation zone (DZ) are shown, and the neuropil with interstitial edema (asterisks) in all regions of both treated groups. (Bielschowski, x 20)

Fig. 6-II. Photomicrographs showing the normal appearance of cell bodies and neuropil of **G)** Th, and **J)** Ce in fetuses of the control group, **H)** Th and **K)** Ce represent the group exposed to 160 ppm of Pb. **I)** Th and **L)**. Ce correspond to the group exposed to 320 ppm of Pb. Hyperchromatic (Hc) and shrunken (S) cells, condensed nuclei (Nc), karyorrhexis (k) and devastation zone (DZ) are shown, and the neuropil with interstitial edema (asterisks) in all regions of both treated groups. (Bielschowski, x 20).

cerebellum) of control rats did not reveal changes (Figs 6-I A, D, and 6-II G, J). Histological analysis in the rat fetuses treated with lead acetate at 160 ppm was as follows: the parietal cortex with few shrunken, hyperchromatic cells. In the group treated with 320 ppm, the same lesions were present but with higher intensity and large devastation zones and condensed nuclei, interstitial edema was present in both treated groups (Figs. 6-I B and 6-I C). The striatum showed few hyperchromatic cells in both lead-treated groups and the same lesions as in cortex (Figs. 6-I E and 6-I F). The immature cells of thalamus and cerebellum showed hyperchromatic some of them shrunken and destroyed fibers in the two groups, and karyorrhexis in the thalamus and devastation zones in cerebellum (figs. 6-II

H, 6-II I and 6-II K, 6-II L). Interstitial edema was observed in all regions studied from both lead-exposed groups (Figs. 6-I B, 6-I C, 6-I E, 6-I F, 6-II H, 6-II I, 6-II K, 6-II L).
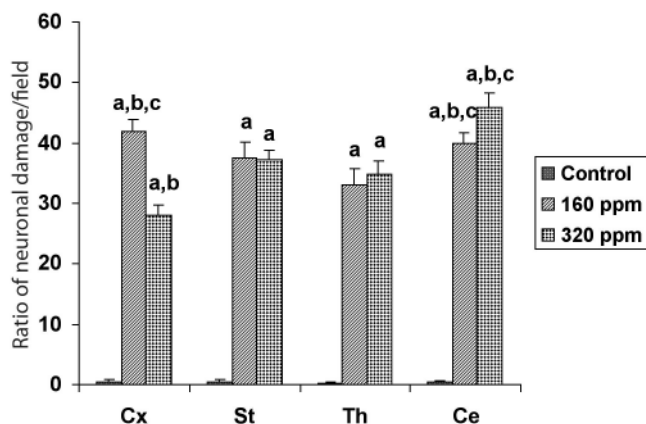
Morphometric analyses

There was no cell death in the group administered with deionized water. The groups treated with 160 and 320 ppm Pb revealed extensive cell loss and atrophy in Cx, St, Th, and Ce in rat fetuses exposed to lead acetate at 160 and 320 ppm. Figure 7 shows the effects of Pb on the ratio of cells damaged per field. Figure 8 shows that interstitial edema increased in all brain regions of rats treated with 160 and 320 ppm as compared to the control group. These findings were statistically significant Cx and Ce. Total numerical neuron density was not significantly changed in the striatum and thalamus between the treated groups, however, all brain regions showed elevated levels of lead and high LFP. We consider that there was no significant direct correlation between neuron death and the content of lead in the brain regions. But it is important to take into account that the pathological changes in brain regions may be related to increased LFP detected in the brain regions and the content of lead in the placenta and umbilical cord.
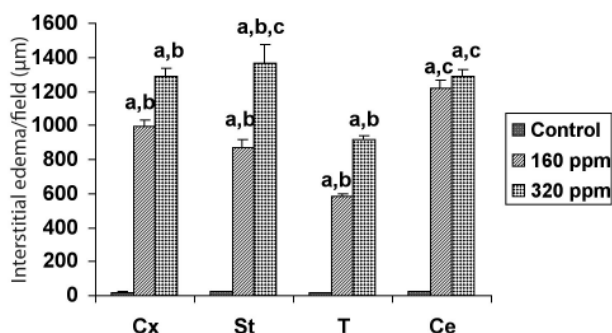

Fig. 7. The ratio of neuronal damage per field in Cx, St, Th, and Ce of rats exposed to 160 and 320 ppm of Pb in drinking water during 21 days of gestation. Results are means ± S.E.M. of n=10 fields per section. [a]p<0.05 differences vs control; [b]p<0.05 160 ppm different vs 320 ppm; [c]p<0.05 different vs regions. ANOVA followed by Tukey's test.

