Life Sciences 144 (2016) 113–120

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Pre-administration of BAX-inhibiting peptides decrease the loss of the nigral dopaminergic neurons in rats

Chi Ma [a,1], Yujun Pan [a,*,1], Zizhen Yang [b], Zhaojun Meng [c], Ruohan Sun [a], Tianyu Wang [a], Yiping Fei [a], Wenhui Fan [a]

[a] Department of Neurology, First Hospital and Clinical College of Harbin Medical University, Harbin, Heilongjiang Province 150001, China
[b] Department of Neurology, Qingdao Central Hospital, Qingdao, Shandong Province 266000, China
[c] Department of Ophthalmology, Fourth Hospital and Clinical College of Harbin Medical University, Harbin, Heilongjiang Province 150001, China

ARTICLE INFO

Article history:
Received 11 June 2015
Received in revised form 11 November 2015
Accepted 19 November 2015
Available online 2 December 2015

Keywords:
Bax-inhibiting peptide
6-Hydroxydopamine
Dopaminergic neurons
Apoptosis
Parkinson's disease

ABSTRACT

Aims: In this study, we investigated the effects of Bax-inhibiting peptide (Bip)-V5, an anti-apoptosis membrane-permeable peptide, on the 6-hydroxydopamine (6-OHDA) induced Parkinson's disease (PD) model rats.
Materials and methods: Rats were randomly divided into five groups: Control, 6-OHDA only, Vehicle + 6-OHDA, zVAD + 6-OHDA, and V5 + 6-OHDA, that rats were preadministrated with different reagents before 6-OHDA administration.
Key findings: The result showed that intrastriatal preadministration of Bip-V5 significantly decreased the amphetamine-induced rotation of the 6-OHDA model rats and the loss of the nigral dopaminergic (DA) neurons. Moreover, Bip-V5 intrastriatal preadministration not only significantly decreased the expression of activated caspase 9 and activated caspase 3 but also decreased the enhanced expression of AIF and its nuclear translocation in the SNpc. The results in our study provide the first experimental evidence that both caspase-dependent and AIF-dependent apoptosis pathways are involved in the loss of the nigral DA neurons caused by intrastriatal administration of 6-OHDA, and intrastriatal preadministration of Bip-V5 can inhibit the above two apoptosis pathways to protect the nigral DA neurons.
Significance: Our results provide a new idea that Bax-inhibiting peptide may be a promising preventive or therapeutic method for PD.

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder caused by the degeneration of the dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) and the consequent loss of associated projecting nerve fibers in the striatum [1]. Mounting evidence has supported that apoptosis contributed to the DA neurons loss [2] and the proapoptotic protein Bax played a key role in the mitochondrion-mediated apoptotic process [3–6]. Under some stimuli, Bax is activated and translocates from the cytosol onto the outer mitochondrial membrane, which permeabilizes the outer mitochondrial membrane by opening mitochondrial permeability transition pore complex (PTPC) or formation of mitochondrial pore. Subsequently the apoptogenic factors such as cytochrome *c*, procaspase 9 and apoptosis inducing factor (AIF) release from the mitochondrial intermembrane space into the cytoplasm, and then cytochrome *c* binds with apoptotic protease activating factor 1 (Apaf-1) and dATP to converse procaspase 9 to activated caspase 9, which in turn activates caspase 3, an executioner in caspase-dependent apoptosis pathway [2]. In addition, AIF directly induces high molecular weight DNA fragmentation and chromatin condensation in AIF-dependent apoptosis pathway [7,8]. The high expression of caspase 3 or the nuclear translocation of AIF has been found in the DA neurons of PD models and PD patients [5,9–12]. Because Bax functions on the upstream of activated caspase 9, activated caspase 3 and AIF nuclear translocation, so we hypothesize that inhibition of Bax may protect the DA neurons of PD models and PD patients by blocking both caspase-dependent and AIF-dependent apoptosis pathways. Bax-inhibiting peptide (Bip), a cytoprotective membrane-permeable peptide, is comprised of five-amino-acid peptides VPMLK (V5) or PMLKE (P5) and synthesis from the Bax-binding domain of Ku70 [13]. Previous studies have shown that Bip application played anti-apoptotic roles in the treatment of several animal models related to human disease including cerebral ischemia, hypoxia-ischemia encephalopathy, Alzheimer's disease and optical nerve transection [14–17]. The catecholamine-selective neurotoxin 6-hydroxydopamine (6-OHDA) intrastriatal injection can induce the slow and retrograde degeneration of the nigral DA neurons and has been widely used in the study of Parkinson's disease [18,19]. The purpose of

