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The combined effects of developmental lead and ethanol exposure on hippocampus dependent spatial learning and memory in rats: Role of oxidative stress

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ABSTRACT

Either developmental lead or ethanol exposure can impair learning and memory via induction of oxidative stress, which results in neuronal damage. we examined the effect of combined exposure with lead and ethanol on spatial learning and memory in offspring and oxidative stress in hippocampus. Rats were exposed to lead (0.2% in drinking water) or ethanol (4 g/kg) either individually or in combination in 5th day gestation through weaning. On postnatal days (PD) 30, rats were trained with six trials per day for 6 consecutive days in the water maze. On day 37, a probe test was done. Also, oxidative stress markers in the hippocampus were also evaluated. Results demonstrated that lead + ethanol co-exposed rats exhibited higher escape latency during training trials and reduced time spent in target quadrant, higher escape location latency and average proximity in probe trial test. There was significant decrease in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities and increase of malondialdehyde (MDA) levels in hippocampus of animals co-exposed to lead and ethanol compared with their individual exposures. We suggest that maternal consumption of ethanol during lead exposure has pronounced detrimental effects on memory, which may be mediated by oxidative stress.

1. Introduction

Lead (Pb) is a heavy metal which is distributed in the environment. Humans may be exposed to lead due to food, water contamination and air pollution caused by industrial emission (Ahamed and Siddiqui, 2007). The lead has been known to cause damage in the nervous system. The developing central nervous system (CNS) is far more vulnerable to lead's toxic effects than the mature brain (Lidsky and Schneider, 2003). Learning and memory impairments in experimental animals exposed to lead during embryonic and gestational stages have also shown continuous brain damage till adult stage (Goyer, 1996; Rice, 1993; Ruff et al., 1996; Shannon and Graef, 1992). Developmental lead exposure has been found to decrease the induction and amplitude oflong-term potentiation (LTP), the cellular model of learning and memory, in the rat hippocampus (Ruan et al., 1998; Yu et al., 2007). Moreover, morphological analysis shows that there is anobvious reduction in the length of dendritic field and the number of dendritic branches ofhippocampal dentate granule cells after developmental lead exposure (Alfano and Petit, 1982; Petit et al., 1983). Previous studies have reported that developmental lead exposure causes alteration of NMDAR subunit ontogeny and disruption of its downstream signaling (Neal et al., 2011; Toscano and Guilarte, 2005), which are associated with deficits in hippocampal LTP (Nihei et al., 2000).

The disruption of the pro- and antioxidant balance is one mechanism of lead neurotoxicity, which can induce brain injury through oxidative damage to critical biomolecules, such as lipids, proteins, and DNA. Previous studies reported that lead exposure can increase the level of reactive oxygen species (ROS) and oxidative stress in the CNS (Adonaylo and Oteiza, 1999; Bondy, 1992; Hermes-Lima et al., 1991; Monteiro et al., 1985). Because the developing brain is rich in unsaturated fatty acids, it is more vulnerable to ROS which can cause lipid peroxidation. Also, excessive ROS generation is considered a trigger of the programmed cell death by apoptosis (Bussche and Soares, 2011). There

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is evidence that the low micromolar doses of lead can impair CNS function in children and alter synaptogenesis in the neonatal rat brain (Oberto et al., 1996).

Alcohol ingestion is a major lifestyle factor which has been reported to influence lead toxicity. An increased blood lead level was reported in wine drinkers compared with beer or spirit drinkers (Dally et al., 1989). The CNS is one of the major targets for alcohol and neuronal degeneration. One of the mechanisms by which ethanol damages the developing CNS is through generation of ROS and by reducing antioxidant level (Flora et al., 1991; Goodlett and Horn, 2001).

The toxic effects of lead increase by alcohol ingestion in part via increasing its absorption and thus it is assumed alcoholics are more susceptible to the toxic effects of lead (Goyer and Mahaffey, 1972). Ethanol consumption was reported to increase body lead burden and essential metals depletion (Dhawan et al., 1992; Flora et al., 1991). Alcohol is known to cross the blood brain barrier might also facilitate the entry of lead in the brain (Flora and Tandon, 1987). Several researches have shown that consumption of ethanol during gestation and lactation impaired learning and memory (Berman and Hannigan, 2000; Gabriel et al., 2002; Vaglenova et al., 2008).

Learning and memory function is primarily governed by the hippocampus, a region shown to be particularly vulnerable to the teratogenic effects of alcohol (Berman and Hannigan, 2000). Exposure to moderate to high levels of alcohol during development could reduce pyramidal cellnumber in the CA1 and CA3 regions of the hippocampus (Livy et al., 2003). Alcohol exposure at the moderate to high levels during gestation could induce mark alterations in the cytoarchitecture of pyramidal cell dendrites within the hippocampus as well as impaired long term potentiation (LTP) induction in CA1 pyramidal cells (An et al., 2013; Hoff, 1988; Tanaka et al., 1991; Tarelo-Acuna et al., 2000). Previous research has demonstrated reductions in dendritic spine density as well as dendritic branching within the hippocampus, particularly within the CA1 region and the Dentate Gyrusm (Gonzalez-Burgos et al., 2006; Hoff, 1988; Tarelo-Acuna et al., 2000).

