Bacoside-A exerts protective effect against Parkinson's disease-induced functional damage in mice via inhibition of apoptosis and oxidative response

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Abstract

Purpose: To determine the effect of bacoside-A on Parkinson's disease (PD) in a rat model, and elucidate its mechanism of action.

Methods: A rat model of PD was established by administration of 5 µL of 6-hydroxydopamine in ascorbic acid (0.1 %). Measurement of serum levels of inflammatory factors was carried out using enzyme-linked immunosorbent assay (ELISA) kits. Western blotting was used to assay Bax, cytochrome c and Bcl-2 in rat hippocampus.

Results: Bacoside-A treatment significantly reduced PD-induced high turning values in rats (p < 0.05). Treatment with bacoside-A reversed PD-mediated suppression of serum activities of CAT and glutathione peroxidase (GPx). In bacoside-A-treated PD rats, dose-dependent suppression of acetylcholinesterase (AChE) and inducible nitric oxide synthase (iNOS) activities were observed (p < 0.05). Bacoside-A-treated PD rats significantly (p < 0.018) reduced interleukin (IL)-1β and IL-6 levels. Treatment of PD rats with bacoside-A effectively reduced levels of tumor necrosis factor (TNF)-α, NF-kB p65, (COX)-2 and p53 protein, and also reversed up-regulations of Bax, cytochrome C, caspase-3 and caspase-9.

Conclusion: Bacoside-A exhibits a protective effect against Parkinson disease-induced oxidative damage and neuronal degeneration in rats through downregulation of iNOS, AChE, inflammatory cytokines and pro-apoptotic proteins. Therefore, bacoside-A has potentials for use in the management of Parkinson disease.

Keywords: Parkinson disease, Neuroprotective, Pro-apoptotic, Cytokines, Neurotoxicity

INTRODUCTION

Parkinson's disease (PD) is the second most frequently diagnosed neurodegenerative disorder after Alzheimer's disease [1, 2]. The pathogenesis of PD starts with degeneration of dopamine neurons present in the substantia nigra pars compacta, followed by aggregation of...
ubiquitinated α-synuclein in the other neurons [1]. Attempts are being made to identify effective and therapeutic agents, and molecular targets for use in the development of treatment strategies for neurodegenerative disorders [3]. Unfortunately, no effective therapeutic agents are available for the treatment of neurodegenerative disorders till date. Thus, there is need for development of novel therapeutic strategies for these disorders.

Parkinson’s disease (PD) ultimately leads to oxidative stress-mediated cell injury which severely affects the lives of patients [4]. It is believed that inhibition of free radical production is of great importance for prevention of neurodegenerative disorders [5]. The hydrolysis of acetylcholine by acetylcholinesterase (AChE) leads to the termination of nerve impulses [6]. Studies have shown that AChE enhances apoptosis of cells, and its inhibition plays therapeutic role in PD by preventing dopaminergic neuronal death [6]. Thus, the inhibition of AChE activity prevents dopaminergic neurotoxicity in vitro and in vivo [6].

*Bacopa monniera* has a history of use in traditional medicine for enhancing memory and preventing epileptic disorders [7]. Phytochemical investigation of *Bacopa monniera* revealed the presence of many potent secondary metabolites such as betulinic acid, stigmasterol and bacosides, and subsequent studies revealed that its cognitive improvement property was associated with bacosides A and B [8]. The present study determined the neuroprotective effect of bacoside-A in a rat model of Parkinson’s disease induced by 6-hydroxydopamine, and investigated its mechanism of action.

**EXPERIMENTAL**

**Animals and experimental design**

A total 50 male Wistar rats weighing 295 - 340 g (8 - 10 weeks of age) were supplied by Vital River Laboratories, Beijing China. The rats were housed in an animal center under controlled temperature of 23 - 24 °C and 65 % humidity. All rats were exposed to 12-h light/12-h dark cycles and given *ad libitum* access to feed and water. The rats were assigned randomly to five groups namely: sham group, model group, and three bacoside-A treatment groups i.e. 5, 10 and 20 mg/kg. The treatment groups were given bacoside-A intraperitoneally at doses of 5, 10 and 20 mg/kg 24 hours prior to PD induction. Rats in sham and model groups received equivalent volumes of normal saline at the same time, in place of drug. The experimental procedures on rats were carried out in accordance with the guidelines of the National Institute of Health, China [6]. The study received approval from the Ethics Committee for Animal Care and Welfare, Tianjin Medical University General Hospital, Tianjin, China (approval no. SU/17/007).

**Establishment of PD rat model**

The rats were anesthetized with pentobarbital sodium at a dose of 30 mg/kg intraperitoneally. A hole was carefully drilled in the cranial cavity of each rat, and a needle was inserted in right side of the substantia nigra pars compacta. Then, 5 µL of 6-hydroxydopamine in 0.1% ascorbic acid (2 µg/µL) was administered to the rats, except those in sham group.

