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Aluminum chloride induces neuroinflammation, loss of neuronal dendritic spine and cognition impairment in developing rat

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HIGHLIGHTS

- $AlCl_3$ caused neuroinflammation in hippocampus.
- $AlCl_3$ caused loss of dendritic spine in hippocampus.
- Loss of spine may result in cognition impairment of $AlCl_3$-treated rats.

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ABSTRACT

Aluminum (Al) is present in the daily life of humans, and the incidence of Al contamination increased in recent years. Long-term excessive Al intake induces neuroinflammation and cognition impairment. Neuroinflammation alter density of dendritic spine, which, in turn, influence cognition function. However, it is unknown whether increased neuroinflammation is associated with altered density of dendritic spine in Al-treated rats. In the present study, $AlCl_3$ was orally administrated to rat at 50, 150 and 450 mg/kg for 90d. We examined the effects of $AlCl_3$ on the cognition function, density of dendritic spine in hippocampus of CA1 and DG region and the mRNA levels of IL-1β, IL-6, TNF-α, MHC II, CX3CL1 and BNDF in developing rat. These results showed exposure to $AlCl_3$ lead to increased mRNA levels of IL-1β, IL-6, TNF-α and MCH II, decreased mRNA levels of CX3CL1 and BDNF, decreased density of dendritic spine and impaired learning and memory in developing rat. Our results suggest $AlCl_3$ can induce neuroinflammation that may result in loss of spine, and thereby leads to learning and memory deficits.

1. Introduction

Worldwide, over 46 million people live with dementia (Martin, 2015). It is estimated that about 75% of these people are affected by Alzheimer's disease (AD) (Morris, 1994). Although the etiological factors of AD are not well understood, many studies suggest Aluminum (Al) is a potential contributing factor (Bhattacharjee et al., 2014; Exley, 2014). Aluminum is an accumulative toxic metal that causes toxic effects on the brain, bone, liver, spleen (Willhite et al., 2014). Brain is the main target organ for Al accumulation (Kaneko et al., 2004). Al can penetrate the blood–brain barrier (Zatta et al., 2002), and accumulate in all brain regions, most being in the hippocampus (Kaur et al., 2006). Epidemiological surveys and animal studies showed that accumulation of Al in the hippocampus causes neurons apoptose, abnormal deposition of β-amyloid and neuroinflammation, resulting in hippocampus-dependent learning and memory ability impairments (Flaten, 2001; Kiesswetter et al., 2009; Wang et al., 2014b; Oshima et al., 2013; Zaky et al., 2013). Al was widely used in water purifiers, food additives and pharmaceuticals (Tony et al., 2008; Fung et al., 2009; Anderson and Berkowitz, 2010; Bondy, 2014; Zhu et al., 2014; Gupta, 2014); it is also present in ambient and occupational airborne particulates (Weinbruch et al., 2010; Boullemant, 2011). In addition, the growing prevalence of acid rain and bauxite mines exploitation can result in the discharge of larger amounts of Al salts

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from insoluble minerals, raising the risk of human contact with Al (Smith, 1996; Borgmann, 2007). Therefore, it is necessary to develop further understanding of the mechanism of Al-induced neurotoxicity.

Neuroinflammation is associated with pathogenesis of learning and memory deficits (Clark et al., 2010; Ze et al., 2014). The hippocampus with abundantly expressed receptors for pro-inflammatory cytokines such as Interleukin-1β (IL-1β), Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) is especially vulnerable to injury and inflammation (Teeling and Perry, 2009). Pro-inflammatory cytokines can impair long-term potentiation (LTP), and inhibit neurotrophins which is important for neuronal survival/function, synaptic plasticity and memory formation (Poo, 2001; Lynch, 2002; Tyler et al., 2002; Pickering and O'Connor, 2007; Tong et al., 2008; Minichiello, 2009). In addition, exaggerated pro-inflammatory cytokine response in the hippocampus is associated with altered density of dendritic spine (Bitzer-Quintero and González-Burgos, 2012; Hu et al., 2014; Le et al., 2014; Zou et al., 2015). Changes in dendritic spines govern alterations in synaptic plasticity, which, in turn, influence learning and memory (Segal, 2005). However, it is unknown whether increased pro-inflammatory cytokines is associated with loss of dendritic spine in Al toxicity exposed rats.

In this study we want to determine whether loss of dendritic spine is associated with the neuroinflammation and the cognition impairment in $AlCl_3$-treated rats.

