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Asiaticoside: Attenuation of rotenone induced oxidative burden in a rat model of hemiparkinsonism by maintaining the phosphoinositide-mediated synaptic integrity

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ABSTRACT

Asiaticoside (AS), a triterpenoid saponin isolated from the Indian medicinal herb *Centella asiatica* is known to exert a neuroprotective effect by attenuating the neurobehavioral, neurochemical and pathological changes in animal models. However, its potential neuroprotection in rotenone-induced hemiparkinsonism which implicates phospholipid-mediated neurotransmission remains unclear. Therefore, we have investigated the neuroprotective effects of AS in rat model of ROT-infused hemiparkinsonism with respect to phosphoinositides-assisted cytodynamics and synaptic function. Adult male Sprague-Dawley rats (250–300 g) were distributed randomly into 6 groups, with 6 rats in each group: Sham control, Vehicle control (DMSO-0.1%), ROT-infused group (6 μg/μl/kg), AS-treated group (50 mg/kg/day), Drug (AS) control and Levodopa (L-DOPA)-treated group (6 mg/kg/day). At the end of the experimental period, the rats were sacrificed after performing behavioral analyses and the striatum regions were dissected out. Phosphoinositides (PI) are involved in intrinsic membrane signals that regulate intracellular membrane trafficking vesicle and endocytosis. We have assessed *mRNA* and protein expressions of genes involved in PI-mediated signaling and also in synaptic function (*PI3K*, *PDK 1*, *PEBP*, *Stx 1A* and TH) in addition to the levels of neurotransmitters and the enzymatic antioxidant profile. AS caused an improved working memory and motor co-ordination in the ROT group. It alters the levels of neurotransmitters ($p < 0.01$), the expression of *mRNA* and protein assessed which were significantly affected ($P < 0.001$) by rotenone, thus exhibiting its intervention in the progression of neurodegeneration. We demonstrate that AS can mediate distinct function in PI-assisted vesicle endocytosis, cytoprotective signaling and in the synaptic function thereby mitigating the ROT-infused hemiparkinsonism, however, its specific regulatory role remains to be unraveled.

1. Introduction

Asiaticoside (AS), a major pentacyclic triterpenoid saponin component of *Centella asiatica* (*C. asiatica*), is well documented to possess the properties of learning and memory enhancement (Mukherjee, 1953), wound healing (Singh et al., 2010), anti-inflammatory potentials (Somchit et al., 2004), and is reported to improve the general mental ability of mentally retarded children (Rao and Rao, 1973). *C. asiatica* (Umbelliferae) in which the asiaticoside is present in abundance is a herbaceous plant, found throughout India. The plant is known as Brahmi in *Sanskrit* and is used in the Indian system of medicine, Ayurveda (Kumar, 2000), for improving the mental ability. As a dietary supplement, *C. asiatica* is used to treat sleep disorders in patients with mental health conditions. In pharmacological studies, the plant extract was shown to exert an anti-depressant activity (Chatterjee et al., 1992). In rats, it was shown that long-term pretreatment with *C. asiatica* has improved elevated plus-maze performance, and attenuated the acoustic startle response (ASR) (Bradwejn et al., 2000). Furthermore, it could enhance gamma amino butyric acid concentrations in the brain of rats (Chatterjee et al., 1992). The administration of *C. asiatica* during postnatal developmental stage was reported to influence the neuronal morphology and promote higher brain function of both juvenile as well as young adult mice (Rao et al., 2005). Our earlier lab results have shown

Abbreviations: AS, Asiaticoside; ROT, Rotenone; L-DOPA, Levodopa; DMSO, Dimethyl sulfoxide; PI, Phosphoinositides; PE, Phosphatidylethanolamine; PD, Parkinson's disease; SNpc, Substantia nigra pars compacta; ROS, Reactive oxygen species; ATP, Adenosine triphosphate; DNA, Deoxyribonucleic acid; RNA, Ribonucleic acid; AD, Alzheimer's disease; HD, Huntington's disease; SOD, Superoxide dismutase; CAT, Catalase; LPO, Lipid peroxidase; GPx, Glutathione peroxidase; GSH, Reduced glutathione; PEBP, Phosphatidylethanolamine binding protein; Syn 1, Synaptojanin 1; Stx 1A, Syntaxin 1A; PI3K, Phosphoinositide 3-kinase; PDK1, Phosphoinositide-dependent kinase-1; VMAT 2, Vesicular monoamine transporter 2; BDNF, Brain-derived neurotrophic factor; TH, Tyrosine hydroxylase; NGF, Nerve growth factor; MAPK, Mitogen-activated protein kinase; mTOR, Mammalian target of rapamycin; NMDA, *N*-methyl-D-aspartate receptor; HCNP, Hippocampal cholinergic neurostimulating Peptide; qRT PCR, Quantitative real-time polymerase chain reaction.

