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Research report

Effects of naringin on learning and memory dysfunction induced by gp120 in rats

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ABSTRACT

The aim of the present study was to investigate the effects of naringin on learning and memory dysfunction induced by HIV-1-enveloped protein gp120 in rats, and to identify its potential mechanisms of action. Learning and memory ability was evaluated via Morris water maze test, $P2X_7$ receptor and P65 protein expressions in the rat hippocampus were detected by western blot analysis, and $P2X_7$ mRNA expression in the hippocampus was measured by RT-PCR. We also recorded $P2X_7$ agonist BzATP-activated current in the hippocampus via patch clamp technique. The results showed that naringin treatment (30 mg/kg/day) markedly decreased the escape latency and target platform errors of rats treated with gp120 (50 ng/day), and further, that naringin treatment significantly decreased the expression of $P2X_7$ and P65 protein and $P2X_7$ mRNA in the hippocampus of gp120-treated rats. In addition, naringin treatment reduced BzATP-activated current in the hippocampus of gp120-treated rats. These results altogether demonstrated that naringin can improve gp120-induced learning and memory dysfunction via mechanisms involving the inhibition of $P2X_7$ expression in the hippocampus.

1. Introduction

Human immunodeficiency virus (HIV)-associated dementia (HAD), also known as AIDS-dementia complex (ADC), is associated with cognitive impairments, movement disorders, and mental and behavioral abnormalities (Lucas and Nelson, 2015; Peluso and Spudich, 2014). Learning and memory dysfunction is one of the core symptoms of HAD, and the neuroinflammatory response induced by HIV-1 is probably a major contributor to HAD-related cognitive and behavioral changes (Fitting et al., 2013; Hong and Banks, 2015). In the brain, the multiplication of HIV in infected macrophages/microglia releases HIV proteins such as gp120 and Tat; the activation of microglia may then result in increased HIV replication, as well as increased production of inflammatory mediators such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), which play an important role in neuronal injury and death caused by HAD (Brabers and Nottet, 2006; Purohit et al., 2011).

$P2X_7$ receptor has been implicated in the release of cytokines and in the induction of cell death. It was originally identified in macrophages, microglia, and certain lymphocytes, and its activation is known to have dramatic cytotoxic properties. Due to its ability to regulate the release of proinflammatory cytokines, $P2X_7$ receptor can affect neuronal cell death and may be involved in the pathophysiology of neurodegeneration (Skaper et al., 2010). $P2X_7$ receptor activation on the microglia appears to be necessary for the microglial-mediated injury of neurons, and further, $P2X_7$ receptor activation is able to initiate the ATP-induced chemotaxis of microglial cells (Monif et al., 2009). To this effect, targeting $P2X_7$ receptors may be a helpful approach to the treatment of acute and chronic neurodegenerative disorders (Skaper et al., 2006).

Naringin (4′,5,7-trihydroxyflavanone-7-rhamnoglucoside), a flavonoid naturally existing in grapefruit and other citrus fruits, has been shown to possess numerous biological benefits such as antioxidant, anti-inflammatory, and anti-apoptotic properties. Preclinical evidence has demonstrated that naringin exerts favorable effects on atherosclerosis, cardiovascular disorders, diabetes mellitus, neurodegenerative disorders, osteoporosis, and rheumatological disorders (Bharti et al., 2014). Naringin has been shown

Abbreviations: HAD, HIV-1 associated dementia; LV, lateral ventricle; MWM, Morris water maze; $P2X_7R$, $P2X_7$ receptor; HIV, human immunodeficiency virus; ADC, AIDS-dementia complex; SD, Sprague-Dawley; ACSF, artificial cerebrospinal fluid; PMSF, phenylmethanesulfonyl fluoride.

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to protect against neurodegeneration induced by 3-nitropropionic acid via modulating oxidative stress and inflammatory responses, as well (Gopinath et al., 2011; Gopinath and Sudhandiran, 2012). Protection against HIV-associated neurodegeneration by naringin has not yet been reported, however. In this study, we investigated the ameliorative effect of naringin on HIV-1 envelope glycoprotein gp120-induced learning and memory deficits in addition to $P2X_7$ receptor expression in the hippocampus to explore the underlying mechanisms of naringin.

2. Materials and methods

2.1. Experimental animal grouping and study design

All studies were performed in accordance with the guidelines set by the Ethical Committee of Nanchang University. Healthy Sprague-Dawley (SD) rats (4–6 weeks old, weight 125–175 g) were provided by the Laboratory Animal Science Department of Nanchang University. Animals were kept under constant conditions ($22 \pm 2\,^{\circ}C$ and 12-h light/dark cycle) with free access to food and tap water. Rats were treated with different doses of gp120 (0, 50, 70, 100 ng/day) by lateral ventricle (LV) perfusion for three days to observe the effects of gp120 on learning and memory deficits. The rats were then randomly divided into five groups: a control group (Ctrl), sham surgery group (Sham), model group (gp120 50 ng/day), naringin treatment group (gp120 50 ng/day + naringin 30 mg/kg/day), and $P2X_7$ receptor antagonist BBG treatment group (gp120 50 ng/day + BBG 50 mg/kg), with six rats in each group.

