Neuroprotection of pramipexole in UPS impairment induced animal model of Parkinson's disease

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ABSTRACT

Parkinson's disease (PD), a progressive neurodegenerative movement disorder, is known to be caused by diverse pathological conditions resulted from dysfunction of the ubiquitin-proteasome system (UPS), mitochondria, and oxidative stress leading to preferential nigral dopamine (DA) neuron degeneration in substantial nigra. To slow the neurodegeneration in PD, several pathogenetic pathways leading to this disease should be intervened. In the present study, the primary goal of the proposed study is to investigate whether PPX possesses neuroprotection against UPS impairment induced nigro-striatal DA neuron degeneration and to determine the role of autophagy in the neuroprotective effects of PPX in this model of PD. The results of the study may provide us new insight into the potential novel mechanisms for the treatment of PD.

BACKGROUND

Pramipexole, a DA receptor D3 preferring agonist, is used to treat PD by acting directly on the dopamine receptors with significant improvement and mild side effects. Several studies have showed that promipexole possessed neuroprotective properties in acute MPTP or 6-OHDA lesioned animal model of PD. Recently, we have developed a new animal model of UPS impairment that mimic a progressive neurodegeneration in nigro-striatal pathway and display some cordial features of PD. Using this model we tested the neuroprotective effects of pramipexole against nigral neurodegeneration and determine the possible underlying mechanisms.

METHODS

Animals and treatment

C57BL/6 mice (Male, age of 12 weeks) were randomly divided into eight groups of 5 mice each. Intraperitoneal administration of PPX at two doses twice a day started 7 days before microinjection with lactacystin, up to the end of the study (28 days after microinjection of lactacystin), while the microinjection of a same volume saline was served as a control.

Immunohistochemistry

Serial frozen sections were subjected to free-floating immunohistochemistry with primary antibody: rabbit anti-tyrosine hydroxylase (TH, 1:1500) and rat anti-CD11b (1:50) et al. The secondary biotinylated anti-rabbit or -rat IgG antibody (1:200) was added followed by ABC elite kit and DAB.

Measurement of BDNF and GDNF by ELISA

Samples were weighed and added 100-200 µl lysis buffer, sonicated, centrifuged at 14,000 × g for 30 min at 4° C. The levels of BDNF and GDNF were measured by using the BDNF or GDNF Emax ImmunoAssay System.

Proteasome activity assay

Samples were centrifuged and the supernatants were assayed for protein concentrations. The 20S Proteasome Activity kit was carried out with 50 µg of midbrain lysates and the appropriate substrate for incubation. The activity was measured by detection of the fluorophore AMC.

Electron Microscopy

The tissue was postfixed in 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer for 2 days and punched out the SN area with the Palkovits method. The sections were postfixed with 1% osmium tetroxide, and stained en bloc with 1% uranyl acetate. The samples were dehydrated in increasing concentrations of ethanol, infiltrated, and embedded in LX-112 medium. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a electron microscope.

FIGURES

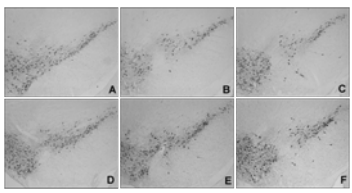


Fig. 1. PPX reduced lactacystin induced loss of DA neurons in SN. The mice were sacrificed at the end of the study (day 28). Representative photomicrographs of SN with TH immunohistochemistry (10X). A–F) control, lactacystin, PPX low dose+lactacystin, PPX high dose+lactacystin , PPX, U99194+PPX high dose+lactacystin, respectively. PPX at two doses: 0.1 mg/kg (low) and 0.5 mg/kg (high).

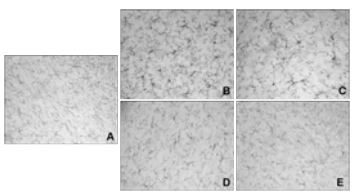
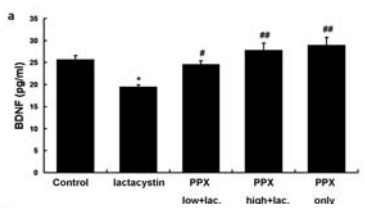


Fig. 2. PPX decreased microglial activation induced by lactacystin. The changes of microglial activation in SN are demonstrated by microglia with CD11b immunohistochemistry (40X). A–E) control, lactacystin, PPX low dose+ lactacystin, PPX high dose+lactacystin, and PPX groups, respectively. PPX at two doses: 0.1 mg/kg (low) and 0.5 mg/kg (high).