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that the AS (Fig. 1) could exert significant neuroprotection against MPTP-induced neurotoxicity (Sampath and Janardhanam, 2013); this inspired us to carry out further investigations on the effect of AS against hemiparkinsonism.

Parkinson's disease (PD) is characterized by selective degeneration of A9 dopaminergic (DA) neuron loss of neuromelanin-containing neurons substantia nigra pars compacta (SNpc) (Rinne et al., 1989). PD is a unique human disease that is clinically characterized by cardinal motor symptoms such as postural instability, bradykinesia and resting tremor (Savitt et al., 2006). PD is characterized by non-motor symptoms such as sleep disorders, depression, hyposmia and autonomic dysfunction (Comella, 2007; Dubow, 2007; Ziemssen and Reichmann, 2007).

The cause and mechanisms of pathological complications of PD in the majority of cases remain largely unknown. Rotenone (ROT), a commonly used natural pesticide extracted from the roots of tropical plants, such *as Derris elliptica*, is able to freely cross the blood brain barrier, plasma membrane and mitochondrial membranes thus inducing PD-like symptoms. Radiolabeled (3H) dihydrorotenone was found to bind striatal sections from rodent brains with a Kd of ~55 nM (Higgins and Greenamyre, 1996). Dopaminergic neurodegeneration and the occurrence of cytoplasmic inclusions similar to Lewy bodies (Betarbet et al., 2000) on ROT exposure have been reported. ROT inhibits mitochondrial complex I and generates ROS (Li et al., 2003; Panov et al., 2005) and dopamine localization from vesicles to the cytosol which is involved in ROT-infused apoptosis. Dopamine itself can be autoxidized to form a reactive species of dopamine which covalently binds to a variety of molecules including proteins and nucleic acids (Fornstedt, 1990).

The membrane phospholipid, phosphatidylinositol is phosphorylated at different positions thereby producing distinct second-messengers known as phosphoinositides (PIs). PIs regulate cell homeostasis and mutations in the enzymes that are involved in the metabolic cycling of the PIs have been reported in the diseased conditions (Wen et al., 2011). PIs were initially characterized as mediators of growth factor-induced signal transduction events at the plasma membrane (Toker and Cantley, 1997). Their involvement in vesicular trafficking was first understood from the identification of Vps34, a PtdIns 3-kinase (PI3K) that regulates transport between the Golgi and lysosome (Schu et al., 1993). A study had shown that synaptic vesicle trafficking pathways are implicated in neurodegeneration (Esposito et al., 2012) and the implication of α-synuclein in synaptic vesicle exocytosis and synaptic vesicle recycling (Burré et al., 2010; Nemani et al., 2010). PIs are acted upon by PI3K, synthesized at the cytosolic face of cellular membranes where they. The PI3K product, PtdIns 3-phosphate plays a major role in endocytic trafficking (Wurmser et al., 1999; Gillooly et al., 2001) and by recruiting or activating the effector proteins, they mediate signaling (Wurmser et al., 1999). We were much interested to appreciate the *mRNA* and protein expressional variations of enzymes involved in phospholipid assisted trafficking. We used a rat model of hemiparkinsonism for evaluating the anti-PD effect of AS on the PI assisted neuroprotection by assessing the *mRNA* and protein expressions of PI related protein in order to assess the PI-mediated vesicular mechanisms of neuroprotection by AS. The effects of AS on learning and memory activity, motor co-ordination, contents of dopamine, glutamate and redox status in the nigrostriatal system of ROT-infused hemiparkinsonism in rats were also investigated.

