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Protective effects of naringin against gp120-induced injury mediated by $P2X_7$ receptors in BV2 microglial cells

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ABSTRACT. This study was aimed at exploring the effects of $P2X_7$ receptors on gp120-induced injury and naringin's protective effects against gp120-induced injury in BV2 microglia. BV2 microglia injury model was established by gp120 treatment and MTS assay was used to verify whether naringin has a cell-protective effect against gp120-induced injury. Changes in $P2X_7$ receptor expression were assayed using RT-PCR, qPCR, and western blot. Results showed that the ODs of the Ctrl, gp120, gp120+naringin, and gp120+BBG groups were 0.91 ± 0.10, 0.71 ± 0.09, 0.83 ± 0.10, and 0.83 ± 0.10, respectively. Compared to the control group, the gp120 group showed a significantly decreased cell survival rate. Cell survival rates of the gp120+naringin group increased significantly compared to those of the gp120 group, while no difference was observed when compared to the gp120+BBG group. The relative $P2X_7$ mRNA expression levels

in the Ctrl, gp120, gp120+naringin, and gp120+BBG groups were 0.73 ± 0.06, 1.05 ± 0.06, 0.78 ± 0.05, and 0.81 ± 0.04, respectively. The corresponding $P2X_7$ protein expression levels were 0.46 ± 0.04, 0.79 ± 0.04, 0.38 ± 0.07, and 0.42 ± 0.06. $P2X_7$ mRNA and protein expression in the gp120 group increased significantly compared to those in the control group, and declined in the gp120+naringin group compared to those in the gp120 group. Therefore, $P2X_7$ receptors might be involved in gp120-induced injury in BV2 microglia, and naringin might play a protective role by inhibiting the up-regulated expression of $P2X_7$ receptors.

Key words: AIDS-dementia complex; BV2 microglial cells; Naringin; Gp120; $P2X_7$ receptor

INTRODUCTION

Human immune deficiency-associated dementia (HAD), also referred to as AIDS-dementia complex (ADC), includes core symptoms of learning and memory dysfunction (Lucas and Nelson, 2015). Its specific mechanisms are not well understood (Zhou et al., 2012). However, it is commonly accepted that ADC is associated with the degree of immune suppression and the activation of macrophages and microglia cells (Harezlak et al., 2014). Various harmful substances can be produced by activated macrophages or microglia, which go on to damage other cells in the central nervous system (CNS) or induce apoptosis (Fu et al., 2014). Envelope glycoprotein gp120 (or gp120) is a glycoprotein found on the surface of the HIV envelope, and is essential for the virus' entry into cells as it plays a vital role in their attachment to specific cell-surface receptors (de Witte et al., 2007). Nuclear factor kappaB (NF-kappaB or NF-κB) proteins comprise a family of structurally related eukaryotic transcription factors that are involved in the control of a large number of normal cellular and organismal processes. Research studies suggested that intrathecal gp120 may lead to an activation of NF-κB within the spinal cord in exaggerated pain states (Ledeboer et al., 2005), and gp120 has been shown to promote tissue and cell injury by inducing NF-κB activation (Kapasi et al., 2006). Previous researchers have found that $P2X_7$ receptors play a vital role in regulating the function of microglia, and can be activated by adenosine 5'-triphosphate (ATP), thus releasing inflammatory cytokines to mediate chronic inflammatory responses in neurons, ultimately resulting in neurotoxicity (Feng et al., 2015; Lioi et al., 2015). The $P2X_7$ receptor, which is involved in inflammatory and immune responses and is closely associated with neurodegenerative diseases, may represent a new target for the prevention and treatment of ADC. Brilliant Blue G (BBG), a selective $P2X_7$ receptor antagonist, may block the interactions between ATP and $P2X_7$ receptors, thus suppressing microglial activation in the hippocampus and alleviating neural injury and consequent functional deficits (Deng et al., 2015; Xu et al., 2015), as well as decreasing the expression levels of $P2X_7$ receptor immunoreactivity (Zou et al., 2016). In addition, naringin, a naturally-produced falconoid, possesses numerous biological benefits including anti-inflammatory and antioxidant properties, metabolic regulation of sugars and lipids, and myocardial protection (Li et al., 2014), indicating that it may remedy ADC to some extent. In this study, we explored the potential protective effects of naringin against gp120-induced microglial cell injury at the cellular level by establishing a representative

microglial cell-injury model. The findings presented here may be of considerable significance in terms of both broadening the application scope of naringin and exploring potential ADC treatment methods.