2.2. Model preparation

All rats apart from those in the control group underwent cannula fixation surgery of the LV. Rats were anesthetized by intraperitoneal (i.p.) injection of 10% chloral hydrate (0.3–0.35 mg/kg). The fur on the head of the rat was removed, and a 2 cm incision was made in the middle of the top of the head to expose the anterior fontanel. According to the rat brain orthostatic atlas, the animal's skull was opened with a dental drill 2.0 mm; stereotaxic coordinates for the lateral ventricles were: anterior/posterior: −1.0 mm, medial/lateral: ±1.5 mm, dorsal/ventral: −3.5 mm from bregma; then a sterilized polyethylene plastic tube was immediately inserted into the skull and fixed with dental powder. The animal's head skin was thoroughly disinfected with iodine and the incision was sutured. Amoxicillin was given daily to prevent infection. LV infusion was performed on the fourth day after stereotaxic surgery was restored, and the rats were anesthetized repeatedly with ether during the process. Sham group rats were given 5 μl artificial cerebrospinal fluid (ACSF). Rats from gp120, naringin treatment, and $P2X_7$ receptor antagonist BBG groups were given corresponding 5 μl gp120 by intracerebroventricular (ICV) injections at a speed of 0.5 μl/min, while the LV perfusion was not performed in the control group rats. The experimental drug was administered for three consecutive days.

2.3. Methods of administration

After LV administration of gp120, rats in the naringin treatment group were given naringin 30 mg/kg/day repeatedly by gavage for 14 consecutive days. Rats in the $P2X_7$ receptor antagonist BBG group were treated with BBG via i.p. injection every 48 h with a dose of 50 mg/kg for 14 consecutive days. The rats in control, sham, and gp120 groups were gavaged with a mixture of equal volumes of polyethylene glycol 400 (PEG400) and normal saline for 14 consecutive days.

2.4. Morris water maze behavioral test

Behavioral testing was conducted using a water maze apparatus (diameter 120 cm, height 110 cm, water depth 25 cm) containing a submerged platform (13 × 13 cm, 2 cm below water level) and an arm. A small fixed light source and intra-maze cues were used for spatial orientation. Room and water temperature were respectively maintained at $22\,^{\circ}C$ and $26 \pm 1\,^{\circ}C$. Two 3-day protocols (consisting of five blocks of three trials each for a total of 15 trials per day) were performed on the 6th–8th days and 13th–15th days after intragastric administration. Two measurements were recorded during each 60 s trial: incorrect arm choices (i.e., spatial exploration tests) and the escape latency required to discover the hidden platform (i.e., place navigation tests). After successfully locating it, the rats were kept on the platform for 10–15 s before being removed from the apparatus, dried, and given food and water. For each block, rats performed in cohorts of three to allow for extended periods of rest between blocks. The target arm for each individual animal, which was set for a unique 3-day protocol, was allocated randomly while the starter arm varied stochastically with each trial. In subsequent tests, each individual rat was randomly assigned a different goal arm in order to evaluate the changes in their learning and memory. The rats' swimming speeds were measured with a Noldus EthoVision video tracking system (Leesburg, VA, USA). Before recording escape latency and arm-choice errors, rats were allowed to swim for 2 min in order to adapt to the new environment, then gently taken into the tank facing the pool wall. If the rats failed to find the platform in 60 s, they were placed on the platform for 15 s–20 s and their escape latency was recorded as 60 s.

2.5. Western blot experiment

After the water maze experiment, rats were anesthetized by i.p. injection of 10% chloral hydrate (Shanghai Xingya Medical Company, Batch No: 050101, China) then decapitated to remove the hippocampus. Tissues were quickly frozen in tubes on dry ice; all specimens extracted at different time points were frozen and stored at $-4\,^{\circ}C$. The hippocampus tissues of each group were positioned in the spherical part of a 2 ml homogenizer, in which 1 ml detergent lysis buffer containing phenylmethanesulfonyl fluoride (PMSF) was homogenized. The tissues were then put back on ice and ground repeatedly until the organization was fully crushed after 50 min of clevage. The lysate was then moved to a 1.5 ml centrifuge tube with a transferpettor, which was centrifuged at $4\,^{\circ}C$ at the speed of 12000 rpm for 5 min. The supernatant was harvested and preserved at $-20\,^{\circ}C$. The quantity of total protein associated with the supernatant was measured via Lowry method. After having been diluted with sample buffer and heated to $95\,^{\circ}C$ for 10 min, samples containing equal amounts of protein (20 μg) were separated by SDS-polyacrylamide gel (10%) electrophoresis with a Bio-Rad electrophoresis device, then transferred onto nitrocellulose (NC) membrane in the same system. The labeled proteins were then visualized with enhanced chemiluminescence on high-performance film (Shanghai Pufei Biotech Co., China); chemiluminescent signals were observed on autoradiography film, and the band intensity was quantified in AlphaImager 2200 software (Alpha Innotech, USA). The primary antibodies included rabbit polyclonal anti-$P2X_7$ (Chemicon International Co.), rabbit polyclonal anti-P65 (Chemicon International Co.), and β-actin (Advanced Immunochemicals, Long Beach, CA). The secondary antibody was goat anti-rabbit IgG (Beijing Zhongshan Biotech Co., China). Band densities were normalized to each β-actin internal control.