**Rotational behavior test**

Rotational testing of the rats was performed over a duration of 30 min 14 days after surgery. The rotational test and measurement of lesion volumes were performed to determine the severity of behavioral disorder. In this test, the rats were rotated through 360˚ to the ipsilateral and contralateral sides.

**Measurement of oxidative stress**

Blood samples of the rats were taken on day 31 of treatment and centrifuged at 4 °C for 15 min at 5,000 x g to obtain sera. Microplate readers (LLC, Sunnyvale, CA, USA) were used for measurement of absorbance of the serum samples at 455 nm. Assays of serum CAT, GSH-Px, TNF-α, IL-1β, IL-6 and NF-κB p65 were carried out using assay kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to manufacturer’s instructions.

**Determination of activities of iNOS and AChE**

The rats were sacrificed on day 31 of treatment following administration of chloral hydrate at a dose of 350 mg/kg through the intraperitoneal route. The rat brains were excised carefully to isolate the hippocampal tissues for determination of activities of iNOS and AChE. The hippocampal tissues were rinsed in PBS and homogenized with ice-cold RIPA buffer (Beyotime Institute of Biotechnology, Nanjing, China) containing protease inhibitors. The homogenates were subjected to centrifugation for 20 min at 1200 g, and the protein contents of the supernatants were measured with bicinchoninic acid assay kit. The activities of...
iNOS and AChE in supernatants were assayed with ELISA kits (Nanjing Jiancheng Bioengineering Institute) as per the supplier’s protocols. Absorbance was read at 465 nm in a microplate reader (Molecular Devices LLC).

**Western blotting**

Portions of hippocampal tissues were homogenized with ice-cold RIPA buffer in combination with protease inhibitor mixture. The homogenates were subjected to centrifugation for 20 min at 1200 g, and the protein contents of the supernatants were measured with bicinchoninic acid assay kit. Then, 30-µg protein samples were resolved on SDS-polyacrylamide gel (10 - 12%) and subsequently transferred to PVD membranes. Incubation was performed overnight at 4˚C with primary antibodies against Bax (1:500 dilution), cytochrome c (1:500 dilution) and β-actin (1:4,000 dilution), all from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA).

After washing with TBS-Tween-20, the membranes were incubated with ig-conjugated secondary antibody (1:5,000 dilution) at room temperature for 1 h. Chemiluminescence detection kit was used for visualization of the immunoreactive bands, while ImageJ software v3.0 was employed for quantification.

**Statistical analysis**

The data are presented as mean ± standard deviation of three measurements, and statistical analysis was carried out using SPSS 20.0 (IBM Corp., Armonk, NY, USA). Differences were determined statistically using one-way analysis of variance, followed by Tukey’s post hoc test. Differences were considered statistically significant at \( p < 0.05 \).

**RESULTS**

**Bacoside-A improved PD-induced rotational behavioral changes**

In PD rats, the turning value increased significantly \((p < 0.049)\) compared to the sham group (Figure 1). Bacoside-A treatment significantly \((p < 0.049)\) decreased the PD-induced higher turning values in rats in a dose-based manner. The PD-induced higher turning values were significantly suppressed by bacoside-A treatment at doses of 5 and 10 mg/kg. However, at 20 a dose of mg/kg, bacoside-A completely eliminated the PD-induced increase in turning values in the rat model.

**Bacoside-A elevated CAT and GSH-PX activities in PD rats**

The activity of CAT in PD rats was suppressed significantly \((p < 0.049)\), when compared to that in the sham control group (Figure 2 A). In addition, GPx level was reduced significantly \((p < 0.049)\) in PD rats relative to sham group (Figure 2 B). However, bacoside-A mitigated PD-mediated suppression of CAT as well as GPx levels in a dose-dependent manner. In rats treated with 20 mg/kg bacoside-A, the PD-mediated suppressions of CAT and GPx activities were effectively reversed.

**Bacoside-A inhibited activities of AChE and iNOS in PD rats**

The activities of AChE and iNOS in PD rats were markedly increased \((p < 0.049)\), relative to those in sham group (Figure 3). In PD rats, bacoside-A suppressed the activities of AChE and iNOS dose-dependently. Bacoside-A treatment at a dose of 20 mg/kg suppressed AChE and iNOS activities in PD rats to levels close to those of the sham group.
Bacoside-A suppressed inflammation in PD rats

In PD rat model, marked elevations were observed in the levels of IL-1β and IL-6, relative to the sham group (Figure 4). However, bacoside-A significantly and dose-dependently decreased the levels of IL-1β and IL-6 (p < 0.018). The PD-mediated elevations in IL-1β and IL-6 were effectively reversed by bacoside-A at a dose of 20 mg/kg. Moreover, TNF-α and NF-κB p65 levels were significantly increased in PD rats, relative to the sham group (p < 0.049). However, treatment of PD rats with bacoside-A caused reductions in TNF-α and NF-κB p65 levels in a dose-based manner.