MATERIAL AND METHODS

Cell culture and grouping

BV2 microglial cells (No. GDC0311) were obtained from the China Center for Type Culture Collection. They were incubated in a constant-temperature incubator at 37°C (Sanyo, Japan; 5% CO_2) and cultured in a DMEM high-glucose medium (HyClone, USA) supplemented with 10% FBS (TransGen Biotech, China) and 1% penicillin-streptomycin solution (Solarbio, China). The cells which underwent cryopreservation and resuscitation served as the first generation, then after a passage ratio standing at 1:6 and a 3- to 4-day generation cycle, stable cells of the third to fourth generation respectively were used for the experiment. All cells used for the experiment were controlled within 30 generations. Gp120, naringin, and Brilliant Blue G (BBG) were all provided by Sigma (USA).

In order to screen naringin performance, the cells were treated with different doses of naringin (0, 10, 20, 40, 80, 160 μM/L). In the cell-damage-detection experiment, the cells were randomly divided into four groups: a control group (Ctrl), the gp120 model group (gp120), the naringin treatment group (gp120+naringin) and the $P2X_7$-receptor-antagonist BBG treatment group (gp120+BBG). Besides the Ctrl group, all the other groups were given 2.0 mg/L gp120 for 24 h. The gp120+naringin and the gp120+BBG groups were also simultaneously given naringin (80 mm/L) and BBG (50 mm/L) respectively. Pharmaceutical interference was conducted until the cells were inoculated into plates and grew to about 70-80%, after which gp120 was dissolved with sterile water, naringin with DMSO, and BBG with PBS buffer. All drugs were diluted in a DMEM high-glucose medium.

Drug concentration screening and cell damage detection by MTS colorimetric assay

When the cells in the culture flask grew to 80-90%, cell passage was performed with 2 mL DMEM high-glucose medium, which was used to suspend the cells after centrifugation, prior to a cell count. An appropriate volume of cell suspension was siphoned and diluted with complete culture media, which were subsequently mixed sufficiently to achieve a density of approximately 1 x 10^5/mL, before being added to a 96-well plate (100 μL per well). When the cells grew to about 60%, the medium was replaced by a basic culture medium that had been incubated for 24 h with drugs. An MTS storage fluid (Promega, USA) was then diluted with a DMEM high-glucose medium in a ratio standing at 1:10 for 24 h, after which the medium was changed. The DMEM high-glucose medium containing no cells served as an empty control with 100 μL MTS diluent per well and was incubated at 37°C under 5% CO_2 atmosphere for 4 h. The optical density (OD) of each well, which represented the cell survival rate, was measured by a multifunctional microplate reader (PerkinElmer, USA) at 490 nm. The optimal drug concentration of gp120 was used as a model to screen naringin performance. Six suspensions with different naringin drug concentrations were prepared: 0, 10, 20, 40, 80, and 160 μM/L, and each concentration was set with 5 parallel wells.

Oxidative damage index detection

Cells were inoculated into the 24-well plate at a density of approximately 1 x 10^5/mL, and each group was set with 3 parallel wells. After being treated with drugs for 24 h, the supernatant was taken from each BV2 microglial cell group to measure NO levels with a Nitric Oxide Test Kit (Nanjing Jiancheng Bioengineering Institute, China). Oxidative damage was measured by a multifunctional microplate reader (PerkinElmer, USA), and the OD index was calculated according to the manufacturer's instructions.