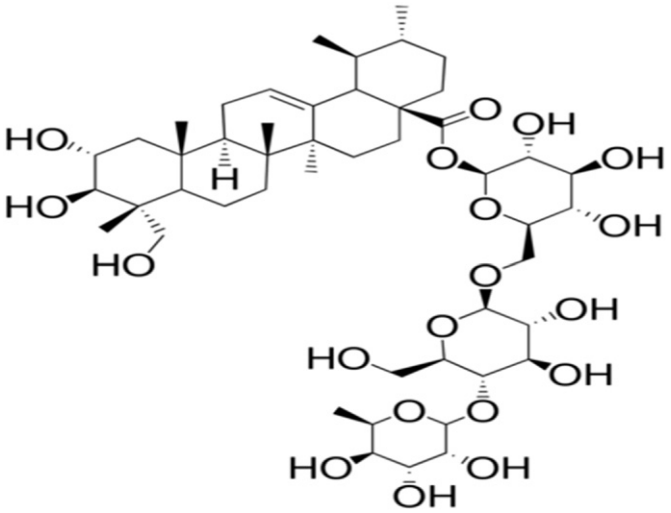

Fig. 1. The structure of asiaticoside.

2. Materials and methods

2.1. Drugs and chemicals

All drugs and chemicals used in the study were of molecular and analytical grade and they were purchased from Sigma Chemical Company (St. Louis, MO, USA), Amersham Biosciences and Sisco Research Laboratories (Mumbai, India). The following antibodies were used in our study: PI3K-#4292, PDK1-#3062, MAPK-#9212 and mTOR-#2972 purchased from Cell Signaling Technology (Danvers, MA, USA), Anti-Tyrosine hydroxylase (TH-SAB 4,502,964) obtained from Sigma Aldrich (USA) and NGF (sc-548), α-Tubulin (sc-5286) procured from Santa Cruz Biotechnology, Inc. (USA). ROT was dissolved in 1:1 DMSO and polyethylene glycol. AS was dissolved in hot water and given orally.

2.2. Collection of plant materials and extraction

Wild *C. asiatica* (*Umbelliferae*) was collected from the Kanchipuram, Tamil Nadu, India. The plant was identified and authenticated by Department of Medical Botany, National Institute of Sidda, An Autonomous body under the Ministry of AYUSH, Govt. Of India, Tambaram Sanatorium, Chennai (Certificate Number: NISMB1462014). The fresh leaves with petioles were air dried in shadow and ground by mechanical grinder. Fine plant powder was then used for the exhausted extraction by Soxhlet apparatus for four repeated cycles using 100% ethanol, 50% ethanol and water as an extraction solvent. During extraction the solute-solvent ratio was 10:1 and extraction temperature was 45° ± 2 °C. The extracts were then filtered, evaporated using Rota evaporator to a thick residue at 45 °C and stored at 4 °C. Then amount of dried leaves used were 588 g and the obtained crude extract was 292.50 g. This crude extract was used for further analyses (Rahman et al., 2013).

2.3. Fractionation, chromatography and isolation of compounds

The (100 g) residue was portioned in an Ethyl acetate (EtAc) water mixture (1:1) ratio. (100 g) EtAc extract was mixed with silica gel to make a slurry, separate the sections and then were subjected to column chromatography [silica gel (mesh 70–230, 250 g), column, 5 × 50 cm] eluted with 1 l eluent of a polarity started from 85% $CHCl_3$ to 75% MeOH, The fractions

were 100 ml with 5 μl/min flow rate. The fractions were checked on TLC (triterepnoids contents) using a mobile phase ($CHCl_3/CH_3OH/H_2O$ 10/3/0.2 respectively) and vanillin reagent as the detecting agent (data not shown) the most interesting compound-AS. The yield of extraction is 3500 mg.

2.4. High Performance Liquid Chromatography (HPLC) analysis

Triterpene standard (asiaticoside) stock was made using 1 mg (Sigma, France) dissolved in 2 ml HPLC grade methanol and then diluted further to obtain concentrations of 50, 100 μg/ml, respectively. HPLC analysis was carried out in Shimadzu Europe, plus detector connected to computer with LC solution software. The column used was a brand new product of