* Corresponding author at: Department of Neurology, First Hospital and Clinical College of Harbin Medical University, Room 501, Building 3, 23 Youzheng, Harbin 150001, China.
E-mail address: panyujun2015@sina.com (Y. Pan).
[1] This two authors are the co-first authors.

http://dx.doi.org/10.1016/j.lfs.2015.11.019
0024-3205/

our current study was to investigate the effects of Bax-inhibiting peptide-V5 (Bip-V5) preadministration on the 6-OHDA induced PD model rats. The result will provide the experimental basis for the future application of Bax-inhibiting peptide for prevention and therapy of Parkinson's disease.

2. Materials and methods

2.1. Animals and ethical approval

Adult male Wistar rats (obtained from the Beijing Vital River Laboratory Technology Co. Ltd., China) weighing between 280 g and 300 g were used for the experiment. All rats were maintained at 25 ± 2 °C, on a 12:12 h light/dark cycle, with water and food provided ad libitum. All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Experimental Animal Ethics Committee of the Harbin Medical University in China.

2.2. Experimental design

Rats were randomly divided into five groups: Group 1: Control, the rats were without any injection. Group 2: 6-OHDA only, the rats were only administrated with 4 μg/μl 6-hydroxydopamine (6-OHDA, Sigma Chemical Co., MO, USA) solution (dissolved in sterile saline solution containing 0.2% ascorbic acid). Group 3: Vehicle + 6-OHDA, the rats were preadministrated with 0.1% phosphate-buffered saline (PBS) before 6-OHDA administration. Group 4: zVAD + 6-OHDA, the rats were preadministrated with 200 μM carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone (zVAD-fmk, Calbiochem, CA, USA) solution (dissolved in 0.1% PBS) before 6-OHDA administration. Group 5: V5 + 6-OHDA, the rats were preadministrated with 200 μM Bip-V5 (Calbiochem, CA, USA) solution (dissolved in 0.1% PBS) before 6-OHDA administration. The concentrations of the zVAD-fmk solution and the Bip-V5 solution were selected on the basis of experience ([20]). All reagents mentioned above were injected into rat striatum; 12 rats in each group.

2.3. Model establishment

Except the rats in the Control group, each rat in the other groups was anesthetized by intraperitoneal injection of chloral hydrate (0.3 ml/kg) and placed on a stereotaxic apparatus (Jiangwan, China). Then the skull was exposed and the hole was drilled into the right striatum using the coordinates relative to bregma: AP: +1.0 mm, ML: −3.0 mm, DV: −5.0 mm [21]. In the Vehicle + 6-OHDA group, 4 μl PBS was injected into the above coordinates at a rate of 1 μl/min with a 10 μl syringe (Hamilton Company, NV, USA). Upon completion of administration, the 22 gauge injection needle was left in the place for a further 5 min for the reagent's full diffusion away from the injection site and then slowly retracted the needle. One hour later, 4 μl 6-OHDA solution was administrated into the same injection coordinates. Similar as the Vehicle + 6-OHDA group, before 6-OHDA administration, 4 μl zVAD-fmk and Bip-V5 were preadministrated into the right striatum of the rat in the zVAD + 6-OHDA group and the V5 + 6-OHDA group, respectively.