RT-PCR and qPCR experiment

Total RNA was extracted with a Total RNA Isolation Kit (Beijing TiangGen Biotech, China) and PCR amplification was undertaken after reverse transcription (RT) reaction. The upstream primer and downstream primer sequences of the $P2X_7$ mRNA were 5'-CGTCAAGTGGGTCTTGCACA-3' and 5'-CACCCCTTTTTACAACGCCG-3', respectively, and the expected product size was 333 bp. β-actin, as an internal control, was also amplified using specific primers (the forward and reverse primer sequences were 5'-CACCCGCGAGTACAACCTTC-3' and 5'-CCCATACCCACCATCACACC-3', respectively); the expected product size was 240 bp. The multifunctional gel imaging system (Bio-Rad Company, USA) was used to read the spot density scanning value of the objective electrophoresis bands. Band densities were evaluated with the ImageLabC gel imaging system software (Bio-Rad Company, USA) and normalized to each β-actin internal control to show the relative expression of $P2X_7$ mRNA. PCR was performed in over 31 cycles, with the RT reaction being undertaken at 37°C for 60 min and the RTase inactivation being undertaken at 94°C for 3 min, followed by denaturation at 94°C for 45 s, annealing at 60°C for 30 s, and final extension at 72°C for 45 s.

In order to ensure the accuracy of the results, the processes of the $P2X_7$ mRNA expression experiment was repeated by qPCR. After extracting the total RNA and quantifying it by determining its optical density at 260 nm, 25 ng of the total RNA was reverse transcribed. An RNA-specific control was included for each sample to assess potential genomic DNA contamination. SYBR® Green Real-time PCR, using the ABI PRISM® 7500 Sequence Detection System (Applied Biosystems Inc.: Foster City, CA, USA) was performed to quantify the expression of $P2X_7$ at the mRNA level. For the PCR amplification, 2 μL cDNA were used in a total volume of 20 μL, and each sample was assayed in triplicate. The upstream and downstream primer sequences of the $P2X_7$ mRNA were 5'-GCACGAATTATGGCACCGTC-3' and 5'-CCCCACCCTCTGTGACATTC-3', respectively, and the expected product size was 171 bp. The expression levels of β-actin were selected as the reference and also amplified using specific primers (forward and reverse primer sequences were 5'-TCCTTCCTGGGCATGGAGT-3' and 5'-AGCACTGTGTTGGCGTACAG-3', respectively). The expected product size was 104 bp. Cycling parameters were as follows: 94°C for 30 s to activate DNA polymerase, 40 cycles of 94°C for 5 s and 60°C for 30 s for amplification; 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s to obtain the melt curve. The average threshold cycle (CT) value for β-actin was subtracted from the average $P2X_7$ value to yield the ΔCT value (ΔCT = CT target - CT reference). Result was calculated as follows: ΔΔCT = ΔCT test sample - ΔCT calibrator sample. The levels of $P2X_7$ expression (RQ), representing relative expression results, were compared with those in the independent groups by using the following equation: $RQ = 2^{-\Delta\Delta Ct}$.

Western blot experiment

After being inoculated into a 6-well plate and treated with drugs for 24 h, the cells were disintegrated in a RIPA lysis buffer to extract total proteins, and the lysates were centrifuged at 4°C at a speed of 12000 rpm for 5 min. The supernatant was harvested and preserved at -20°C. After diluting with a sample buffer and heating to 95°C for 10 min, samples containing equal amounts of proteins (20 mg) were separated by SDS-polyacrylamide gel (10%) electrophoresis, using a electrophoresis set-up (Bio-Rad Company, USA), and afterwards transferred onto a PVDF membrane. The chemiluminescent signals were observed using the multifunctional gel imaging system, and the band intensity was quantified in Image-Pro Plus 6.0 software (Media Cybernetics, USA). The primary antibodies included rabbit polyclonal anti-$P2X_7$ (Abcam, UK; 1:1000 dilution), rabbit polyclonal anti-P65 (CST, USA; 1:1000 dilution), and β-actin (Beijing Zhongshan Biotech Co. 1:800 dilution). The secondary antibody was goat anti-rabbit IgG (Beijing Zhongshan Biotech Co.). Band densities were normalized to each β-actin internal control.

Statistical analyses

Statistical analyses of the data were performed with SPSS 21.0 (IBM, USA). All results are reported as means ± SE. Statistical significance was determined by one-way analysis of variance (ANOVA), and significance was defined as $P < 0.05$.

