Novel multifunctional drug D-264 for Parkinson's disease: Evidence of neuroprotective property in PD animal models induced by MPTP and lactacystin

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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 pathologenetic pathways leading to this disease should be intervened. In the present study, we found that D-264 significantly improved behavioral performances and attenuated significantly both MPTP and lactacystin induced DA neuron loss, proteasomal inhibition, and microglial activation in substantia nigra (SN). Furthermore, D-264 treatment was shown to increase levels of brain-derived neurotrophic factor (BDNF) and glial cell line-derived factor (GDNF) in MPTP and lactacystin treated mice, partially indicating mechanism of neuroprotection by D-264. Our study indicates that multivalent drug D-264 can protect neurodegeneration induced by the selective neurotoxin and UPS inhibitor and may serve as an improved and better neuroprotective treatment agent for PD.

BACKGROUND

PD is a progressive disorder that affects locomotor system and other functions. The impairment of movement results from the loss of dopamine neurons in the substantia nigra and neurotransmitter DA in the nigrostriatal dopaminergic pathway. The D3 receptor has been implicated in neuroprotection as D3 receptor-preferring agonists e.g. pramipexole, could protect DA neurons against MPTP and 6-OHDA neurotoxicity more robustly than less selective D3 receptor-preferring agonists. Our goal is to develop selective D3 receptor agonist based on our novel hybrid template, we have recently developed a molecule (-)-N6-(2-(4-(Biphenyl-4-yl) piperazin- 1-yl)ethyl)-N6-propyl-4,5,6,7- tetrahydrobenzo[d]thiazole-2,6-diamine (D-264) which exhibited high affinity and selectivity for D3 receptor both in the binding and functional assays.

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 D-264 at two doses once a day started 7 days before administration of MPTP or microinjection with lactacystin, up to the end of the study (14 days after administration of MPTP or 21 days after microinjection of lactacystin), while the administration of a same volume saline was served as a control.

Immunohistochemistry

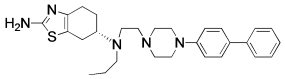
Serial frozen sections were subjected to free-floating immunohistochemistry with primary antibody: rabbit anti-tyrosine hydroxylase (TH, 1:1500) and rat anti-CD11b (1:50) et al. For avidin–biotin-peroxidase method of immunostaining, the secondary biotinylated anti-rabbit or -rat IgG antibody (1:200) 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 at 14,000 g at 4° C for 20 min. The supernatants were assayed for protein concentrations by the Bradford's method. The 20S Proteasome Activity kit was carried out with 50 μg of midbrain lysates and the appropriate substrate at 37° C for 90 min incubation. The activity was measured by detection of the fluorophore AMC. The results are expressed as fluorescence units/mg protein.



D-264

RESULTS

D-264 protected against MPTP and lactacystin induced DA neuron loss in SN

Compared with the vehicle control, the number of DA neurons was reduced in MPTP and lactacystin-injected mice by 51.9% and 47.9% at the end of study. Pretreatment with D-264 at low and high dose protected the DA neurons against neurotoxin MPTP injury at the end of the study with 18.4% and 65.6% reduction in the DA neuron loss; while pretreatment with D-264 at low and high dose showed 54.1% and 77.8% protection against lactacystin-induced DA neuron loss, respectively. Furthermore, compared with low dose of D-264, the high dose was more potent in protecting DA neurons against both MPTP and lactacystin induced injury (Fig. 1).

D-264 decreased microglial activation in MPTP and lactacystin induced 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 administrated with MPTP and injected with lactacystin. A dense deposition of hypertrophic microglia was seen at the immunostain pictures. At the end of the study, D-264 managed to inhibit the microglial activation. Compared with administration of MPTP and microinjection of lactacystin, pretreatment with D-264 at high dose inhibited activation of microglia by 70.4% and 76.3%, respectively (Fig. 2).

D-264 increased the BDNF and GDNF levels in MPTP and lactacystin treated mice

The protein levels of BDNF and GDNF were measured by ELISA. MPTP and lactacystin decrease the expression of BDNF by 23.9%, 24.7% and GDNF by 55.0%, 30.5%, respectively. The pretreatment of high dose of D-264 attenuated the reduction of BDNF by 55.6% in MPTP-lesioned mice; the pretreatment of low and high D-264 increased the level of BDNF by 110% and 117% as compared with the lactacystin-lesioned mice. The pretreatment of low and high dose of D-264 attenuated the reduction of GDNF by 32.4% and 92.7%, respectively, in MPTP-lesioned mice; the pretreatment of low and high D-264 increased the level of GDNF by 150% and 160% as compared with lactacystin-lesioned mice (Fig. 3).