2.4. Amphetamine-induced rotation assessment

The amphetamine-induced rotation assessments were performed one day before and four weeks after the surgery. Each rat was intraperitoneal injected with 2.5 mg/kg D-amphetamine (Sigma Chemical Co., MO, USA) solution (dissolved in 1 ml 0.1%PBS), placed immediately into an automated rotometer bowl (San Diego Instruments, Inc., CA, USA) and filmed for half an hour by video camera. The net rotation number of each rat was analyzed as the number of right turn (the lesioned side) minus the number of left turn (the unlesioned side).

2.5. Immunohistochemistry

Four weeks after the surgery, six rats in each group were anesthetized with lethal dose of chloral hydrate and perfused with 0.9% saline and 4% paraformaldehyde. Then the brains were removed and post-fixed with 4% paraformaldehyde for 24 h. The SNpc and the striatum were paraffin embedded, serially sectioned and coded. The section thickness of the SNpc and the striatum were 8 μM and 40 μM respectively. The striatum sections were incubated with anti-tyrosine hydroxylase (TH) antibody (1:200; GeneTex Inc., TX, USA) and the SNPC sections were incubated with anti-TH antibody, anti-caspase 3 antibody (cleaved, 1:50, Boster Bioengineering Co. Ltd., China), anti-caspase 9 antibody (cleaved, 1:50, Boster Bioengineering Co. Ltd.) and anti-AIF antibody (1:200, GeneTex) overnight at 4 °C. Then the sections were incubated with peroxidase-conjugated affinipure IgG antibody (1:1000, ZhongShan JinQiao Biological Technology Co. Ltd., China) for 20 min. All images were captured with an optical microscope (Nikon 80i, Japan). To evaluate the number of DA neurons in the SNpc (from the Bregma 4.92 mm to 5.52 mm) and the density of DA fibers in the striatum (from the Bregma 0.48 mm to 0.84 mm), every 4th section of the SNpc and every 3th section of the striatum were selected and immunostained with TH antibody. To evaluate the proteins expression of the activated caspase-9, activated caspase-3 and AIF in the SNpc, the other sections of SNpc were immunostained with cleaved caspase-9 antibody, cleaved caspase-3 antibody and AIF antibody, successively. The number of DA neurons (five sections per rat) was analyzed by three experienced pathologist who was blinded to the sample groups. The optical density of DA fibers (three sections per rat) and the expression of the three proteins (three sections per rat) were analyzed using Image-ProPlus 6.0. All the sections for analyze were code-math sections between each group.

2.6. Western blot analysis

Four weeks after the surgery, the other six rats in each group were anesthetized with lethal dose of chloral hydrate and the brains were removed. Both sides of the SNpc regions were dissected and quickly frozen in liquid nitrogen. Then fresh-frozen sample was homogenized using a glass homogenizer in 100 μl RIPA Lysis buffer (Beyotime institute of Biotechnology, China) containing 1 mM PMSF (Beyotime institute of Biotechnology) and protein content was measured using the bicinchoninic acid (BCA) kit (Beyotime institute of Biotechnology). Same amounts of protein (100 μg) were separated by 10%SDS-PAGE and transferred to PVDF. The blots were blocked in non-fat dry milk for 1 h, and then incubated with anti-caspase 9 and anti-AIF antibody (1:1000, GeneTex) and anti-β-actin antibody (1:5000, Santa Cruz Biotechnology Inc., CA, USA) overnight at 4 °C, followed by incubation with peroxidase-conjugated affinipure IgG antibody (1:10,000). Immunoreactive proteins were detected using the enhanced chemiluminescence (ECL) (Invitrogen Biotechnology Inc., CA, USA). The immunoblots were imaged and quantified using Bio-RAD GelDoc XR System (Bio-Rad Laboratories, USA) according to the manufacturer's instruction.

2.7. Statistical analysis

Values are expressed as mean ± standard error of the mean (SEM) and analyzed using the SPSS 19.0 (SPSS Inc., IL, USA). Comparisons between different groups were made using one-way analysis of variance (ANOVA) followed by Least-significant difference (available for equal variances assumed) or Dunnett's T3 (available for variances not assumed) post-hoc analysis. Values of $P < 0.05$ were considered statistically significant.