RESULTS

Drug concentration of naringin

We used 2.0 mg/L gp120 as a model and set concentration gradients between 10 μM/L-160 μM/L to investigate optimal naringin concentration(s). The OD values of the six concentration gradients were 078 ± 0.08, 0.78 ± 0.07, 0.79 ± 0.09, 0.85 ± 0.10, 0.96 ± 0.10, and 0.89 ± 0.09. Statistical analysis indicated that, compared to the control group, 40 μM/L naringin began to provide protection for cell survival with statistical significance ($P < 0.05$); both 80 μM/L and 160 μM/L naringin had more significant effects ($P < 0.01$), albeit 80 μM/L naringin seemed better than 160 μM/L (Figure 1). Accordingly, we chose 80 μM/L naringin for subsequent experiments.

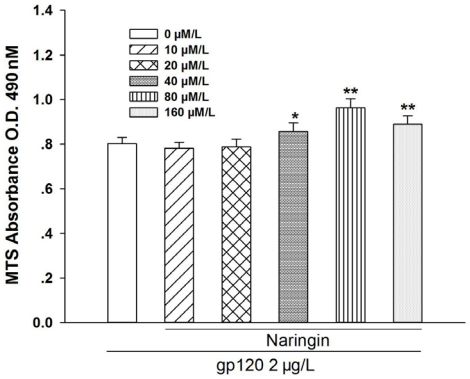

Figure 1. Cell survival rates with different concentrations of naringin treatment. Values are reported as means ± SE of six parallel experiments. *P < 0.05, **P < 0.01 versus 0 μM/L group).

Cell survival rate by drug treatment

After a 24-hour drug treatment, the ODs of the Ctrl, the gp120, the gp120+naringin and the gp120+BBG groups were 0.91 ± 0.10, 0.71 ± 0.09, 0.83 ± 0.10, and 0.83 ± 0.10 respectively. The cell survival rate of the gp120 group declined notably compared to the control group ($P < 0.01$), indicating that gp120 had damaged BV2 microglia cells to some extent. Compared to the gp120 model group, the gp120+naringin group showed an increase in cell survival rate ($P < 0.05$) but had no statistically significant difference compared to the gp120+BBG group ($P > 0.05$), indicating that naringin did have a certain protective impact on gp120-induced injury in BV2 microglial cells (Figure 2).

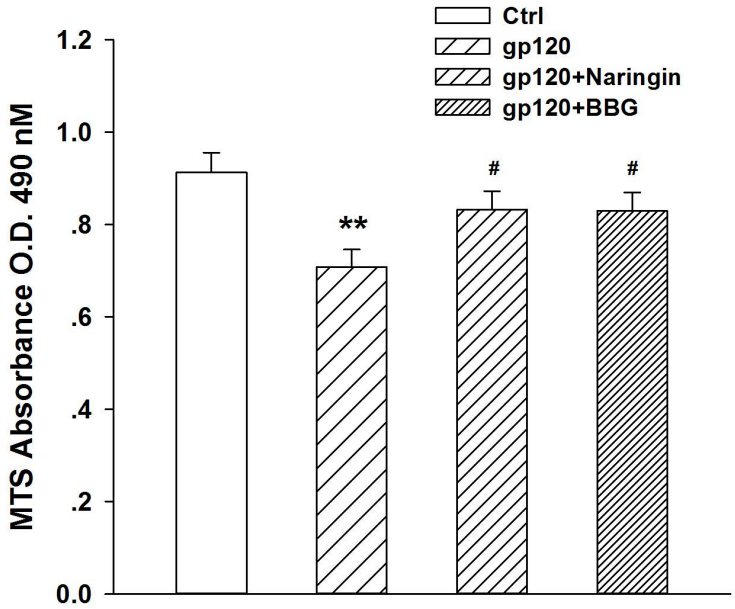

Figure 2. BV2 microglia survival rate with different drug treatment. Values are reported as means ± SE of six parallel experiments. **$P < 0.01$, versus Ctrl group; #$P < 0.05$, versus gp120 group.