D-264 alleviated lactacystin induced proteasomal inhibition

As a proteasome inhibitor, lactacystin caused a 48.5% inhibition of the chymotrypsin-like proteasomal activity in the ventral midbrain 21 days after microinjection of lactacystin. In order to evaluate the level of proteasomal activity upon treatment with D-264 in lactacystin-injected mice, the assay was carried out. It was shown that pretreatment of D-264 at high dose notably attenuated lactacystin-induced proteasomal inhibition by 75.4%, and the low dose of D-264 by 40.8% as determined chymotrypsin-like proteasomal activity assay (Fig. 4).

FIGURES

Figure 1

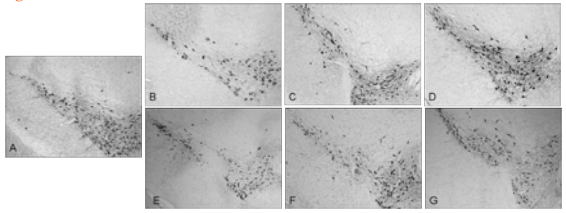
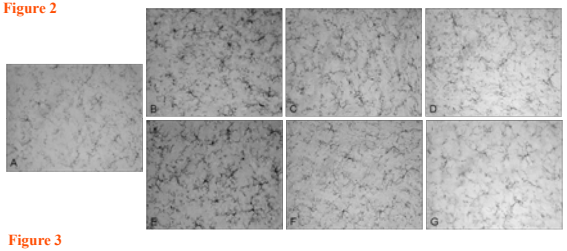


Figure 2



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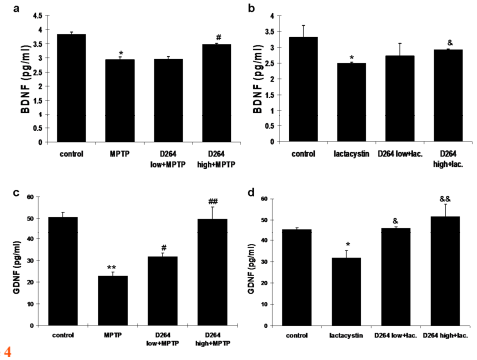
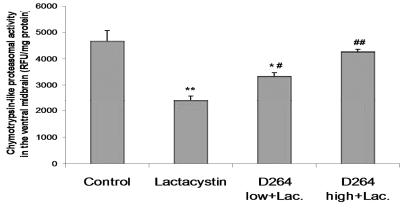


Figure 4



LEGENDS

Fig. 1. D-264 reduced MPTP and lactacystin induced loss of DA neurons in SN. The mice were sacrificed at the end of the study (day 21). a) Representative photomicrographs of SN with TH immunohistochemistry (10X). A–G) Control, MPTP, D-264 low dose+MPTP, D-264 high dose+MPTP, lactacystin, D-264 low dose+lactacystin, and D-264 high dose+lactacystin groups, respectively. D-264 at two doses: 1 mg/kg (low) and 5 mg/kg (high).

Fig. 2. D-264 decreased microglial activation induced by MPTP and lactacystin. a) The changes of microglial activation in SN are demonstrated by microglia with CD11b immunohistochemistry (40X). A–G) Control, MPTP, D-264 low dose+MPTP, D-264 high dose+MPTP, lactacystin, D-264 low dose+lactacystin, and D-264 high dose+lactacystin groups, respectively. D-264 at two doses: 1 mg/kg (low) and 5 mg/kg (high).

Fig. 3. Effects of D-264 on expression of BDNF protein in the striatal after MPTP and lactacystin induced mice. The trend of increased amount of BDNF & GDNF in D-264 treated groups was detected by ELISA. The results were expressed as means ± SE (n=6). *P<0.01, **P<0.001 vs. control, #P<0.01, ##P<0.001 vs. MPTP and &P<0.01, &&P<0.001 vs. lactacystin. D-264 at two doses: 1 mg/kg (low) and 5 mg/kg (high).

Fig. 4. D-264 alleviated lactacystin-induced proteasomal inhibition. The effect of D-264 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. D-264 at two doses: 1 mg/kg (low) and 5 mg/kg (high).

CONCLUSIONS

- D-264 treatment partially rescued the loss of dopaminergic neurons in SN, inhibited the activation of microglia, and improve the impairment of behaviour caused by MPTP and lactacystin in pole and rotarod tests (data not shown here).
- The neuroprotective effects of D-264 shown in the present study may partly due to the upregulation of both BDNF and GDNF and the restoration of proteasomal activity .
- As a drug used in the clinic for other indications, we proposed that further studies on D-264 should be conducted in order to consider it as a novel therapy for PD.

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