Bacoside-A inhibited COX-2 and p53 protein levels in PD rats

The protein levels of COX-2 and p53 were increased markedly in PD rats, relative to the sham group (Figure 5). However, significant reductions in COX-2 and p53 protein levels in PD rats were observed on treatment with bacoside-A. The COX-2 and p53 protein levels in 20 mg/kg bacoside-A-treated PD rats were comparable to those in the sham group.

Bacoside-A inhibited Bax, caspase-3 and -9 activity in PD rats

In PD rats, there were marked increases in the expressions of Bax, caspase-3 and caspase-9, relative to the sham group (Figure 6). However, treatment of PD rats with bacoside-A mitigated the up-regulation of Bax, caspase3 and caspase-9 in a dose-dependent manner. The suppressions were significant in PD rats treated with bacoside doses of 5 and 10 mg/kg, relative to untreated group.

Bacoside-A inhibited cytochrome C in PD rats

Cytochrome c activation was elevated markedly in PD rat model, relative to that in the sham group (Figure 7). Bacoside-A treatment significantly reversed PD-induced elevation of cytochrome c activation in the PD rats. In PD rats treated with bacoside at a dose of 20 mg/kg, the activation of cytochrome c was suppressed to a value close to that of the sham group.
DISCUSSION

Parkinson's disease, also called paralysis agitans, globally affects 10% of people aged >65-years [9,10]. It is a chronic and progressive disorder of the nervous system arising from functional defects in the extrapyramidal system [9,10]. Oxidative response associated with the depletion of antioxidants and over-production of oxidative radicals causes severe damage to cells [11]. It has been found that neurons present in the substantia nigra pars compacta are highly susceptible to oxidative stress-induced cellular damage [4]. In the present study, the turning value of the PD rat model was significantly higher than that of rats in the sham group. However, the turning value was decreased effectively and dose-dependently by bacoside-A. Moreover, the PD rats had suppressed serum levels of CAT and GPx, relative to the vehicle-treated group. These findings reveal that bacoside-A decreased turning value in PD rats through enhancement of the anti-oxidant enzymes CAT and GPx. It has been revealed that AChE contributes significantly to apoptosis [12]. Thus, down-regulation of AChE is vital in neuroprotective therapies through anti-apoptotic mechanism [13]. Imbalance between dopamine and acetylcholine is associated with the pathogenesis of PD [13]. In mice models, the anti-inflammatory properties of therapeutic agents have been linked to inhibition of AChE [14]. Inflammation and disruption of the blood brain barrier enhance the interaction between the CNS and peripheral immune system, resulting in increased accumulation of leukocytes in brain parenchyma [15].

Accumulation of peripheral immune cells in the CNS leads to neuronal degeneration through paracrine and endocrine pathways [16]. In the present study, PD rats had enhanced AChE activity in hippocampal tissues. However, bacoside-A treatment exerted suppressive effect on AChE activities in the hippocampal tissues of PD rats. The serum levels of IL-1β, IL-6, TNF-α, NF-κB p65 and COX-2 in PD rats were also significantly elevated. However, these increases were reversed by bacoside-A. Parkinson's disease (PD) is also influenced by over-production of NO via DNA damage [17,18]. Previous studies have linked neuronal apoptosis with over-expressions of Bax, caspase-3, cytochrome c and p53, as well as suppression of Bcl-2 [19-22]. In the present study, Bax, caspase-3, cytochrome c and p53 were upregulated, whereas Bcl-2 was suppressed in PD rats. However, bacoside-A treatment reversed the PD-induced increases in expressions of Bcl-2, caspase-3, cytochrome c and p53 in the rats.

CONCLUSION

Bacoside-A exhibits protective effect against Parkinson disease-induced oxidative damage and neuronal degeneration in rats via a mechanism involving the suppression of iNOS, AChE, inflammatory cytokines and pro-apoptotic proteins. Therefore, bacoside-A has some promise for use in the management of Parkinson disease.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Binbin Zhang, Jiankuan Shi, and Lei Chang contributed to this work equally. Yijun Song - conceived and designed the study; Binbin Zhang, Jiankuan Shi, Lei Chang, Hong Wang, Yaping Wang, Minxia Li, Yuying Li - collected and analyzed the data; Binbin Zhang, Jiankuan Shi, Lei Chang - wrote the manuscript. All authors read and approved the manuscript for publication.

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