NO levels

The NO level of BV2 microglia was calculated according to the formula provided in the NO test kit after measuring the OD with a microplate reader. Results showed that the NO levels (mm/L) of BV2 microglia in the Ctrl, the gp120, the gp120+naringin and the gp120+BBG groups were 23.15 ± 2.47, 39.76 ± 3.77, 27.86 ± 4.60, and 29.93 ± 4.34 respectively. Statistically, the NO level in the gp120 group was considerably higher than that in the Ctrl group ($P < 0.01$), and the NO level in the gp120+naringin group was lower than that in the gp120 group, while there was no statistical difference between the gp120+naringin group and the gp120+BBG group ($P > 0.05$) (Figure 3).

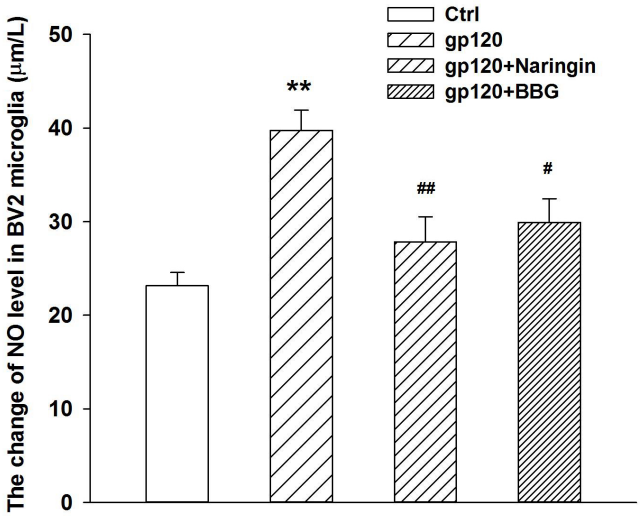

Figure 3. NO level of BV2 microglia in each group. Values are reported as means ± SE of three parallel experiments. **P < 0.01, versus Ctrl group; #P < 0.05, ##P < 0.05, versus gp120 group.

Effect of naringin on $P2X_7$ mRNA expression in gp120-treated BV2 microglia

After total RNA extraction, the $P2X_7$ mRNA expression level in BV2 microglia was detected by RT-PCR. Results showed that the relative expressions of $P2X_7$ mRNA in the Ctrl, the gp120, the gp120+naringin and the gp120+BBG groups were 0.73 ± 0.06, 1.05 ± 0.06, 0.78 ± 0.05, and 0.81 ± 0.04, respectively. The expression level of $P2X_7$ mRNA in the gp120 group was significantly higher than that in the control group, indicating that gp120 can induce up-regulation of $P2X_7$ mRNA expression. The expression level of $P2X_7$ mRNA in the gp120+naringin group was much lower than that in the gp120 group ($P < 0.01$), while no significant difference appeared compared to the gp120+BBG group ($P > 0.05$). In order to ensure the accuracy of the results, we repeated the experiment using qPCR, and the results were similar with that of the RT-PCR. Based on the results obtained, we concluded that naringin was able to inhibit gp120-induced up-regulation of $P2X_7$ mRNA expression in BV2 microglia (Figure 4).

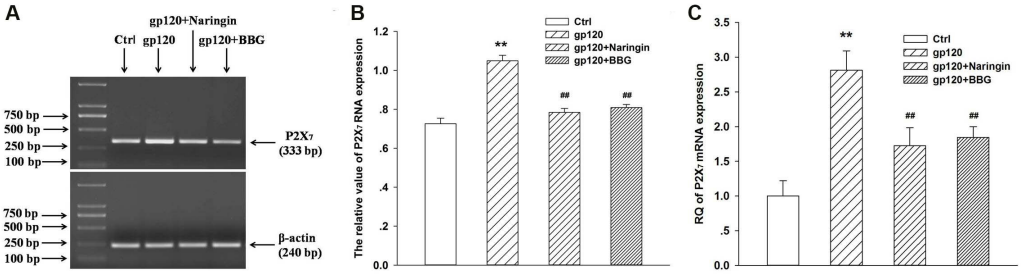

Figure 4. Effects of naringin on $P2X_7$ mRNA expression in BV2 microglia. **A.** agarose gel electrophoresis(AGE) of BV2 microglia PCR products in each group. **B.** The expression level of $P2X_7$ mRNA in BV2 microglia in each group by RT-PCR; **C.** the content of $P2X_7$ mRNA expression experiment repeated by qPCR. Values are reported as means ± SE of five parallel experiments. **P < 0.01, versus Ctrl group; ##P < 0.01, versus gp120 group.

