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The protective role of ascorbic acid on hippocampal CA1 pyramidal neurons in a rat model of maternal lead exposure

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ABSTRACT

Oxidative stress is a major pathogenic mechanism of lead neurotoxicity. The antioxidant ascorbic acid protects hippocampal pyramidal neurons against cell death during congenital lead exposure; however, critical functions like synaptic transmission, integration, and plasticity depend on preservation of dendritic and somal morphology. This study was designed to examine if ascorbic acid also protects neuronal morphology during developmental lead exposure. Timed pregnant rats were divided into four treatment groups: (1) control, (2) 100 mg/kg ascorbic acid once a day via gavage, (3) 0.05% lead acetate in drinking water, and (4) 0.05% lead + 100 mg/kg oral ascorbic acid. Brains of eight male pups (P25) per treatment group were processed for Golgi staining. Changes in hippocampal CA1 pyramidal neurons' somal size were estimated by cross-sectional area and changes in dendritic arborization by Sholl's analysis. One-way ANOVA was used to compare results among treatment groups. Lead-exposed pups exhibited a significant decrease in somal size compared to controls ($P < 0.01$) that was reversed by cotreatment with ascorbic acid. Sholl's analysis revealed a significant increase in apical dendritic branch points near cell body ($P < 0.05$) and a decreased total dendritic length in both apical and basal dendritic trees of CA1 neurons ($P < 0.05$). Ascorbic acid significantly but only partially reversed the somal and dendritic damage caused by developmental lead exposure. Oxidative stress thus contributes to lead neurotoxicity but other pathogenic mechanisms are also involved.

1. Introduction

Lead (Pb), a nonphysiological heavy metal and potent neurotoxicant, has been used by humans since ancient times (Davis et al., 1990). Due to the widespread use of lead in various products, such as batteries and outdoor paint, it is a common environmental contaminant in water and soil (Patrick, 2006). Chronic exposure to low levels of lead is especially toxic to the developing brain. Indeed, chronic lead exposure can produce a variety of cognitive and behavioral deficits in children and experimental animals (Davis et al., 1990). Environmental lead exposure is associated with higher risk of irreversible neurodevelopmental, learning, and behavioral deficits in children (Costa et al., 2004a,b). Neurodevelopmental deficits associated with lead exposure, such as attention deficit hyperactivity disorder and impaired cognitive function, may stem from disruptions in glutamatergic, cholinergic, and dopaminergic transmitter system function (Cory-Slechta, 1995a,b).

Recent findings suggest that the developing brain is particularly sensitive to low levels of Pb from gestation through lactation, leading to lifelong cognitive deficits (Basha et al., 2012; Ryzhavskii et al., 2008), due to the myriad development processes occurring during this period (Bayer et al., 1993). Several clinical studies have demonstrated that increased maternal blood lead during pregnancy and lactation is associated with a significant decrease in subsequent intelligence, attention, and other brain functions in infants and adolescents (Ris et al., 2004; Schnaas et al., 2006). In experimental animals, there is a well-established association between hippocampal dysfunction and behavioral deficiencies following lead exposure (Antonio-García and Massó-Gonzalez, 2008a; Cory-Slechta, 1995a,b; Lukawski and Sieklucka-Dziuba, 2007). Some reports have suggested that lead tends to accumulate selectively in the hippocampus, thereby causing particularly severe morphological and functional damage to hippocampal neurons (Alfano et al., 1983; Bielarczyk et al., 1994; Booze and Mactutus, 1990; Costa et al., 2004a,b). Numerous toxic mechanisms are purposed to explain lead-induced injury to the developing brain (Bellinger, 1994; Gomaa et al., 2002). One current theory, with a large body of supporting evidence, suggests that

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lead-induced oxidative stress contributes to the pathogenesis of lead toxicity by disrupting lipid and protein function (Baranowska-Bosiacka et al., 2011; Hermes-Lima et al., 1991; Monteiro et al., 1985). Lead can both reduce the cellular activity of free radical scavengers such as catalase, superoxide dismutase, and glutathione (Hsu and Guo, 2002) and enhance production of reactive oxygen species (ROS) (Monteiro et al., 1991; Ribarov and Bochev, 1982). Small molecule antioxidants such as ascorbic acid scavenge aqueous ROS by rapid electron transfer and thereby inhibit membrane lipid peroxidation (Patra et al., 2001a). A previous study demonstrated that ascorbic acid administration during pregnancy and lactation reversed lead-induced suppression of antioxidant enzyme activities (Antonio-García and Massó-Gonzalez, 2008b). Neurotransmission and synaptic integration depend critically on somal and dendritic morphology, so we designed the present study to examine if ascorbic acid can protect hippocampal CA1 pyramidal neurons against lead-induced morphological injury.

