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Saikosaponin D acts against corticosterone-induced apoptosis via regulation of mitochondrial GR translocation and a GR-dependent pathway

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ABSTRACT

Saikosaponin D is an agonist of the glucocorticoid receptor (GR), and our preliminary study showed that it possesses neuroprotective effects in corticosterone-treated PC12 cells. However, further proof is required, and the molecular mechanisms of this neuroprotection remain unclear. This study sought to further examine the cytoprotective efficiency and potential mechanisms of action of Saikosaponin D in corticosterone-treated PC12 cells. The cells were treated with 250 μM corticosterone in the absence or presence of Saikosaponin D for 24 h; cell viability was then determined, and Hoechst 33342/propidium iodide (PI) and annexin/PI double staining, and TUNEL staining were performed. Next, mPTP, MMP, $[Ca^{2+}]i$, translocation of the GR to the nucleus and Western blot analyses for caspase-3, caspase-9, cytochrome C, GR, GILZ, SGK-1, NF-Kb (P65), IκB-α, Bad, Akt, Hsp90 and HDAC-6 were investigated. The neuroprotective effects of Saikosaponin D were further confirmed by Hoechst 33342/PI, annexin/PI and TUNEL staining assays. These additional data suggested that Saikosaponin D partially reversed the physiological changes induced by corticosterone by inhibiting the translocation of the GR to the mitochondria, restoring mitochondrial function, down-regulating the expression of pro-apoptotic-related signalling events and up-regulating anti-apoptotic-related signalling events. These findings suggest that SSD exhibited its anti-apoptotic effects via differential regulation of mitochondrial and nuclear GR translocation, partial reversal of mitochondrial dysfunction, inhibition of the mitochondrial apoptotic pathway, and selective activation of the GR-dependent survival pathway.

1. Introduction

Major depression (MD) is frequently combined with mental and endocrine disorders and is a common and public disease that ranks among the leading causes of disability worldwide (Murray and Lopez, 1997; Nemeroff, 1998). The neurobiological explanations of current treatments for MD are inadequate, and recent research has looked beyond the monoamine hypothesis to other pathological processes, such as stress and neuroplasticity (Delgado, 2000; Nestler et al., 2002). Exposure to psychological and physiological-related chronic stress is one cause of the activation of the hypothalamic–pituitary–adrenal (HPA) axis, which initiates a sustained rise in glucocorticoids that is involved in the pathogenesis of depression.

Glucocorticoids are a family of steroid hormones that are relevant to energy metabolism and are secreted from the adrenal cortex under the control of the HPA axis; this release principally accompanies episodes of stress (Aihara et al., 2007; Sapolsky, 2000). As a part of the stress response, glucocorticoid levels seem to regulate hippocampal positive feedback on HPA axis activity; however, sustained elevations of glucocorticoids can trigger dysfunction of the HPA axis and reduce the birth of new granule dentate gyrus cells (Fuchs and Gould, 2000; Gregus et al., 2005; McEwen, 2000). Moreover, post-mortem analyses have revealed a reduction in the numbers of neuronal cells in the hippocampi of patients with MD (Stockmeier et al., 2004). Therefore, the progression of glucocorticoid-induced apoptosis within the central nervous system (CNS) might be a significant cause of MD, and glucocorticoids have also been proven to modulate certain key cellular events within the CNS that include synaptic plasticity (Kim et al., 2001), neurotransmitter

Abbreviations: MD, major depression; HPA axis, hypothalamic–pituitary–adrenal axis; CNS, central nervous system; GC, glucocorticoid; GR, glucocorticoid receptors; mPTP, mitochondrial permeability transition pore; MMP, mitochondrial membrane potential; GILZ, glucocorticoid induced leucine zipper; SGK-1, serum and glucocorticoid inducible kinase-1; NF-κB, nuclear transcriptional factor kappa B; IκB-α, NF-kappa B inhibitor alpha; Akt, serine/threonine protein kinase; Hsp90, heat shock protein 90; HDAC-6, histone deacetylase-6; CCK-8, Cell Counting Kit-8; TUNEL, terminal-deoxynucleotidyl transferase mediated nick end labelling.

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receptor expression (Meijer et al., 1997), and the actions of neurotoxins (Brooke et al., 1998). Thus, this growing body of results suggests that an anti-depressant effect may result from mitigating glucocorticoid-induced neurotoxicity.

GC-induced apoptosis is a complex process that is tightly regulated via intracellular GC receptors, and these glucocorticoid receptors (GR) are necessary for the initiation of the multi-component process that requires contributions from both genomic and cytoplasmic signalling events (Beato and Sanchez-Pacheco, 1996; Chen and Qiu, 1999). In the cellular resting state, GR are localised primarily within the cytosolic fraction, but in situations of transactivation and transrepression, the activated GRs modify gene transcription, and these changes in expression may underlie the induction of apoptotic or anti-apoptotic signals (Grugan et al., 2008; McEwan et al., 1997; Morsink et al., 2006). Furthermore, the translocation of GRs to the mitochondria and protein–protein interactions in the non-genomic pathway are both essential and sufficient for inducing the expression of pro-apoptotic proteins and inducing apoptosis (Sionov et al., 2006a,b). Taken together, these findings suggest that it is likely that interference with these GR-relative signal transduction pathways might contribute to the suppression of GC-induced neuronopathies in chronic stress during the MD.