Effect of naringin on $P2X_7$ protein expression in gp120-treated BV2 microglia

We used Western blot to measure the relative expression of $P2X_7$ and P65 proteins in BV2 microglia after extracting the total proteins. Results showed that the relative expression of $P2X_7$ receptor proteins in BV2 microglia in the Ctrl, the gp120, the gp120+naringin, and the gp120+BBG groups were 0.46 ± 0.04, 0.79 ± 0.04, 0.38 ± 0.07, and 0.42 ± 0.06 respectively. Statistical analysis revealed that the level of expression of $P2X_7$ receptor proteins in the gp120 group was significantly higher than that in the control group, indicating that gp120 can induce the up-regulation of $P2X_7$ receptor proteins expression. The expression of $P2X_7$ receptor proteins in the gp120+naringin group was much lower than that of the gp120 group ($P < 0.01$), while no significant difference appeared compared to the gp120+BBG group ($P > 0.05$), indicating that naringin was able to inhibit gp120-induced up-regulation of $P2X_7$ receptor protein expression in BV2 microglia (Figure 5).

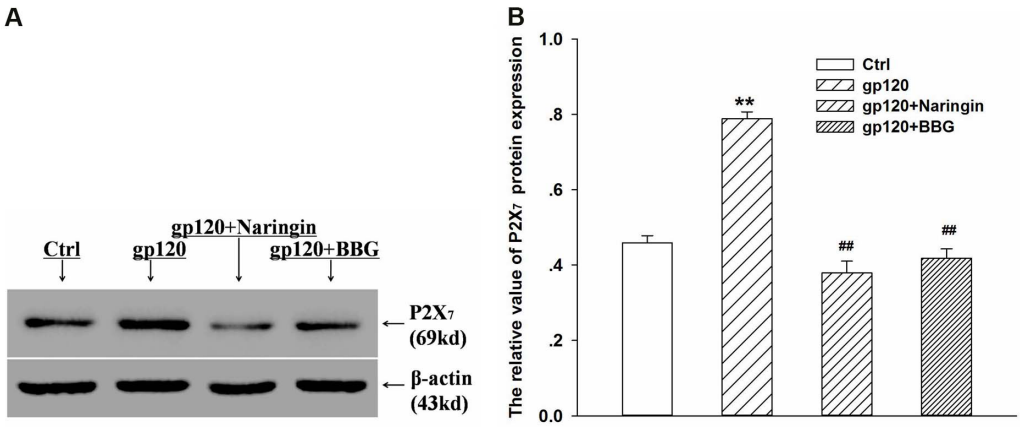

Figure 5. Effects of naringin on $P2X_7$ protein expression in BV2 microglia. **A.** SDS-PAGE electrophoresis of BV2 microglia $P2X_7$ receptor proteins in BV2 microglia in each group. **B.** The relative expression level of $P2X_7$ receptor proteins in BV2 microglia in each group detected by Western blot. Values are reported as means ± SE of five parallel experiments. $^{**}P < 0.01$, versus Ctrl group; $^{\#\#}P < 0.01$, versus gp120 group.

Effect of naringin on P65 protein expression in gp120-treated BV2 microglia

NF-κB with a sequence-specific dimeric structure is an important type of transcription factor existing widely in mammalian cells, which can bind specifically to a variety of cellular gene-promoter and -enhancer sequences (Kwak et al., 2011; Yamanaka et al., 2011; Byeon et al., 2012). Its activation, as a regulator of inflammation and oxidative stress reaction, is also involved in the process of cell proliferation and apoptosis (Goldin et al., 2006; Gratas-Delamarche et al., 2014). Hence, we made further investigations into the role(s) of P65 participation in gp120-induced cell injury in BV2 microglia. The relative expression levels of P65 proteins in the Ctrl, the gp120, the gp120+naringin and the gp120+BBG groups in the BV2 microglia cells were 0.47 ± 0.10, 0.81 ± 0.15, 0.57 ± 0.08, and 0.53 ± 0.06 respectively. Compared to the control group, the expression level of P65 proteins in the gp120 group increased

remarkably, indicating that gp120 can induce the up-regulation of P65 protein expression in BV2 microglia. The expression level of P65 proteins in the gp120+naringin group was much lower than that of the gp120 group ($P < 0.01$), indicating that naringin was able to inhibit gp120-induced up-regulation of P65 protein expression in BV2 microglia (Figure 6).

