

OBJECTIVE

To systemically evaluate a ubiquitin proteasome system (UPS) impairment mouse model for Parkinson's disease (PD) research

BACKGROUND

UPS impairment has been proposed to play an important role in the pathogenesis of PD. Mice with UPS impairment in the nigra have been used for investigating mechanisms underlying dopamine (DA) neuron degeneration and for testing preclinical drugs to treat PD. However, the pathological, biochemical and behavioral features of UPS impairment animal model of PD have not been systematically evaluated.

METHODS

Animal treatment and proteasome inhibitors application: C57BL/6 mice were microinjected with proteasome inhibitors (lactacystin, PSI or MG-132) into the medial forebrain bundle (iMFB), either unilaterally or bilaterally using stereotaxic coordinates to develop a UPS impairment model of nigral DA neuron degeneration.

Neuropathological examination: Immunohistochemical and immunofluorescent staining and cell counting on brain sections were used to evaluate nigral DA neuron loss, nigral glial activation, α -synuclein intensity and inclusion-like granule in nigral DA neurons; electron microscopy, iron concentration assay and western blot assays were performed on nigral tissue to determine apoptosis, insoluble ubiquitin-conjugates level, intracytoplasmic inclusion and total iron level.

Biochemical assay: High performance liquid chromatography for detection of levels of the striatal DA and its metabolites; Western blot for assaying the nigral-striatal TH level; proteasome activity was determined by cytofluorescent assays.

Motor activity tests: Locomotion, skilled motor activities (vertical pole test, grip strength, suspension test and dowel test)

RESULTS

Neuropathological examination:

- Lactacystin iMFB induced dose-dependent, preferential and progressive DA neurodegeneration in the SN of mouse (Fig. 1-1).
- Activated caspase 3 and apoptotic nuclear were shown in the SNc of lactacystin iMFB mice (Fig. 1-2).
- Activation of astrocytes and microglia at the early stage of DA neurodegeneration in the SNc of lactacystin iMFB mice (Fig. 1-3)
- An elevated nigral protein level of insoluble ubiquitin conjugates, increased intensity of α -synuclein in the nigral DA neurons, and a few DA neurons contained inclusion-like granule (Fig. 1-4).
- Iron accumulation in the SNc of lactacystin iMFB mice (Fig. 1-5).

Biochemistry assays:

- Progressive deficiency of striatal DA (Fig. 2-1), decrease in the nigral-striatal TH level (Fig. 2-2) and sustained impairment of nigral proteasome activity (Fig. 2-3) in the lactacystin iMFB mice.

Reproducibility of UPS impairment model:

- DA neurodegeneration and striatal DA deficiency were replicated in mice treated with PSI or MG-132 (Fig. 3).

Motor activity tests:

- A sustained decrease in locomotor activities and skilled motor activities (Fig. 4-1)
- The decreased locomotor activities can be reversed by chronic application of dopamine receptor type 2/3 agonist pramipexole (Fig. 4-2)

RESULT I

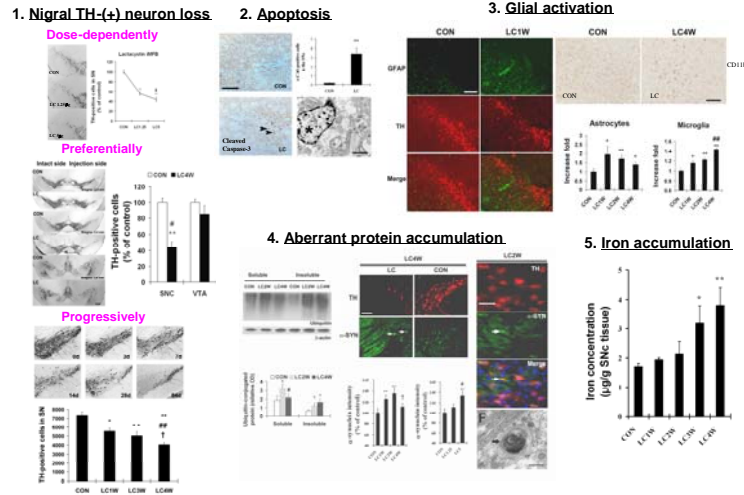


Figure 1. Neuropathology of UPS impairment mouse model. 1. Infusion of lactacystin into MFB induces a dose-dependent, preferential and progressive nigral DA neuron injury in mouse model. 2. Activation of apoptosis in the mouse SNc. 3. Glial activation in the lactacystin-iMFB mice. 4. Aberrant protein aggregation in the lactacystin-iMFB mouse model (inclusion-like granule in F). 5. Iron accumulation in the SNc of the lactacystin-iMFB mice.

RESULT II

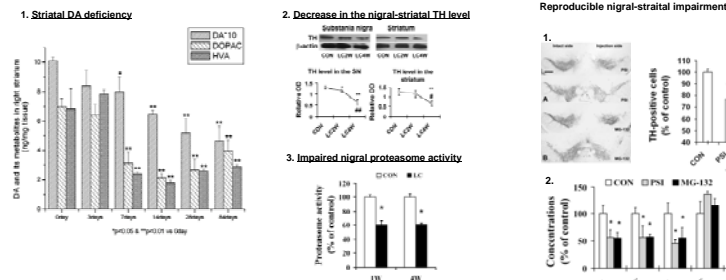


Figure 2. Biochemical changes in the UPS impairment mouse model. 1. Progressive decrease in the level of striatal dopamine (DA) and its metabolites. 2. Decreased nigral-striatal protein levels of tyrosine hydroxylase (TH). 3. Sustained impairment of nigral proteasome activity.

Figure 3. Reproducible impairment of the nigral-striatal system by other proteasome inhibitors. 1. Loss of nigral DA neurons in the SNc of the PSI-iMFB (A) and MG-132-iMFB (B) mice. 2. Decrease in the level of striatal DA and its metabolites (Lower)

RESULT III

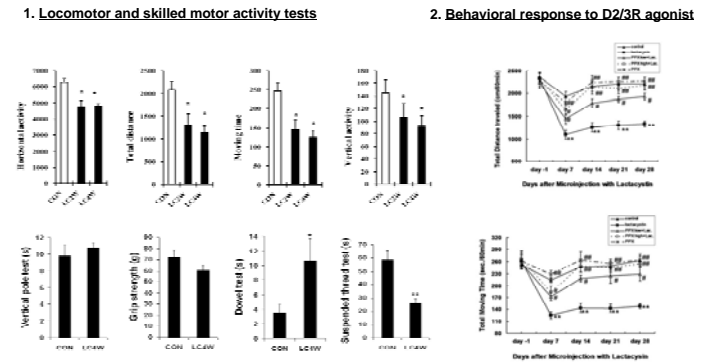


Figure 4. Behavioral changes in the UPS impairment mouse model. 1. Locomotor activities (horizontal activity, total distance, moving time and vertical activity) and skilled motor activities (vertical pole, dowel test and suspended thread test) were examined in the lactacystin-impaired mouse model (left). 2. The decreased locomotor activity was reversed by chronic administration of DA receptor type 2/3 agonist Pramipexole (PPX, right, Chao Li et al., unpublished data).

CONCLUSIONS

These pathological, biochemical and behavioral characters in this model, show progressive DA neurodegeneration with increased ubiquitin conjugates and α -synuclein aggregate, mimicking some features of PD at earlier stage. This model may be suitable to investigate the molecular mechanisms of nigral degeneration and to evaluate neuroprotective medications

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