Discussion

The aim of the present study was to assess the effects of lead exposure in several brain regions of the fetuses of rats exposed during pregnancy to low levels of Pb, and were correlated with oxidative damage in the placenta and umbilical cord, also was examined the lipoperoxidation of brain regions associated with the lead levels of the umbilical cord and the placenta.

A variety of biological markers of dose have been used or proposed, including umbilical cord lead level, maternal blood level at different times during pregnancy, amniotic fluid lead level, and the concentrations of lead in umbilical cord or placental tissues (Korpela et al., 1986). In our study examined the lead level of such markers (umbilical cord tissue and placental tissue), because the kinetics of lead in the maternal-fetal unit are incompletely understood (Gomaa et al., 2002) These correlation suggest that these biological markers provide largely nonredundant information about fetal exposure. It is important the utility of such biomarkers be compared as predictors of fetal risk for adverse health outcomes. Our results report a significant association between lead levels found in the umbilical cord, the placenta and brain regions of fetuses treated with 160 and 320 ppm of lead. The present study are supported by previous studies (Furman and Laleli, 2001; Gomaa et al., 2002) they compared umbilical cord lead levels, maternal blood lead levels and maternal blood lead levels. In this research lead concentration in umbilical cord are significantly correlated with a are lower than,

Fig. 8. Interstitial edema per field in, Cx, St, Th, and Ce of rats exposed to 160 and 320 ppm of Pb in drinking water during 21 days of gestation. Results are means ± S.E.M. of n=10 fields per section. [a]p<0.05 differences vs control; [b]p<0.05 160 ppm different vs 320 ppm; [c]p<0.05 different vs regions. ANOVA followed by Tukey's test.

concentrations in maternal venous blood, Our results demonstrate that pre-natal lead exposure induces a significant increase in the lipid peroxidation and lead high levels were associated with the morphological changes in immature neurons of the Cx, St, Th and Ce, in placenta and umbilical cord were observed. This findings support that the placenta is not a very effective biological barrier for hinder much of the lead transport.

Rat dams blood Pb levels in the intoxicated group ranged between 32.3 µg/dl and 52.4 µg/dl (Fig. 1), these values are comparable to those found in the brain regions of fetuses of rats after treatment with 160 and 360 ppm. Previous studies have shown an increase in Pb concentrations in the placental body (Invengar and Rapp, 2001) and have observed a strong correlation between maternal and umbilical cord lead levels (Hernández-Avila et al., 1997). Our results indicate that lead is highly concentrated in brain, placenta and umbilical cord with both treatments. We believe that the placenta and umbilical cord could be good biomarkers of fetal lead exposure.

Pb exerts its most severe consequences in the developing brain due to intense cellular proliferation, differentiation and synaptogenesis. Moreover, the developing organism presents a five-fold higher absorption of Pb (Kern et al., 1993; Moreira et al., 2001 a,b), lead exposure in embryonic brain also show that not only the proliferation was retarded, also affected the maturation and the migration of the immature cells (Jaako-Movits et al., 2005). Previous studies have shown that lead is capable of causing oxidative stress in liver, kidney and brain (Daggett et al., 1998; Adonaylo et al., 1999). There is some data demonstrating that in rats exposed to lead (2% w/v lead acetate in drinking water for 10 days) the levels of phospholipids and lipid peroxides in brain is increased (Shafiq-Ur-Rehman, 1984). Previous our studies have demonstrated the enhanced brain regional lipid peroxidation in developing rats exposed to low level (0.01% and 0.05% w/v orally for 45 Days) (Villeda et al., 2001). In our results we show the increase of lipid peroxidation and higher lead levels in rat fetuses pre-natal lead exposure.

