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Amelioration of cerebellar dysfunction in rats following postnatal ethanol exposure using low-intensity pulsed ultrasound

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ABSTRACT

Background: The neonatal development stage of the cerebellum in rats is equivalent to a human foetus in the third trimester of pregnancy. In this stage, cell proliferation, migration, differentiation, and synaptogenesis occur. Clinical and experimental findings have shown that ethanol exposure during brain development causes a variety of disruptions to the brain, including neurogenesis depression, delayed neuronal migration, changes in neurotransmitter synthesis, and neuronal depletion.During postnatal cerebellar development, neurons are more vulnerable to the destructive effects of ethanol. The effects of low-intensity pulsed ultrasound (LIPUS) on the number of cells and thickness of the cell layers within the cerebellar cortex were examined during the first two postnatal weeks in rats following postnatal ethanol exposure.

Method: Postpartum rats were distributed randomly into six groups. Normal saline was injected intraperitoneally into control animals and ethanol (20%) was injected into the intervention groups for three consecutive days. Intervention groups received LIPUS at different frequencies (3 or 5 MHz), after administration of ethanol. After transcardial perfusion, the rat's brain was removed, and a complete series of sagittal cerebellum sections were obtained by systematic random manner. Photomicrographs were made with Motic digital cameras and analysed using Nikon digital software.

Results: The numbers of granular cells decreased in ethanol-treated rats compared to the control group. LIPUS, administered at (3 or 5 MHz), combined with ethanol administration resulted in a reduction of ethanol's effects. Using 5 MHz LIPUS resulted in significantly higher numbers of granular cells in the internal layer compared to the control rats. Using 3 or 5 MHz LIPUS alone resulted in a significant enhancement in the granular cells of the molecular layer. A significant reduction was seen in the thickness of the external granular layer in ethanol-treated rats.

Conclusion: This study showed that exposure to LIPUS can affect the number of granular cells and thickness of the cell layer within the cerebellar cortex in neonatal rats. LIPUS also could attenuate ethanol toxicity effects on the cerebellum.

1. Introduction

The cerebellum is the motor coordination centre for cognitive processing and sensory discrimination. Alcohol abuse gives rise to cerebellar dysfunctions such as cerebellar ataxia. Children with foetal alcohol spectrum disorder show cerebellar deficits (Luo, 2015; Sullivan et al., 2002; Sullivan and Pfefferbaum, 2005). Neuropathological effects of alcohol consumption include shrinkage of grey matter, enlargement of ventricles, and degeneration of white matter (Harper, 2009; Harper and Kril, 1990; Laas and Hagel, 2000; Samantaray et al., 2015).

Ethanol prevents proper brain development, especially in the cerebellum. The cerebellar neurons with compare to other brain ones are more vulnerable to the damaging effects of ethanol (Kumar et al., 2013). The toxic effects of alcohol on brain are affected by developmental stage, age, dose of ethanol, and duration

Abbreviations: LIPUS, low-intensity pulsed ultrasound; EGL, the external granular layer; MAP, microtubule-associated proteins; Eth, ethanol-treated.

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of ethanol exposure. Alcohol also has destructive effects on the functions of neurons including survival, neurogenesis and cell migration (De la Monte and Kril, 2014). The effects of ethanol are especially pronounced especially during the first postnatal week, when exposure can inhibit brain activity, suppress of neuronal activity, alter synaptic transmission, resulting in damage to the hippocampus (Morton and Valenzuela, 2016; Lebedeva et al., 2015). Other studies have shown that ethanol consumption leads to the depletion of Purkinje cells, granule cells, and deep nuclear cells, in addition to causing deficits in motor coordination. In some studies has shown that prenatal exposure to ethanol can result in cognitive and behavioural deficits known as foetal alcohol syndrome (Green, 2004).

Acute ethanol exposure in neonatal rats causes transient gliosis. Previous studies have shown that during central nervous system development, astroglial cells are important targets in confronting with of ethanol toxicity. Clinical and experimental findings have shown that ethanol exposure during brain development period causes a variety of disruptions in normal brain e.g. neurogenesis depression, delayed neuronal migration, neurotransmitter synthesis alterations, and neuronal depletion. Evidence from in vivo and in vitro researches supports the idea that ethanol exposure changes astrogliogenesis in humans and experimental animals (Guerri and Renau-Piqueras, 1997). The neonatal rat cerebellum development stage is equivalent to a human foetus in third-trimester of pregnancy. In this stage has occurring cell proliferation, migration, differentiation and synaptogenesis (Green, 2004).

