

Original Research Article

Protective effect of liquiritin on corticosterone-induced neurotoxicity in PC12 cells

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Sent for review: 29 May 2018

Revised accepted: 23 September 2018

Abstract

Purpose: To determine the protective effects of liquiritin on corticosterone-induced neurotoxicity in rat pheochromocytoma (PC12) cells.

Methods: Neurotoxicity in PC12 cells was induced by different concentrations of corticosterone. Proliferation of PC12 cells was evaluated using CCK8 assay kits, while apoptosis was determined by flow cytometry.

Results: The results indicate that corticosterone inhibited the proliferation of PC12 cells time- and dose-dependently. The inhibitory effect (0.2 mM) was ameliorated by liquiritin. Furthermore, the cell apoptosis rate and protein level of caspase 3 in PC12 cells induced by corticosterone were ameliorated by liquiritin (1 and 2 mg/mL) treatment. Moreover, the protective effect of liquiritin (2 mg/mL) on corticosterone induced neurotoxicity in PC12 cells was weakened by K252a (the specific TrkB inhibitor) treatment. In addition, the protein level of brain-derived neurotrophic factor (BDNF) and (tyrosine-kinase receptor) TrkB showed a reverse trend to caspase 3.

Conclusion: Liquiritin shows protective effects against neurotoxicity induced by corticosterone in PC12 cells, and these effects are exerted via up-regulating BDNF/TrkB signaling.

Keywords: Liquiritin, Antidepressant, Corticosterone, Neuroprotection, PC12 cells, BDNF/TrkB signaling

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INTRODUCTION

Depression is a debilitating, life-threatening and frequently occurring psychiatric disorder. In addition to emotional abnormalities, most patients with depression suffer from organic damage to the nervous and mental system [1]. In recent years, a great number of studies have reported that corticosterone at high levels could

induce depressive-like behavior in rats, which can be improved by acupuncture and antidepressant treatment [2,3]. Moreover, corticosterone induce changes in brain anatomy and neurochemistry, both of which are closely associated with depression [4]. Brain-derived neurotrophic factor (BDNF) is one kind of major neurotrophic factors in adult brain. A large number of studies show that BDNF expression

were reduced in depressed patients or rodents after corticosterone treatment [4,5]. Meanwhile, the expression of BDNF were up-regulated by classical antidepressants in depressed patients and rats brain [6]. This means that BDNF is an important target of the classical antidepressants.

Pheochromocytoma (PC12) cell is the most commonly used neuronal cell lines for neuroscience research [7]. Previous study indicated that corticosterone at high concentration induces cell injury and cell death in pheochromocytoma PC12 cells and hippocampal neurons, and these neurotoxicity caused by corticosterone were ameliorated by classical antidepressants [8]. These reports indicate that antidepressants possesses neuroprotective and neurotrophic activities in PC12 cells. Liquiritin is the main content extracted from *Glycyrrhiza Radix*, and it has been reported to possess anticancer and anti-inflammatory abilities [9,10]. Liquiritin also showed antidepressant-like effects in the tail suspension test and forced swimming test in mice [10]. Whereas, the specific molecular mechanism is not yet clear. Thus, this study has tested the neuroprotective effects of liquiritin on corticosterone-induced neurotoxicity *in vitro*, and investigated the involvement of BDNF signaling pathway in this process.

EXPERIMENTAL

Chemicals and reagents

Corticosterone and liquiritin were obtained from the Aladdin company (Shanghai, China). CCK-8 and Annexin V-FITC apoptosis detection kits were purchased from Beyotime Biotechnology (Shanghai, China). The primary antibodies of BDNF, TrkB and Caspase 3 were purchased from Abcam (MA, USA). The primary antibody of GAPDH was purchased from CST (MA, USA).