2. Materials and 'methods

2.1. Animals and drug treatments

Timed pregnant Wistar rats (Pasteur's Institute, Tehran, Iran) were housed individually in plastic cages with free access to food and water. The animal room was maintained at 22–24 °C under a 12-h light/12-h dark cycle. The study was performed according to the guidelines for laboratory animal use and care set forth by the research council at Golestan University of Medical Sciences (Gorgan, Iran). On the 5th day of gestation, dams were randomly divided into four groups of five: Group 1 received distilled water as a control, Group 2 received fresh 100 mg/kg ascorbic acid in saline administered orally once a day, Group 3 received distilled water containing 0.05% lead acetate, and Group 4 received 0.05% lead acetate in drinking water plus ascorbic acid (100 mg/kg in saline) administered orally once a day. Dosing was continued until postnatal day (PND) 25. Solutions were made fresh daily. Water consumption by dams and water consumption and body weight of dams and pups were recorded every 3 days. The dose of Pb^{2+} was based on the results of a preliminary study in which we exposed pregnant rats to 0.05%, 0.75%, and 1% lead acetate in drinking water. The dose of ascorbic acid was chosen according to (Chang et al., 2012). At 0.05%, lead acetate from the 5th day of gestation to PND 25 did not induce severe Pb intoxication, but plasma lead levels were substantially higher in Pb-treated pups compared to those from the control maternal group (Table 1).

At birth, eight pups were left with each dam. On PND 25, eight male pups in each maternal treatment group (32 in total) were used for Golgi staining and seven pups per group (28 in total) to evaluate blood Pb concentrations. Briefly, blood samples were collected by cardiac puncture, immediately centrifuged at 14,000 × *g* for 10 min at 4 °C without anticoagulants, and then stored at −70 °C until analysis using an anatomic absorption spectrometer (AA-7000 Shimadzu Corporation).

2.2. Golgi staining

A modified Golgi – Hortega staining technique was used to assess pyramidal cell morphology in each treatment group (Sutton and Brunso-Bechtold, 1991). Briefly, each pup was deeply anesthetized with chloroform and then perfused transcardially with 50 – 70 ml normal saline. Saline perfusion was followed by 50–100 ml of the first fixative (1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4) and then by 50 – 100 ml of the second fixative (6% potassium bichromate, 6% chloral hydrate, and 10% formaldehyde in 0.1 M phosphate buffer). After perfusion, the brain was removed. Hippocampi were isolated, cut into small blocks, and transferred into a freshly prepared solution of 3.5% potassium bichromate in distilled water for 48 h. The blocks were then washed several times with a 1% silver nitrate solution in distilled water before incubation in 0.75% silver nitrate solution in distilled water for another 48 – 72 h. Tissue blocks were then dehydrated in an ascending alcohol series, cleared in xylene, and embedded in paraffin. Paraffin-embedded blocks were cut into 75-μm sections using a rotary microtome. The tissue sections were floated in xylene to remove paraffin, transferred onto microscopic slides using a paintbrush, and immediately coverslipped. After drying at room temperature, the microscopic slides were photographed using a DP71 digital camera mounted on a BX51 Olympus microscope. The cross-sectional area of all well impregnated pyramidal neurons in CA1 subregions of the hippocampus were measured using using a computer based image analysis system (analysis starter soft imaging solutions, Japan). In each treatment group, 45–52 Golgi impregnated neurons with well-defined dendritic processes were chosen for dendritic analysis using Sholl's concentric circles technique (Ristanović et al., 2006).

2.3. Sholl's concentric circles method

Hippocampal pyramidal neurons from CA1 subregion with well branched dendrites, dark and consistent silver impregnation throughout the dendrites, and that were relatively separated from neighbouring neurons were selected for Sholl's analysis. Sholl's concentric circles were drawn for each neuron using ImageJ software (Fig. 2). The mean number of intersections of dendrite branches with consecutive 10-μm-spaced concentric circles was calculated.

2.4. Statistical analysis

The data was normally distributed (Kolmogorov–Smirnov test) and was analysed using parametric tests.Values are expressed as mean ± SEM. Mean values of each maternal treatment group were treated as single measurements for data analysis. Weight, serum lead levels, cell body size, dendritic length, and Sholl's analysis parameters were compared using one way ANOVA followed by Dunnett's or Tukey's post hoc tests. Values of $P < 0.05$ were considered significant. In Sholl's analysis, the number of concentric ring intersections was used as an estimate of total branch length. Statistical analyses were performed using SPSS 19.

3. Results

3.1. Verification of lead exposure

Serum lead levels in rat pups were measured at PND 25 in all groups. Ascorbic acid, Pb and Pb + ascorbic acid exposure had no effect on the normal weight gain of rat pups (data not shown). However, serum lead levels were significantly higher in the pups of dams administered lead ($P < 0.005$) and this was not reversed by co-administration of ascorbic acid (Table 1).

Table 1
Mean ± SEM of blood leas concentrations.

Groups	Blood lead concentration (μg/dl)
Control	0.78 ± 0.08
Ascorbic acid	0.72 ± 0.05
Pb	28.3 ± 3.2***
Pb + ascorbic acid	26.7 ± 2.1***

Mean ± SEM.
*** $P < 0.005$ compared to the control group.