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Effects of catalpol on mitochondrial function and working memory in mice after lipopolysaccharide-induced acute systemic inflammation

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Abstract

The aim of this study was to investigate whether catalpol could facilitate recovery from lipopolysaccharide (LPS)-induced cognitive deficits and protect brain mitochondrial function from LPS-induced acute systemic inflammation. In the study, except control group, mice were challenged with a single dose of LPS (100 μg/mouse, i.p.) to mimic an acute peripheral infection. The results showed that LPS enhanced nuclear factor kappa B (NF-κB) activation and induced a loss in mitochondrial integrity as shown by a significant decrease in membrane potential and increase in mitochondrial permeability transition pore opening. Pretreatment with catalpol (10 mg/kg d, i.p.) for 10 d before injection of LPS reversed the memory deficits induced by LPS, protected brain mitochondrial function, and attenuated LPS-induced NF-κB activation. Taken together, these data indicate that catalpol may possess therapeutic potential against LPS-induced acute systemic inflammation by attenuating NF-κB activation and protecting mitochondrial function in cerebral cortex and hippocampus.

Keywords: Catalpol; Inflammation; Mitochondria; NF-κB; Lipopolysaccharide

Introduction

Increase in life expectancy has resulted in an increase in the prevalence of age-dependent diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). The symptoms of these diseases include progressive memory loss and impairment in spatial and perceptual recognition, as well as in daily living. Brain inflammation probably plays an important role in the pathogenesis of chronic neurodegenerative disorders like AD and PD (Nelson et al., 2002; Liu and Hong, 2003). Neurodegeneration caused by inflammation involves activation of the brain's resident immune cells and the microglia, which produce a large number of pro-inflammatory factors (Pocock and Liddle, 2001; Hanisch, 2002). Lipopolysaccharide (LPS) is a highly conserved cell wall component of gram-negative bacteria that is recognized by the immune system of higher vertebrates as a pathogen-associated molecular pattern (PAMP). LPS binds to the Toll-like receptor 4 (TLR4)/CD14 complex on the surface of mononuclear myeloid cells (Laflamme and Rivest, 2001) and activates the

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transcription factor NF-κB to up-regulate expression of, among other genes, the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α. Recently, peripheral immune activation with LPS has been reported to exacerbate neuroinflammation and prolong sickness behavior in aged mice (Godbout et al., 2005a). Florence et al. (2007) have recently shown that acute systemic inflammation caused by LPS induces central mitochondrial damage and mnesic deficit in adult Swiss mice. Intracellular reactive oxygen species (ROS), generated by highly respiring mitochondria, and peroxides are also well documented to serve as intracellular second messengers to induce signal transduction and activate transcription factors such as NF-κB (Haddad et al., 2002; Zhang et al., 2001).

Catalpol, an iridoid glucoside separated from the roots of *Rehmannia glutinosa*, possessing a wide range of biological and pharmacological activity, including anti-tumor, anti-inflammation, and anti-apoptosis properties, has been reported to significantly improve the cognitive impairment in many animal models of neurodegenerative disease (Li et al., 2004, 2005; Zhang et al., 2007) and protect mice brain from oxidative damage and mitochondrial dysfunction induced by rotenone (Mao et al., 2007). Furthermore, a recent study suggests that catalpol is neuroprotective in vitro since it significantly reduced the release of ROS, TNF-α, NO, and iNOS expression after LPS-induced microglial activation (Tian et al., 2006). Because inflammation and mitochondrial dysfunction are important players in the pathogenesis of neurodegenerative diseases, the neuroprotective action of catalpol has been measured in LPS-induced acute systemic inflammation. The aim of the present study was to investigate whether catalpol could facilitate recovery from LPS-induced cognitive deficits and protect brain mitochondrial function.

Materials and methods

Reagents and drugs

Catalpol, separated and purified according to our previous report (Zhang et al., 2007, 2008), was diluted in physiological saline for treatment. LPS (*Escherichia coli*, 055:B5), obtained from Sigma, was dissolved in phosphate-buffered saline (PBS). The commercial kit used for determination of mitochondrial permeability transition pore (MPTP) opening was purchased from GENMED Company (Shanghai, China). NF-κB p65 antibody and 2′, 7′-dichlorofluorescin diacetate (DCFH-DA) were purchased from Beyotime.

Animals and drug treatment

The Kunming mice (obtained from the Experimental Animal Center, Dalian Medical University, China), weighing 35–37 g, were housed in cages in an air-conditioned room with controlled temperature (24 ± 1 °C) for 5 d before the experiment and were maintained on a 12:12 h light cycle (07:00 h on, –19:00 h off). They were allowed free access to food and water. All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals in Dalian Medical University, Dalian, China.

The mice were randomly divided into three groups: control group ($n = 10$), model group ($n = 10$), and therapy group ($n = 10$). Control group mice were injected with sterile physiological saline (i.p.). Model group mice were treated with sterile physiological saline for 10 d, and then they were treated with LPS (100 μg/mouse, i.p.) for the last day. Therapy group mice were treated with catalpol (10 mg/kg body weight, i.p.) for 10 d, and then they were treated with LPS (100 μg/mouse, i.p.) for the last day. Mice were sacrificed after 4 h from the last drug treatment, and their brains were quickly removed. The cerebral cortex and hippocampus were isolated, weighed, frozen on ice, and stored at −80 °C until assay.

Behavioral testing

A water maze (Morris) was constructed to evaluate mice working memory, which consisted of a black circular tank, 100 cm in diameter and 50 cm in depth. The tank was divided virtually into four equal quadrants and an escape platform was hidden 1.5 cm below the surface of the water in a fixed location in the third quadrant of the pool. After 1-d training, a trial was started by placing the mice into the pool close to the rim, facing the wall of the tank in one of the four quadrants. For the experiment, training took place during a 10-d acquisition phase with two sessions of three massed trials administered each day. A single injection of saline or LPS was administered on the test day (d 11) to determine treatment effects on an animal's ability to integrate new information with existing memories to complete a task. To begin each trial, a mouse was pseudorandomly placed in an arm not occupied by the platform facing the wall. Mice were allowed to swim freely for a maximum of 60 s or until the platform was located. After the mouse reached the platform it was required to remain there for 30 s. If the platform was not located during the 60 s, mice were guided to the platform and allowed to remain for 30 s. After completion of three consecutive trials, mice were placed in their home cage under a heat lamp for approximately 10 min. Performance parameters that were determined included swim speed, latency to the platform, and distance swam.