Evaluating the role of novel ASK1 Inhibitors in preclinical models of Parkinson's disease

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BACKGROUND

Apoptosis signal-regulating kinase 1 (ASK1) is a redox-sensitive MAP3K that serves as a pivotal mediator linking oxidative stress to neuronal apoptosis and neuroinflammation. Activation of ASK1 under pathological oxidative stress triggers phosphorylation of downstream JNK and p38 MAPKs, leading to mitochondrial dysfunction, cytokine release, and glial activation—hallmarks of neurodegenerative processes observed in Parkinson's disease (PD). Despite its well-established pathological role, there has been limited progress in developing potent and selective ASK1 inhibitors with in vivo efficacy in PD models. To address this gap, we designed and characterized two novel ASK1 inhibitors with improved drug-like properties and investigated their effects on neuroinflammatory and neurodegenerative mechanisms. Using both cellular systems and two complementary mouse models—acute MPTP and progressive mThy1-α-synuclein transgenic PD—we aimed to elucidate whether ASK1 inhibition can confer disease-modifying neuroprotection by simultaneously targeting oxidative stress and inflammation.

METHODS

Primary astrocyte cells culture, cell viability, & ROS protection

- Primary rat astrocytes were isolated from the cortex of P0 rats. Brain tissues were dissected in ice-cold Hibernate medium, and the cortex was separated in PBS and cultured in B27-supplemented medium. After 1 week, astrocytes were seeded onto poly-D-lysine–coated plates. Cell viability was measured using a CCK-8 assay, and oxidative stress protection was assessed following H_2O_2 exposure.

MPTP-induced acute PD model generation

- Seven-week-old male C57BL/6 mice were used. All groups except the control received four intraperitoneal injections of MPTP-HCl (20 mg/kg, free base) at 2-hour intervals on day 0 to induce acute dopaminergic neurodegeneration.

mThy1-α-synuclein transgenic (line61, male) PD model

- Drug treatment (4–9 months): ASK1 inhibitors or vehicle were administered once daily by oral gavage for 5 months, beginning at 4 months of age and continuing until necropsy at 9 months.
- Behavioral assessments: Motor performance was evaluated at 4, 7, and 9 months to monitor disease progression and therapeutic response.
- Necropsy and tissue analysis (9M): At 9 months, mice were euthanized, and brains (striatum, substantia nigra, and cortex) were collected for histological and biochemical analyses.

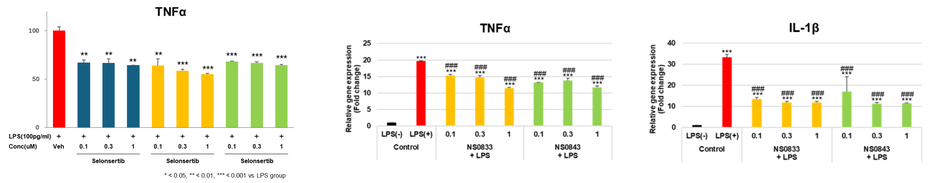
Behavioral assessments

- In the pole test, mice were trained to descend an 80 cm vertical pole, and total descent time was recorded on the test day.
- In the challenge beam test, mice traversed a narrowing Plexiglas beam covered with a mesh grid. Step errors were recorded across five trials by a blinded investigator.