Studies in experimental animals have reported that lead and cadmium alter the lipid metabolism and enhance lipid peroxidation, through the inhibition of superoxide dismutase in all brain regions (Sukla et al., 1987; Antonio et al., 2003). Pb treatment is also associated with alterations in the oxidant defense system, this was shown by the higher activities of glutathione reductase and glutathione peroxidase found in the brains from Pb-treated rats, which may reflect a protective response of the tissue to increased oxidative damage associated to Pb neurotoxicity (Adonaylo et al., 1999; Ping-Chi and Yueliang, 2002).

Oxidative damage associated with the presence of lead in the brain has been proposed to indicate a possible role of free radicals in the pathogenesis of lead toxicity (Adonaylo et al., 1999). From our results we can conclude that lead caused dramatic biochemical changes

in all brain regions studied in the immature brain that were exposure to low levels of lead, also demonstrated that, at birth, lead has an important neurotoxic effect, because has the capacity of crossing the placenta and the blood-brain barrier. The increase in peroxidation seems to be localized in specific cerebral areas, indicating that lead may not distribute uniformly within the central nervous system (Mameli et al., 2001).

Lead-induced oxidative damage could result from direct interaction of Pb with biological membranes, inducing lipid peroxidation (Ding et al., 2000; Antonio et al., 2003). In this work, we found that lead causes a dramatic increase in lipid peroxidation mainly in cortex, striatum and cerebellum, which were also the most damaged brain regions after morphometric evaluation. This correlation suggests that free radical over-production is a likely mechanism of cell damage by lead exposure

Morphological changes observed in brain tissue in the present study are supported by previous neuropathology findings showed lesions in the cerebral cortex (Hirano and Iwata, 1989), mainly in areas V1 and V2 of the occipital cortex of monkeys following moderate level exposure (Finkelstein et al., 1998) cytoplasmic vacuolization and hyperchromatic cells, chromatolysis and interstitial edema (Hirano and Iwata, 1989). We also found light microscope lesions in the four brain regions of fetuses perinatally exposed to Pb, which included, shrunken and hyperchromatic cells, devastation zone, slight interstitial edema or between fibers mainly in the cortex, striatum, thalamus and Cerebellum of the rat fetuses treated with 320 ppm of Pb. Our results show that morphological changes in rat brain are associated with increased lipid peroxidation, however it is not clear whether oxidative stress is the cause or the consequence of these toxic effects of lead, but lead exposure may increase susceptibility of membranes by altering the major constituents of biological membranes are fatty acid that are good sites for lipid peroxidation reactions and are more sensitive to oxidative damage (Sandhier et al., 1994; Soltaninejad et al., 2003). This is probably a response to the cytotoxic effects of lead and also seems to be related to the stimulation of peroxidative reactions occurring in the cell membrane (Donaldson and Knowles, 1993). As for the cells of other tissues, it has been shown that, in neurons, lead produces an increase in lipid peroxidation, which in turn determines alterations in the composition and function of membranes causing damage malfunction and eventually cell death (Shafiq-Ur-Rehman, 1987).

The content of lead in the placenta and umbilical cord showed a direct correlation with brain morphological changes. The morphometric evaluation suggested absolute increases in the number of damaged cells in all brain regions of both treated groups with Pb and intense interstitial edema, present findings indicated more interstitial than intracellular edema, these changes are associated with lead levels and LFP of the umbilical cord and placenta.

In summary, the results presented in this work showed the toxic effects in the four brain regions of fetuses exposed prenatally to low lead acetate, and that low doses of Pb are able to induce biochemical and morphological changes in the studied brain regions, placenta and umbilical cord. We demonstrated that the morphological changes is associated with increased lipid peroxidation, particularly in the parietal cortex, the striatum and the cerebellum of rat fetuses, suggesting that cellular damage mediated by free radicals is involved in the pathology and abnormalities of the placenta and umbilical cord and is also associated to neurotoxicity induced by low levels of Pb exposure during pregnancy.

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