2. Methods

2.1. Animals and treatment

One hundred and twenty male Sprague Dawley (SD) rats (SPF, three-week old) were purchased from the Yisi Experimental Animal Technology (Jilin, China). The license number was SCXK-2011-00004. The rats were kept in SPF animal laboratory of Northeast Agricultural University under controlled temperatures at 23 ± 1 °C, relative humidity at 55 ± 5% and in a 12 h light/dark cycle (lights on between 08:00 a.m and 20:00 p.m). The rats were kept in individual ventilated cages (Suhang Technology Equipment, China) with wood shavings (Xietong Organism, China). The size of the cages is 470 mm × 315 mm × 260 mm, and large enough for the growth of five rats. The cage was polypropylene PP material and stainless steel wire without aluminum. Throughout the experiment, wood shavings were renewed every three days and all rat were allowed *ad libitum* access to food (Xietong Organism, China) and water. The water, food and wood shavings were sterilized when these were used.

After 72 h of acclimatization, the rats (62±5 g) were randomly divided into the control group and three $AlCl_3$ treatment groups (n = 30 per group). In $AlCl_3$ treatment groups, the rats were treated daily with $AlCl_3$ (Aladdin, China) at doses of 50 mg/kg, 150 mg/kg and 450 mg/kg for 90d (8:00 a.m–10:00 a.m), respectively. In control group, the rats were treated daily with deionized water instead of $AlCl_3$. The rats were treated daily with $AlCl_3$ or deionized water at a volume of 5 ml/kg by gavage in SPF animal laboratory. Gavage was performed using a syringe with a ball-tipped gastric-feeding needle. The $AlCl_3$-exposure is based on our previous study (Zhu et al., 2013). However, we modified the amount of $AlCl_3$ administered according to European Food Safety Authority recommended Al doses for children (26.9–286.8 mg/kg-week) (EFSA, 2013) and previous reported values of oral uptake that promoted neurotoxicity (Zhang et al., 2014). To maintain a constant $AlCl_3$ intake, we measured the body weight every five days and then adjusted the dose accordingly. The experiment was carried out according to the Guiding Principles in the Use of Animals in Toxicology, adopted by the Chinese Society of Toxicology. The animal procedures were approved by the Animal Ethics Committee of the Northeast Agricultural University (Harbin, China).

2.2. Morris Water Maze

We used Morris Water Maze (MWM) to evaluate learning and memory of control and $AlCl_3$-exposed rats as described previously (Abdel-Aal et al., 2011). Briefly, the water maze was a black circular pool (160 cm in diameter, 50 cm high) filled with water (30 cm in depth) at 22 ± 1 °C. The pool was set in a moderately lit, circular enclosure made with black curtains. The pool was surrounded by two sets of cues. One set consisted of four cues were placed within the pool, a blue circle at the west wall, a red square at the south wall, a green triangle at the east wall, and a golden cross at the north wall; the other set consisted of two cues were placed externally on the curtains, a white five-pointed star at the south, and a white crescent at the north. These cues remained unchanged throughout the testing period. To assess spatial learning the rats went through an acquisition trial for 1–5 days, followed by 6th d probe trial to assess spatial memory. In the acquisition trial, we placed a transparent round platform below the water surface of northeast quadrant in a circular pool. We then placed the rats (n = 10 per group and saved other 20 per group for other studies) for 30s on this platform. We then placed the rats at a starting point in the middle of the rim of a quadrant with their face to the wall. Rats swam freely until they reach platform where they stayed for 30s. If the rats failed to reach the platform during the 90s, rats we guided the rats to the platform and allowed to remain for 30s. We trained the rats for 5 days with three trials per day (8:00 a.m–12:00 p.m). We kept the trials 10 min apart. We chose the start point of quadrant (except northeast quadrant) in a quasi-random manner. We used video camera in conjunction with a computerized animal tracking system (Xinruan Information Technology, China) to record latency to the platform. We removed the escape platform in the probe trial and placed the rats gently at the start point of southwest quadrant and allowed to swim freely for 90s. We recorded the number of times crossing original platform location and the time that the rats spent swimming in each quadrant.

2.3. Tissue sample preparation

Because spatial learning increases the number of dendritic spines (Tronel et al., 2010), we chose the rats that were not exposed to the MWM to subsequent study. The rats were deeply anesthetized by sodium pentobarbital (50 mg/kg, i.p.) (Lockman et al., 2005). The brain (n = 10 per group) was quickly removed for Golgi-Cox staining. The hippocampus (n = 10 per group) was removed and stored at −80 °C for qRT-PCR. The body and brain weight (n = 20 per group) were measured. The brain index was calculated by multiplying the brain weight (g)/body weight (g) with 100%.

2.4. Golgi-Cox staining

We processed the fresh brains from each group for Golgi-Cox staining (FD NeuroTechnologies, USA) according to the manufacturer's instructions. Briefly, we submerged the fresh brains in mixed solution A and B for 14 days and solution C for 3 days. After that, we cut the brains into 150 mm thick sections (3 sections per brain) and mounted them on the gelatin-coated slides. We then stained the sections with solution D and E, dehydrated in graded ethanol, cleared in xylene, and covered with a coverslip. Finally, we used a Nikon E800 bright-field microscope (Nikon, USA) to visualize the Golgi-Cox stained sections at a magnification of 1000× for