Effects of corticosterone on the proliferation of PC12 cells

PC12 cell line was offered by Shanghai Institute of Biochemistry and Cell Biology (SIBCB, Shanghai, China). This study was approved by the Ethics Committee of the hospital. PC12 cells were treated with corticosterone (0, 0.2, 0.4 and 0.8 mM) for 72 h. The proliferation of PC12 cells were determined by CCK-8 assay kit at 0, 24, 48 and 72 h after treatment.

Effect of different concentrations of liquiritin on proliferation of corticosterone treated PC12 cells

PC12 cells were seeded into 96-well culture plate

(2×10^4 cells per well), and cultured with corticosterone (0.2 mM) at 37 °C for 48 h. Then, liquiritin (0, 0.25, 0.5, 1, 2 and 4 mg/mL, in dimethyl sulfoxide, DMSO) was added. Those cells were cultured for 72 h at normal condition, and the proliferation of PC12 cells was measured using CCK-8 assay kit at 0, 24, 48 and 72 h after treatment.

Evaluation of protective effects of liquiritin

PC12 cells were divided into 5 groups: control group, corticosterone (0.2 mM) treatment group, corticosterone (0.2 mM) + liquiritin (1 mg/mL) group, corticosterone (0.2 mM) + liquiritin (2 mg/mL) group, corticosterone (0.2 mM) + liquiritin (2 mg/mL) group and corticosterone (0.2 mM) + liquiritin (2 mg/mL) + K252a (10 nM) (the BDNF/TrkB signaling inhibitor [11]) group.

The cells in the latter four groups were cultured with corticosterone (0.2 mM) at normal condition. Then, the cells were cultured with corresponding drugs for another 48 h. Cell samples were harvested at the end. The cell apoptosis rate was detected by flow cytometry using Annexin V-FITC/PI apoptosis detection kit. Moreover, proteins levels of BDNF, TrkB and caspase3 were tested by Western blotting.

Cell proliferation and apoptosis

PC12 cells were seeded in 96-well culture plates at a concentration of 2×10^4 per well. Then, the cell proliferation of PC12 cells were determined using CCK-8 assay kit according to the manufacturer's instructions. The PC12 cells apoptosis was determined using FITC-labelled annexin-V/PI assay kit. Cells in each group (1×10^6) were cultured with FITC-labelled annexin-V and PI for 20 min at 25 °C. The intensity of PI or annexin-V fluorescence was determined by FACScan (Becton-Dickinson, CA, USA); 10,000 cells were assessed in each sample.

Western blotting assay

The protein expressions of BDNF, TrkB and caspase3 were measured by western blotting.

The total protein was extracted from PC12 cells, separated by SDS-PAGE, then transferred onto the PVDF membranes. After incubation with the primary antibody at 4 °C for 15 h, the membranes were further incubated with a secondary antibody at 4 °C for 2 h. Protein bands were determined using an ECL-detecting kit (Beyotime Biothech, Shanghai, China); GAPDH protein served as loading control.

Statistical analysis

Data are expressed as mean ± standard deviation. The statistical analysis was performed by Student's t-test and ANOVA using SPSS 20.0 software (IBM, Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Effect of corticosterone on proliferation of PC12 cells

The proliferation of PC12 cells was measured at 0, 24, 48 and 72 h after treatment using CCK-8 assay kit. As shown in Figure 1, the proliferation of PC12 cells was time and dose-dependently decreased by corticosterone treatment. In addition, corticosterone, at a low dose (0.2 mM), inhibited the proliferation of PC12 cells. Thus, this concentration was used in subsequent experiments.

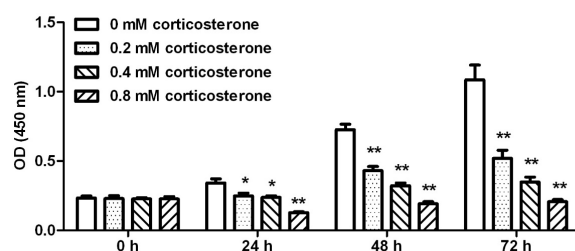


Figure 1: Effects of corticosterone on the proliferation of PC12 cells (testing by CCK-8 assay); $p < 0.05$ and $p < 0.01$, compared with 0 mM corticosterone group

Effect of concentration of liquiritin on proliferation of corticosterone-treated PC12 cells

The proliferation of PC12 cells was unchanged by liquiritin at lower concentrations in a short time (Figure 2). However, at longer treatment times and doses, the proliferation of corticosterone treated PC12 cells was increased by liquiritin (2 and 4 mg/mL) after 48 and 72 h (1, 2 and 4 mg/mL) of incubation (Figure 2).

