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Lithium ameliorates lipopolysaccharide-induced neurotoxicity in the cortex and hippocampus of the adult rat brain

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ABSTRACT

Lithium an effective mood stabilizer, primary used in the treatment of bipolar disorders, has been reported as a protective agent in various neurological disorders. In this study, we examined the neuroprotective role of lithium chloride (LiCl) against lipopolysaccharide (LPS) in the cortex and hippocampus of the adult rat brain. We determined that LiCl -attenuated LPS-induced activated toll-like receptor 4 (TLR4) signalling and significantly reduced the nuclear factor-$_{k}$B (NF-$_{K}$B) translation factor and various other inflammatory mediators such as interleukin-1 beta (IL-1β) and tumour necrosis factor alpha (TNF-α). We also analyzed that LiCl significantly abrogated activated gliosis via attenuation of specific markers for activated microglia, ionized calcium-binding adaptor molecule (Iba-1) and astrocytes, glial fibrillary acidic protein (GFAP) in both the cortex and hippocampus of the adult rat brain. Furthermore, we also observed that LiCl treatment significantly ameliorated the increase expression level of apoptotic neurodegeneration protein markers Bax/Bcl2, activated caspase-3 and poly (ADP-ribose) polymerase-1 (PARP-1) in the cortex and hippocampus regions of the LPS-treated adult rat brain. In addition, the morphological results of the fluoro-jade B (FJB) and Nissl staining showed that LiCl attenuated the neuronal degeneration in the cortex and hippocampus regions of the LPS-treated adult rat brain. Taken together, our Western blot and morphological results indicated that LiCl significantly prevents the LPS-induced neurotoxicity via attenuation of neuroinflammation and apoptotic neurodegeneration in the cortex and hippocampus of the adult rat brain.

1. Introduction

Neuroinflammation has a key role in the pathology of several neurological disorders such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD) (McGeer and McGeer, 1998; Akiyama et al., 2000; Mhatre et al., 2004). The central nervous system (CNS) is sensitive to systemic inflammation which induces the activation of gliosis and circulating cytokines and chemokines which lead to neuroinflammation (Perry, 2004). Mounting studies have shown that in rodents, systemic administration of lipopolysaccharide (LPS), is an integral part of the cell wall of gram negative bacteria which activates the innate immune system, induces neuroinflammation, memory impairment, neuronal cells death particularly in the cortical and hippocampal regions of the rodent brain (Qin et al., 2007; Lee et al., 2008; Noh et al., 2014; Badshah et al., 2015, 2015a, 2016; Czapski et al., 2016 et al., Khan et al., 2016). The toll-like receptor 4 (TLR4), is pattern-recognition receptors (PRR) that recognize and bind with cytokines as well as LPS (Lien et al., 2000). Toll-like receptors (TLRs) are key mediators for neuroinflammation and neurodegeneration in various CNS diseases and initiates different signalling pathways particularly regulates the nuclear factor kappa B (NF-$_{k}$B) downstream and microglia activation. It has been also reported that activation of TLR4 in the macrophage-derived glial cells in the CNS in response to LPS produces a deleterious effects via activation of

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NF-$_k$B, tumour necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and various neuroinflammatory mediators and apoptotic markers and neurodegeneration in various neurological disorders (Lien et al., 2000; Carpentier et al., 2008; Cheong et al., 2011; Kettenmann et al., 2011; Skelly et al., 2013; Dong et al., 2014; Cho et al., 2015; Badshah et al., 2016).

Lithium was approved and considered an effective therapeutic agent in various mood abnormalities of the bipolar disorders. It is well known for its strong anti-manic, antidepressant and anti-suicidal properties (Baldessarini et al., 1999; Marmol, 2008; Malhi et al., 2012). In addition, lithium ameliorates neuroinflammation and neurotoxicity by preventing activated and accumulated gliosis such as microglia and astrocytes (Yuskaitis and Jope, 2009; Li et al., 2016). Furthermore, numerous established studies reported that lithium modulates inflammatory cascade, prevents mitochondrial apoptosis, and reduce oxidative stress in several acute brain injuries e.g. ischemia and chronic neurodegenerative diseases such as AD, ALS and PD (Wada et al., 2005; Caldero et al., 2010; Ferrucci et al., 2010; Diniz and Teixeira, 2011; Forlenza et al., 2014; Fan et al., 2015a, 2015b; Nunes et al., 2015). Interestingly, cognition-based studies suggested that lithium activate various pathways and transcription factors inside the brain and reduce memory impairments (Klein et al., 1996; Fan et al., 2015). It has been reported that administration of acute and chronic lithium reaches to the brain due to its hydrophilic property it does not bind to plasma proteins and crossed the blood brain barrier and accumulated to the brain. In clinics, it is used in the form of lithium salts e.g. lithium chloride (LiCl) because in the salt form the kidney does not metabolised and about 80% of the lithium reabsorbed in the proximal tube of the kidney (Hillert et al., 2012; Malhi et al., 2012; Nunes et al., 2015). Here, we hypothesized that intraperitoneal (I.P.) administration of LiCl at a dose of 0.08 g/kg/day to the adult rats could prevent LPS-induced neurotoxicity in the cortex and hippocampus via attenuation of the neuroinflammation and apoptotic neurodegeneration in the adult rat brain.