Bupleurum yinchowense is a famous medicinal plant in China. The total saikosaponins from *B. yinchowense* have been proven to possess antidepressant-like effects in both acute and chronic unpredictable mild stress models in vivo (Sun et al., 2012) and neuroprotective effect against corticosterone-induced apoptosis in PC12 cells in vitro (Li et al., 2013). Saikosaponin D is one of the main constituents of the total saikosaponins, and its structure is shown in Fig. 1. Japanese scholars have demonstrated that saikosaponins exhibit a correlative relationship with steroids and exhibit GR agonist-like actions (Hattori et al., 2008; Hiai et al., 1981). Our preliminary experiment revealed that Saikosaponin D prevents the neurotoxicity induced by corticosterone in PC12 cells, which have been widely used as an in vitro experimental model of depression (Mao et al., 2011; Zheng et al., 2011). However, further proof regarding the protective activity of Saikosaponin D against corticosterone-induced damage is necessary, and the mechanisms by which SSD suppress apoptosis remain unclear.

The aim of this study was to further prove the neuroprotective effects of Saikosaponin D against corticosterone-induced damage in PC12 cells and to investigate whether the GR agonist-like action contributes to the anti-apoptotic effect of Saikosaponin D. Our results suggest that the anti-apoptotic effects of Saikosaponin D are mediated by the partial inhibition of the mitochondrial apoptotic pathway and the selective activation of the GR-dependent survival pathway.

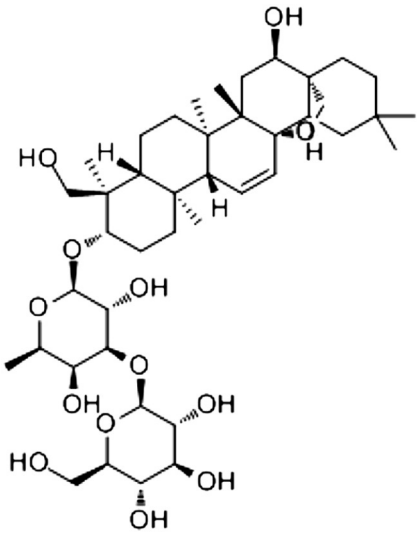

Fig. 1. Chemical structure of Saikosaponin D.

2. Materials and methods

2.1. Chemicals and reagents

Saikosaponin D was prepared by our laboratory. The compound was a white powder with a purity over 98% as verified with high performance liquid chromatography (HPLC). Foetal bovine serum, heat-inactivated horse serum, penicillin and streptomycin were purchased from Gibco (Grand Island, NY, USA). Corticosterone, Dulbecco's Modified Eagle Medium (DMEM) and thiobarbituric acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Primary antibodies for GAPDH (1:200), caspase-3 (1:200), caspase-9 (1:200), cytochrome C (1:200), GR (1:200), GILZ (1:200), SGK-1 (1:200), NF-Kb (1:200), IκB-α (1:200), bad (1:200), phosphorylated-bad (1:200), phosphorylated-Akt (1:200), Akt (1:200), Hsp90 (1:200),and HDAC-6 (1:200) and the secondary antibodies labelled with horseradish peroxidase-conjugated goat anti-mouse or goat anti-rabbit IgG (1:1000) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other chemicals and reagents were of analytical grade.

2.2. Cell culture and treatment

PC12 cells were grown in DMEM supplemented with 10% (v/v) heat-inactivated foetal bovine serum, 5% (v/v) heat-inactivated horse serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were cultured in a humidified 95% air and 5% CO_2 atmosphere at 37 °C. Cells in the exponential phase of growth were used for all experiments. To study the neuroprotective effects of Saikosaponin D, PC12 cells were divided into non-treated control, corticosterone (250 μmol/ml) and corticosterone (250 μmol/ml) plus Saikosaponin D groups for all experiments. Saikosaponin D was applied 24 h prior to treatment with corticosterone and was also present in the medium during the incubation with corticosterone.

2.3. Cell viability assay

Cell viability was evaluated with the Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Kumamoto, Japan). In brief, PC12 cells cultured in 96-well plates were treated as above and then gently washed with PBS. After washing, 90 μl of culture medium and 10 μl of CCK-8 solution were added to each well. The plate was maintained at 37 °C for 2 h, and the absorption was measured at 450 nm on a microplate reader (Spectrafluor, TECAN, Sunrise, Austria). Cell viabilities are expressed as percentages of the control.

2.4. Assessment with Hoechst 33342 and propidium iodide (PI) double staining

To further investigate the protective effects of SSD, Hoechst 33342 and PI double fluorescent staining was assayed. Annexin V/propidium iodide (PI) assay kits were purchased from Invitrogen (Eugene, OR, USA). The PC12 cells were cultured on coverslips in 24-well plates for 24 h. After the this treatment, the cells were incubated with 5 mg/ml Hoechst 33342 for 15 min, washed twice with PBS, incubated with 1 μg/ml PI working solution for an additional 15 min (Saravia et al., 2009), and then visualised by inverted fluorescence microscopy (Leica, Germany). The apoptotic nuclei were counted in at least 200 cells from five randomly selected fields in each treatment and are expressed as percentages of the total numbers of counted nuclei.

2.5. Assessment with annexin V and PI double staining

To corroborate the protective effect of Saikosaponin D, annexin V and PI double staining were detected with flow cytometry. An early indicator of apoptosis is the translocation of the membrane phospholipid phosphatidylserine from the cytoplasmic interface to the extracellular