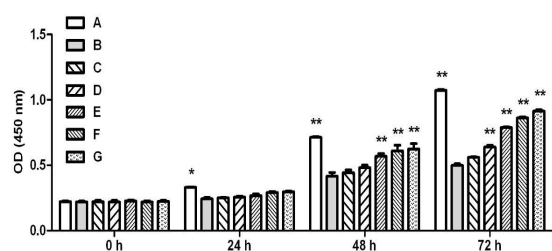


Figure 2: Effect of different concentration of liquiritin on the proliferation of corticosterone treated PC12 cells. A: control group, B: corticosterone (0.2 mM) +

liquiritin (0 mM) group, C: corticosterone (0.2 mM) + liquiritin (0.25 mM) group, D: corticosterone (0.2 mM) + liquiritin (0.5 mg/mL) group, E: corticosterone (0.2 mM) + liquiritin (1 mg/mL) group, F: corticosterone (0.2 mM) + liquiritin (2 mg/mL) group, G: corticosterone (0.2 mM) + liquiritin (4 mg/mL) group; $p < 0.05$ and $p < 0.01$, compared with group B

Protective effects of liquiritin against corticosterone-induced neurotoxicity

To clarify the involvement of BDNF in the process of neuroprotection, BDNF/TrkB signaling inhibitor, K252a, was used [11]. The apoptosis rate in each group was measured by flow cytometry and proteins levels of BDNF, TrkB and the pro-apoptotic cleaved form caspase3 [12] were tested by western blotting.

The results indicate the cell apoptosis rate as well as the protein expression of caspase3 were increased by corticosterone-induced neurotoxicity in PC12 cells, and the increased effects were ameliorated by liquiritin (1 and 2 mg/mL) at 48 h after treatment (Figure 3 and Figure 4). Whereas, this protective effect of liquiritin (2 mg/mL) was abated by K252a treatment. In addition, the proteins level of BDNF and TrkB in PC12 cells were negative correlation with the expression of caspase 3.

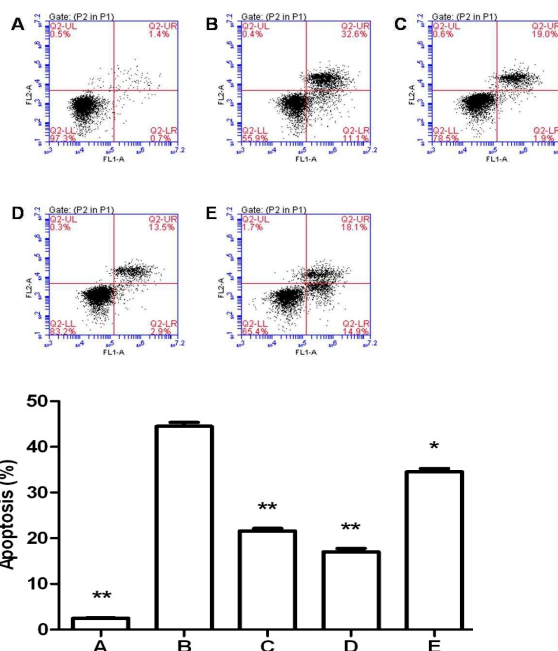


Figure 3: Liquiritin protected against corticosterone-induced increased cell death in PC12 cells. A: control group, B: corticosterone (0.2 mM) treatment group, C: corticosterone (0.2 mM) + liquiritin (1 mg/mL) group, D: corticosterone (0.2 mM) + liquiritin (2 mg/mL) group, E: corticosterone (0.2 mM) + liquiritin (2 mg/mL) + K252a (10 nM) group; $p < 0.05$ and $p < 0.01$, compared with group B