The cerebellar cortex is organized in four layers during the first three postnatal weeks: the external granular layer (EGL), the molecular layer, the Purkinje cell layer, and the internal granular layer. Granular cells precursors proliferate in the EGL during the first two postnatal weeks (Bénard et al., 2015).

The biological effects of LIPUS and the human upper auditory range have been studied sporadically since 1917. LIPUS was widely used in industry and interest in its biological effects in medical diagnosis has recently increased rapidly (Warwick and Pond, 1968).It has also been used widely in clinical settings for repairing pseudarthrosis, bone fractures, and micromechanical stress in some tissues, as well as differentiation of certain cells and healing in various soft tissues. Some reports indicate that LIPUS accelerates peripheral nerve regeneration including Schwann cells and injured nerve cells (Jiang et al., 2016; Mundi et al., 2009). Some studies show that LIPUS can give rise to cytomechanical perturbations such as membrane and nucleus disturbances. LIPUS could result in membrane contraction and a concomitant decrease in the volume of nucleus. These results together indicate that LIPUS application could disturb the physical and sub cellular structures in living cells (Fowlkes and Holland, 2000; Hu et al., 2014). The present study examined the possible effects of LIPUS on neuronal amounts and thickness of the cell layer within the cerebellar cortex during the first two postnatal weeks for rats following ethanol exposure.

2. Materials and methods

2.1. Animals

Wistar rats (200–250 g) were prepared from the animal laboratory of the Pasteur Institute, Karaj, Iran. All animal experiments were approved by the Animal Ethics Committee (approval license 911014.91) and kept in accordance with the Ilam university guidelines. They were kept at a standard temperature (22 °C–25 °C) and 12 h light-dark cycles. The rats had free access to tap water and standard food. Then, the animals were distributed randomly into four groups that included two females and one male rat in each cage. Female rats were separated from male ones after vaginal plaque confirmation. Postpartum, neonatal rats were randomly distributed into six groups, with six animals per group.

2.2. LIPUS exposure

For ultrasound exposure, was used of an ultrasound system that has been routinely applied in medical clinics (Ligiq 200 PRO series, General electric's, Solingen, Germany). Poppy rats in the four intervention groups received LIPUS at days 10, 11, and 12 after birth at either 3 or 5 MHz frequencies, at an intensity of 65 mW/cm^2 at the occipital zone for 10-min durations each day. Two groups received intraperitoneal ethanol (Fig. 1).

2.3. Tissue preparation

Animals were anaesthetized with intraperitoneal ketamine 70 mg/kg and xylazine 10 mg/kg, and transcardial perfusion were performed with buffered formaldehyde. The brains were exposed using an incision along the midline of the skull. After that, the

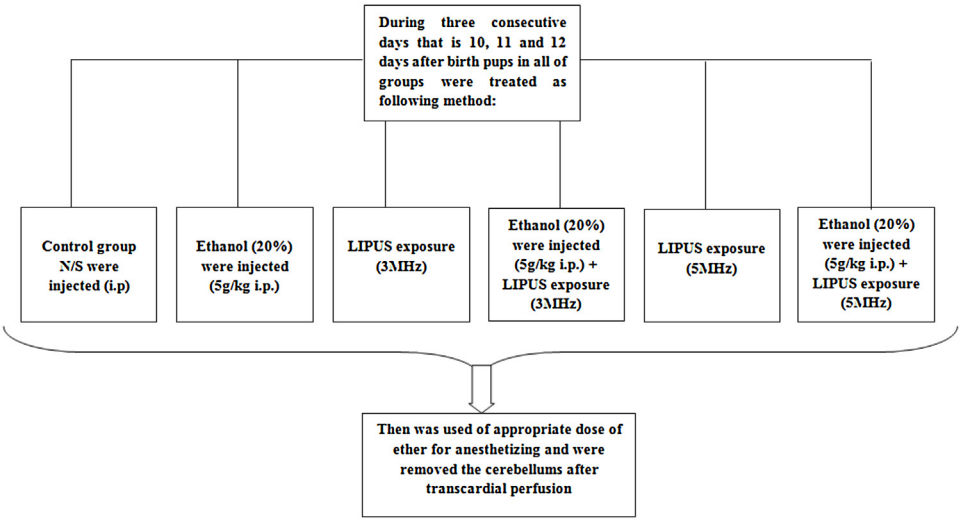

Fig. 1. The experimental protocol.Ethanol was injected in the morning and LIPUS was delivered at the evening for 5 min durations, and n = 6 for each group. Wave intensity was 65 mW/cm^2.