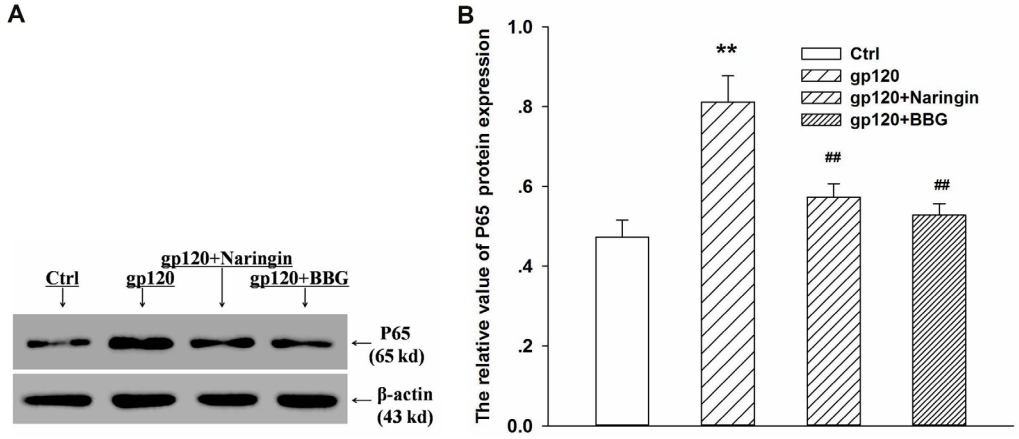

Figure 6. Effects of naringin on P65 protein expression in BV2 microglia. **A.** SDS-PAGE electrophoresis of BV2 microglia P65 proteins in BV2 microglia in each group; **B.** the relative expression level of P65 receptor proteins in BV2 microglia in each group detected by Western blot. Values are reported as means ± SE of five parallel experiments. **$P < 0.01$, versus Ctrl group; ##$P < 0.01$, versus gp120 group.

DISSUSSION

Presently, as mentioned above, the specific mechanism of ADC remains unclear - as such, there is no drug universally accepted as effective in treating ADC in clinical practice (Goodkin et al., 2014). Previous studies suggest that HIV freely crosses the blood-brain barrier or the blood cerebrospinal fluid barrier or via infected immune cells into the central nervous system, indirectly causing ADC and other neurodegenerative diseases (Wrasidlo et al., 2014). The main function of the HIV-1 envelope glycoprotein gp120 is to bind or attach the virus envelope to complementary surface receptors of the host cell, including the CD4, the B7 of T lymphocytes and other chemokine receptor complexes (Lynch et al., 2015). Subsequently, it activates the macrophages and microglia to produce a mass of chemokines and cytokines (Denieffe et al., 2013), such as inflammatory cytokines (e.g., TNFα and IL-1β) and arachidonic acid metabolites. These products can lead to cell injury and interfere with neurotransmitter functions, influencing learning and memory abilities and causing memory deficits (Kesby et al., 2015). To this effect, gp120-induced activation of macrophages/microglia is a critical factor in the occurrence of ADC or other neurodegenerative diseases (Lisi et al., 2012; Emileh et al., 2014). Studies have shown that gp120 can cause toxicity in rodent hippocampus neurons and human embryonic neurons. Similar results have been confirmed in animal experiments as well, where severe brain damage was observed when neonatal rats were given gp120 *in vivo* or adult rats were given ICV injection of gp120 (Potter et al., 2013). More importantly, gp120 may influence glutamate uptake in astrocytes and block glutamine supply for neurons, severely impacting learning and memory (Yu et al., 2007).