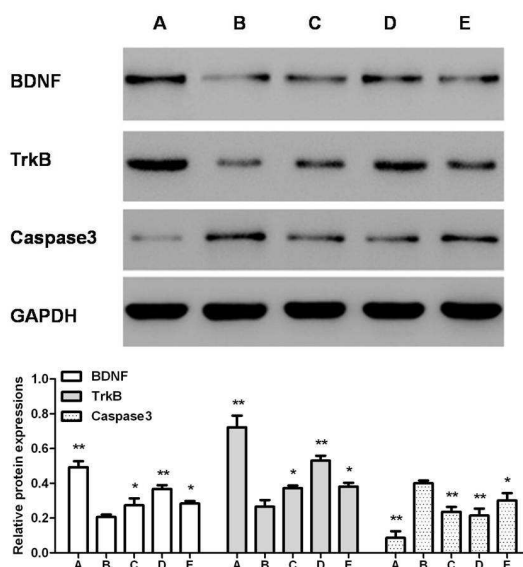


Figure 4: Effect of liquiritin on the protein expression of BDNF, trkB and caspase3 in PC12 cells. A: control group, B: corticosterone (0.2 mM) treatment group, C: corticosterone (0.2 mM) + liquiritin (1 mg/mL) group, D: corticosterone (0.2 mM) + liquiritin (2 mg/mL) group, E: corticosterone (0.2 mM) + liquiritin (2 mg/mL) + K252a (10 nM) group; $p < 0.05$ and $p < 0.01$, compared with group B

DISCUSSION

Depression is one of mental disorders characterized by decreased interest and pleasure and lowered mood. More serious is that the morbidity will increase, if there is no remedy, according to WHO's prediction [13]. PC12 cell line is the most commonly used neuronal cell lines for neuroscience research *in vitro* studies [7,14]. Liquiritin is the main content extracted from *Glycyrrhiza Radix*, and it has been reported to possess a variety of biological activities [9,10]. In the present study, liquiritin showed protective effects on corticosterone-induced neurotoxicity in PC12 cells via up-regulation of BDNF/TrkB signaling.

In recent years, a neurotrophic hypothesis has been used to explain the mechanisms of antidepressant drugs and the pathophysiology of depression. According to this hypothesis, the vulnerability to stress-induced depression is as a result of changes in expression levels of trophic factors in neurons which are innervated by monoaminergic projections [15]. Furthermore, BDNF hypothesis postulates that stress decrease the expression of BDNF and this could be reversed by anti-depression therapy [16,17]. In particular, BDNF is a member of the neurotrophin family which promotes neuronal viability in adulthood [18]. The functions of BDNF are modulated by the activation of intracellular

signaling pathways upon high affinity binding to the trkB receptor [19,20]. The present results indicate that the protein expressions of BDNF and trkB were down-regulated in corticosterone treated PC12 cells. This symptom was ameliorated by liquiritin (1 and 2 mg/mL).

Thus, liquiritin treatment protects against corticosterone-induced decreased cell proliferation and increased cell death in PC12 cells. Liquiritin also shows antidepressant-like effects on corticosterone-induced neurotoxicity in PC12 cells and the possible mechanism of which involves the up-regulation of BDNF/trkB signaling. Therefore liquiritin is a potential therapeutic drug for depression.

DECLARATIONS

Acknowledgement

This work was funded by Zhejiang Science and Technology Program of Traditional Chinese Medicine (nos. 2015ZB093), Hangzhou City Health Science and Technology Plan Project (nos. 2016A58), Hospital Pharmacy Special Research Project of Zhejiang Pharmacy (nos. 2014ZYY19) and Health Science and Technology Program of Hangzhou Obstetrics & Gynecology Hospital (nos. 2015YJB05 and 2017YJA03).

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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