The molecular mechanism for the toxic effects of lead and ethanol may differ but both are known to induce oxidative stress, ROS generation, in hippocampus. Increase of brain oxidative stress seems to have an important role in cognitive impairment caused by normal aging and neurodegenerative diseases. Oxidative damage to the synapses in the rat cerebral cortex and hippocampus is reported to contribute to the deficit of cognitive functions (Fukui et al., 2002; Gispen and Biessels, 2000). Administration of antioxidant agents could improvesuch deficits (Komatsu and Hiramatsu, 2000; Markesbery, 1997). With respect to, similar mechanisms of lead and ethanol neurotoxicity (oxidative stress) and contribution of oxidative damage of hippocampal synapse with cognition deficits, we examined the effects of chronic co-exposure to lead and ethanol on markers of oxidative stress in the hippocampus in the weaned pups on postnatal days 21, whose dams were exposed to lead and ethanol during pregnancy and lactation. Also, we examined the effects of chronic co-exposure to lead and ethanol on spatial learning and memory in rat offspring by Morris water maze task.

2. Methods

2.1. Drugs and chemicals

Absolute alcohol, Lead acetate, 2-Thiobarbituric acid (TBA), 1.1.3.3 tetramethoxypropan, nitro blue tetrazolium (NBT), trichloro acetic acid (TCA), were all purchased from Sigma-Aldrich chemicals.

2.2. Hippocampal lead concentration

At the end of the treatment (On PD 21), pups were sacrificed by decapitation under ketamine–xylazine anesthesia and hippocampi were removed. For hippocampus metal determination, wet tissue weight was recorded. After digestion with concentrated nitric acid using a microwave digestion system (model MDS-2100, CEM, USA), samples were brought to a constant volume and determination of tissue lead contents was performed using an atomic absorption spectrophotometer (AAS, Perkin Elmer model AAnalyst 100) (Jacob Cholak and Henderson, 1971).

2.3. Experimental animals and treatment

All experiments were done in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 23–80, revised 1996) and were approved by the research ethical standards for the care and use of animals in Damghan University. Adult female and male Wistar rats were obtained from the breeding colony of the Pasture Institute of Iran. The animals were housed at four animals per cage and acclimated for one week in an animal room and handled for reduce of stress. Animal rooms were maintained at 21 ± 0.5 °C with 45–70% relative humidity with alternating 12 h light and dark cycles with minimal noise. Animals were given water and food ad libitum.

After one-week acclimatization in the laboratory conditions, female rats were housed overnight with males and checked on the following morning for the presence of copulation plugs. The day at which a vaginal plug found was used to define the beginning of gestation (day 0). Pregnant females were individually housed in plastic cages. Pregnant rats were randomly divided into four different treatment groups of 5 rats: control, lead, ethanol, and lead + ethanol co-exposed groups.

Group I was the control group that animals were administered distilled drinking water in place of lead and distilled water by oral gavages once daily. Group II animals were administered only lead acetate (0.2% in distilled drinking water). Group III animals were administered 4 g/kg of ethanol solution in distilled water (38% v/v) by oral gavages once per day. Group IV animals were administered lead (0.2% in distilled drinking water) along with ethanol (4 g/kg; oral gavage).

All groups were treated from the 5th day of gestation until weaning (PD 21). The dose for lead acetate (0.2% in distilled drinking water) and ethanol (38%) was selected on the basis of some previous reports (An et al., 2013; Chang et al., 2006; Gilbert et al., 1996; Vaglenova et al., 2008). At birth, eight pups were left with each dam. Whenever possible, only male rats were kept within the litters and females were kept only if necessary to maintain equal litter sizes. Co-exposure with lead and ethanol was stopped after weaning and 5–6 pups (per group) were killed after the last dose under ketamine-xylasine anesthesia at PD 21. Hippocampi were removed, weighed and were used to evaluate lead levels, MDA levels, SOD, CAT and GPx activities. The other 13 pups (per group) were tested for learning and memory task in Morris water maze on PD 30–37. The design of experiment is shown in Fig. 1.

2.4. Morris water maze (MWM) task

The MWM used in our study was a black circular pool (140 cm diameter, 45 cm high) filled with water (30 cm depth) at 24 ± 2 °C. The pool was divided into four quadrants of equal size. An invisible escape platform (10 cm diameter) was placed in the middle of one of the quadrants (2 cm below the water surface) equidistant from the side wall and middle of the pool. The behaviour of the animal