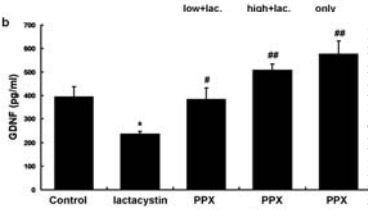


Fig. 3. Effects of PPX on expression of BDNF protein in the striatal after lactacystin induced mice. The trend of increased amount of BDNF & GDNF in PPX treated groups was detected by ELISA. The results were expressed as means ± SE (n=6). *P<0.05 vs. control, **P<0.01 vs. control and #P < 0.05, ##P < 0.01 vs lactacystin. PPX at two doses: 0.1 mg/kg (low) and 0.5 mg/kg (high).

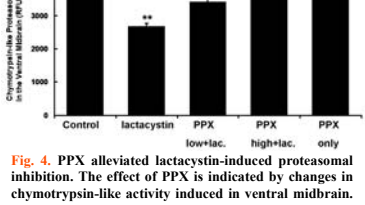


Fig. 4. PPX alleviated lactacystin-induced proteasomal inhibition. The effect of PPX is indicated by changes in chymotrypsin-like activity induced in ventral midbrain. Results are expressed as means ± SE (n=6). *P<0.05, **P<0.01 vs. control and #P<0.05, ##P<0.01 vs lactacystin. PPX at two doses: 0.1 mg/kg (low) and 0.5 mg/kg (high).

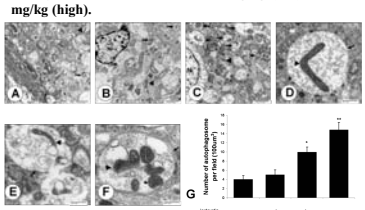


Fig. 5. EM images of the control mice (A) and PPX treated mice (B and C). The arrowheads show the mitochondria and arrows show autophagosome/autophagic vacuoles (AVs) and double membrane structures in D-F. (bars = 2 µm in A-C and 0.5 µm in D-F; nucleus, N).

RESULTS

➢Effects of PPX against lactacystin induced DA neuron loss in SN Compared with the vehicle control, the number of DA neurons was reduced in lactacystin lesioned mice by 49.1% at the end of study. Pretreatment with PPX at low and high doses protected the DA neurons against lactacystin-induced injury at the end of the study with 24.5% and 60.6% reduction in the DA neuron loss, respectively. There was no difference between PPX treatment in non-lesioned mice and the vehicle controls (Fig. 1).

➢PPX reduces microglial activation in lactacystin lesioned mice Glial activation and possible inflammation in the SN were studied by immunohistochemistry. Microglia was detected by CD11b staining and morphological characterization. Compared with vehicle control, an increase in microglial profile was evident in SN in mice injected with lactacystin. A dense deposition of hypertrophic microglia was seen in the CD11b immunostaining profiles. Compared with microinjection of lactacystin, pretreatment with PPX at high dose significantly inhibited activation of microglia by 56.3% (Fig. 2).

➢PPX increases the BDNF and GDNF levels in lactacystin-lesioned mice The protein levels of BDNF and GDNF were measured by ELISA. Lactacystin lesion decreased the protein levels of BDNF by 25.2% and GDNF by 39.4%, respectively. The pretreatment with high dose of PPX significantly increased the levels of BDNF and GDNF by 44.5% and 113% as compared with the vehicle treated lactacystin-lesioned mice (Fig. 3).

➢PPX alleviated lactacystin induced proteasomal inhibition As a proteasome inhibitor, lactacystin caused a 39.1% inhibition of the chymotrypsin-like proteasomal activity in the ventral midbrain 28 days after microinjection of lactacystin. PPX was used in lactacystin-injected mice to observe the reverse effects on proteasomal activity. It was shown that pretreatment of PPX at low and high dose significantly attenuated lactacystin-induced proteasomal inhibition by 42.0% and 80.5% (Fig. 4).

➢PPX enhances autophagosomes in SN cells The accumulation of autophagosome was obvious in SN in PPX treated mice. We detected the mitochondria, ribosome and lysosome engulfed by the autophagosome with characteristic of double membranes. Two or three autophagosomes were in the process of fusion into a late autophagic vacuole. The numbers of autophagosome/autophagic vacuoles were significantly increased by 260.9% in the PPX treated mice compared with vehicle controls (Fig. 5).

CONCLUSIONS

➢PPX treatment partially rescued the loss of dopaminergic neurons in SN, inhibited the activation of microglia, and improve the impairment of behaviour caused by lactacystin in rotarod and locomotor activity tests (data not shown here).

➢The neuroprotective effects of PPX shown in the present study may partly due to enhance the neuroprotective function of ALP.

➢These results suggest that pramipexole possesses a strong neuroprotection against UPS impairment induced dopamine neuron degeneration, and multiple molecular pathways may be attributed to the neuroprotective effect of pramipexole in the animal model of PD.

ACKNOWLEDGEMENT

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