Our gp120 concentration screening experiment revealed that as gp120 concentrations increased, BV2 microglia cell-survival rate gradually declined. The cell injury experiment also indicated that the cell survival rate of BV2 microglia in the gp120 group was much lower than that of the control group. Taken together, these results suggest that gp120 exerts damaging effects on BV2 microglia. Furthermore, there was no apparent difference between the gp120+BBG and the control groups, indicating that $P2X_7$ receptors played a certain role in gp120-induced cell injury. This is possibly because the ATP produced by injured cells activated microglia through $P2X_7$ receptors, inducing the release of ATP in microglia through autocrines and enlarging ATP signals via cascade processes to over-activate the microglia and ultimately cause altered ion permeability and cell death (Burnstock et al., 2011; Kim et al., 2015).

The NF-κB transcription complex, which includes a series of homotype and heterotype dimers comprised of P50, P52, RelA (P65), RelB, RelC, and other subunits, can activate the expression of specific target genes after combining at DNA regulatory regions called JB loci (Chen and Chen 2015). NF-κB plays a critical role in immune response, inflammatory reaction, and cell growth regulation by controlling the expression of various crucial cytokines, adhesion molecules, and chemokines (Kwak et al., 2011; Yamanaka et al., 2011; Byeon et al., 2012). Moreover, NF-κB is also the main regulator of inflammation and oxidative stress reactions (Goldin et al., 2006; Gratas-Delamarche et al., 2014), and its activation is also involved in cell proliferation and apoptosis. Our western blotting results revealed that the expression of P65 proteins in BV2 microglia in the gp120 group increased markedly compared to other groups and was analogous to that of $P2X_7$ proteins, indicating that gp120 may induce cell injury and upregulate the expression of $P2X_7$ receptors, and P65 may be involved in the process.

$P2X_7$ plays a vital role in regulating the functions of microglia (Eyo et al., 2013), which can express $P2X_7$ receptors; the physiological functions of which are quite diverse, including the promotion of oxide and NO generation, T cell activation and maturation, and inflammatory cytokine production (Ficker et al., 2014). Previous research has shown that $P2X_7$ receptors can mediate the lengthy signal response of cells to extracellular proliferation, differentiation, and death by activating mitogen-activated protein kinase (MAPK) or other signaling pathways (Skaper et al., 2010). In this study, results showed that the expression of $P2X_7$ mRNA and proteins were both higher in the gp120 group than that in the control group. Hence, gp120 can induce the up-regulated expression of $P2X_7$ mRNA and proteins, thus resulting in cell injury or death most likely through signaling pathways mediated by $P2X_7$ receptors. Cell viability tests revealed that the cell survival rate of the gp120+naringin group increased over the gp120 group, showing that naringin exerted protective effects against gp120-induced injury.

Because naringin possesses anti-inflammation, anti-oxidative, and anti-stress properties among others, it is likely to alleviate HIV-induced ADC. In this study, the cell survival rate of the gp120+naringin group was similar to that of the gp120+BBG group. According to the results of the RT-PCR, the qPCR and the western blotting, naringin may exert protective effects against cell injury by inhibiting the gp120-induced up-regulated expression of $P2X_7$ receptors at the gene transcription and the protein translation levels. In our NO detection test, we found that compared with control group, NO levels of other three groups were increased, and the impact of which was analogous to the damage generated by the release of NO and other harmful factors in activated microglia (Lenglet et al., 2014). Because naringin and BBG possess analogous inhibitive effects on the gp120-induced release of NO in cells, it can be inferred that the protective effects of naringin on gp120-induced cell injury may be linked to its inhibition of NO release. It thus appears that naringin may affect the activity of

NF-κB via its inhibition effect on the release of NO.

In summary, gp120 does indeed damage cells by inducing the up-regulated expression of $P2X_7$ receptors, while naringin exerts protective effects against the said damage. Though quantifiable results were achieved in this study, the specific mechanisms at work in the above-mentioned damages and protective effects require further research. For instance, whether gp120 causes damage via cell signaling pathways mediated by $P2X_7$ or not, and whether naringin protects against cell injury by regulating inflammatory factors (such as IL-1β or TNFα) or by influencing cell signaling pathways remain unclear.

In this study, we investigated the probable mechanisms underlying ADC and other neurodegenerative diseases and presented a novel approach to the prevention and treatment of ADC. We hope that the findings presented here will provide a valuable resource for future research on innovative ADC treatment methods.

Conflicts of interest

The authors declare no conflict of interest.

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