2. Materials and methods

2.1. Chemicals

LPS and LiCl were purchased from Sigma Chemical Co (St. Louis, MO, USA).

2.2. Rats used in this study

Male Sprague-Dawley rats weighing 250–300 g were housed under a 12-h/12-h light/dark cycle at 23 °C, 60± 10% humidity. All rats were provided with food and water ad libitum. All treatments and experimental procedures were carried out according to the animal ethics committee of the Division of Applied Life Sciences, Department of Biology at Gyeongsang National University, South Korea.

2.3. Rats grouping and their treatment

Randomly male Sprague-Dawley rats were divided into the following three groups: (I) Control (Cont) saline treated as a vehicle for 14 days; (II) saline treated for 7 days, following LPS injection of the rats at a dose of 250 μg/kg for an additional 7 days; and (III) rats injected with LPS (250 μg/kg/day) for 7 days and LiCl 0.08 g/kg/day for 14 days 7 days prior to administration of LPS and 7 days co-administered with LPS (LPS + LiCl). The LiCl was I.P. administered to the rats at a dose of 0.08 g/kg/day for 14 days, and similarly, LPS was dissolved in saline and administered I.P. at a dose of 250 μg/kg for 7 days. The schematic presentation of the experimental designing, treatment and sacrificed of the animal is presented in Fig. 1.

2.4. Protein extraction from the rat brain

After completion of the treatment the rats were sacrificed and the brains were immediately removed. The cortex and hippocampus were dissected with care and the tissues were frozen on dry ice and stored at −80 °C until use. The hippocampus and cortex tissue were homogenized in 0.2 M PBS using phosphatase and protease inhibitors cocktail followed by centrifugation at 13 000 rpm at 4 °C for 25 min. Supernatants were collected and stored at −70 °C.

2.5. Western blotting

In order to measure the expression level of different proteins in the hippocampus and cortex regions of the adult rat brain, we performed Western blotting as we described previously with some modifications (Ahmad et al., 2016; Shah et al., 2016). In brief, protein concentration was measured with (Bio-Rad protein assay kit, BioRad Laboratories, CA, USA). Equal amount of proteins (15–30 μg) were electrophoresed using 4–12% BoltTM Mini Gels and MES SDS running buffer 1x (Novex, Life Technologies). Prestained protein ladders (GangNam stainTM, Intron Biotechnology) covering a broad range of molecular weight was used to detect molecular weights of the proteins. To prevent the nonspecific binding, membranes were blocked in 5% (w/v) skim milk and incubate with primary

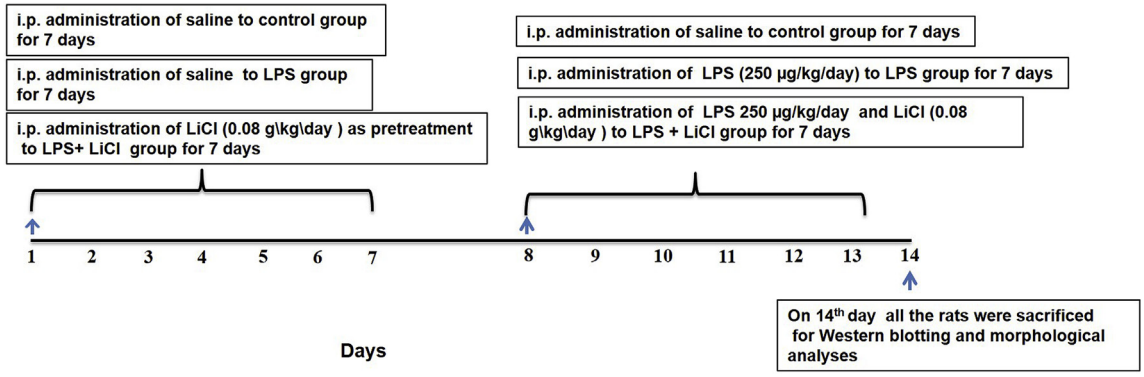

Fig. 1. Diagrammatic representation of the experimental designing, treatment and sacrificed of the animals. Adult male Sprague-Dawley rats weighing 250–300 g were treated with saline for 7 days to control group, saline was injected to LPS group for 7 days and LiCl (0.08 g/kg/day) for 7 days to LPS + LiCl group, further for 7 days saline, LPS (250 μg/kg) and LPS (250 μg/kg/day) + LiCl (0.08 g/kg/day) were administered intraperitoneally (i.p.) to the respective groups of the rats. On 14th day the adult rats were sacrificed and further proceed for Western blotting and morphological assessment.