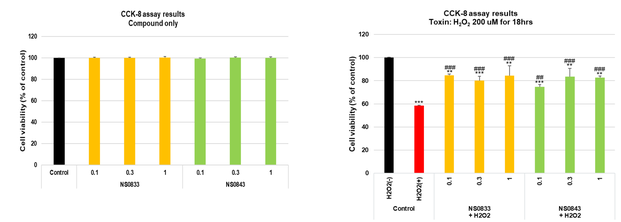
RESULTS

1. In vitro efficacy: Anti-inflammation & Neuroprotection

A. BV-2 microglial cells: Inflammatory marker level and expression

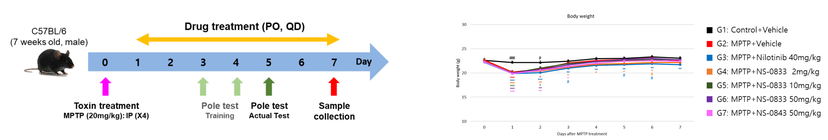


B. Primary astrocytes: Cell protection from oxidative stress

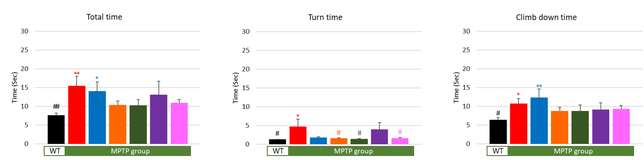


2. In vivo efficacy: MPTP-induced PD

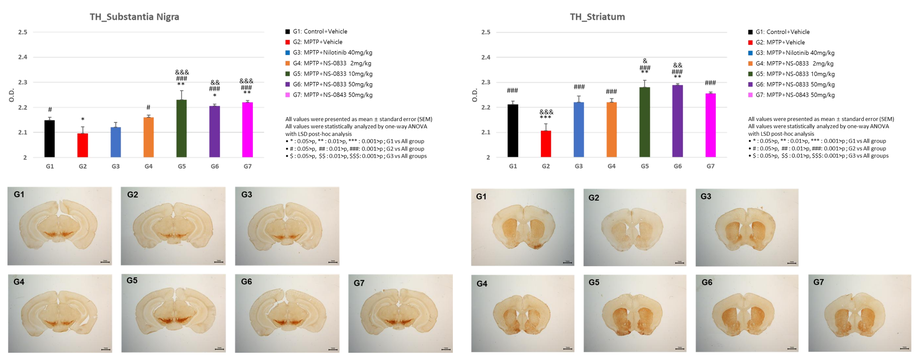
A. Study scheme & Body weight



B. Pole test

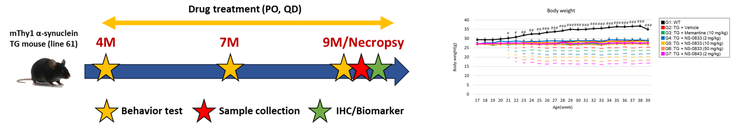


C. TH staining in substantia nigra & striatum

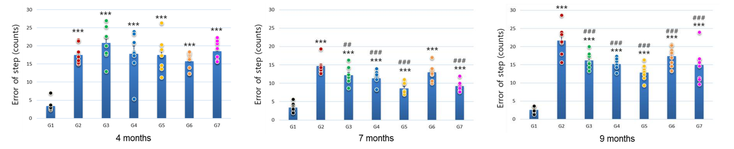


3. In vivo efficacy: α-Synuclein TG PD model

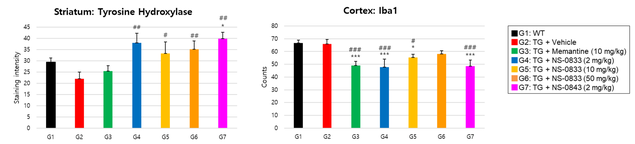
A. Study scheme & Body weight



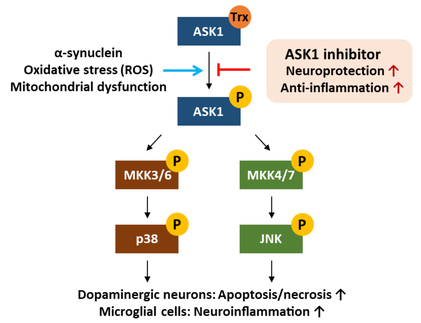
B. Challenge transverse beam test: Error of steps



C. IHC: TH in striatum & Iba1 in cortex



4. ASK1 inhibitors: Mode of Action



* : 0.05>p, ** : 0.01>p, *** : 0.001>p ; G1 vs All, # : 0.05>p, ## : 0.01>p, ###: 0.001>p ; G2 vs All, & : 0.05>p, && : 0.01>p, &&&: 0.001>p ; G3 vs All group

CONCLUSIONS

1. **ASK1 inhibition suppresses neuroinflammation, reducing pro-inflammatory cytokine production in activated microglia.**
2. **Protection of astrocytes against oxidative injury suggests a broad cytoprotective and redox-regulating function.**
3. **Motor and dopaminergic recovery in both acute and progressive PD models demonstrates robust in vivo efficacy.**
4. **Collectively, these findings position ASK1 as a disease-modifying therapeutic target with strong translational potential for Parkinson's disease treatment.**