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Tyrosine kinase inhibition facilitates autophagic SNCA/α-synuclein clearance

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The effects of ABL1/ABL inhibition on clearance of SNCA/α-synuclein were evaluated in animal models of α-synucleinopathies. Parkinson disease (PD) is a movement disorder characterized by death of dopaminergic substantia nigra (SN) neurons and brain accumulation of SNCA. The tyrosine kinase ABL1 is activated in several neurodegenerative diseases. An increase in ABL1 activity is detected in human postmortem PD brains. Lentiviral expression of SNCA in the mouse SN activates ABL1 via phosphorylation, while lentiviral Abl expression increases SNCA levels. Administration of the brain-penetrant tyrosine kinase inhibitor Nilotinib decreases Abl activity and facilitates autophagic clearance of SNCA in transgenic and lentiviral gene transfer models. Subcellular fractionation demonstrates accumulation of SNCA and hyperphosphorylated MAPT/Tau (p-MAPT) in autophagic vacuoles in SNCA-expressing brains, while Nilotinib treatment leads to protein deposition into the lysosomes, suggesting enhanced autophagic clearance. These data suggest that Nilotinib may be a therapeutic strategy to degrade SNCA in PD and other α-synucleinopathies.

Keywords: α-synuclein, dopamine, autophagy, Nilotinib, Tau

Abbreviations: PD, Parkinson disease; SN, substanita nigra; TH$^+$, tyrosine hydroxylase-positive

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ABL1 Activation Leads to Accumulation of SNCA

Stereotaxic injection of male C57BL/6 mice with 1×10^4 multiplicity of infection lentiviral *ABL1* or *SNCA* (or *LacZ*) bilaterally into the SN, significantly increases SNCA levels 6 weeks postinjection and leads to ABL1 activation via tyrosine 412 (T412) phosphorylation. Conversely, lentiviral expression of ABL1 in the (C57BL/6) mouse SN increases T412 phosphorylation and the levels of monomeric and aggregated SNCA. Human postmortem PD striatal extracts also show an association between ABL1 activation and SNCA accumulation. However, Nilotinib decreases monomeric and aggregated SNCA levels in the SN and lowers ABL1/c-ABL activation compared with DMSO. Lentiviral SNCA induces autophagic changes and increases the levels of LC3-II, indicating autophagosome accumulation, whereas Nilotinib decreases the levels of LC3-II relative to actin and LC3-I, suggesting autophagosome clearance. To ascertain whether autophagy mediates SNCA clearance, human M17 neuroblastoma cells were transfected with *LacZ*, *SNCA* or shRNA *BECN1* for 24 h and then treated with 10 μM Nilotinib for an additional 24 h or 100 nM bafilomycin A1 for 3 h before harvest. SNCA is increased in SNCA-transfected cells compared with LacZ. However, Nilotinib reverses the SNCA level, but blocking *BECN1* expression with shRNA attenuates Nilotinib-mediated clearance of SNCA, suggesting autophagic involvement. Bafilomycin A_1 results in Nilotinib failure to clear SNCA, also indicating Nilotinib induces SNCA clearance via autophagy. Additionally, injection of lentiviral SNCA into the SN leads to loss of tyrosine hydroxylase-positive (TH$^+$) neurons and motor impairment; however, Nilotinib decreases human SNCA in SN neurons, protects TH$^+$ neuron and improves motor performance.

Nilotinib Decreases Brain and Blood α-Synuclein Levels

Male 7- to 8-mo-old transgenic A53T SNCA mice were intraperoteneally (I.P.) injected with daily 10 mg/kg Nilotinib for 3 weeks, leading to a significant decrease in SNCA levels and ABL1 activation. The effects of lower dose and longer periods of treatment were evaluated on blood and brain SNCA in 5- to 6 mo old A53T mice, which were injected every other day with 1 mg/kg or 5 mg/kg Nilotinib for 6 weeks. Both concentrations of Nilotinib decrease SNCA levels in the brain as well as the blood. Autophagic markers were examined to determine the role of autophagy in SNCA clearance. Daily I.P. injection of 10 mg/kg Nilotinib for 3 weeks decreases monomeric and aggregated human SNCA and decreases the levels of LC3-II compared with DMSO-treated A53T mice, relative to both LC3-I and actin. Nilotinib significantly increases BECN1 and ATG12 compared with DMSO-treated A53T mice. Isolation of autophagic vacuoles via subcellular fractionation shows human SNCA and p-MAPT accumulation in autophagosomes in A53T mice brains, and this level is increased in older animals. However, Nilotinib decreases human SNCA and p-MAPT in autophagosomes and increases their levels in lysosomes. Nilotinib attenuates SNCA levels in several brain regions, including striatum, cortex and hippocampus.

Transgenic A53T mice express high levels of SNCA in the brain and peripheral organs under the control of a prion promoter, rendering this animal model significant to study the effects of peripheral SNCA accumulation, which is common in PD patients. Nilotinib decreases both brain and blood SNCA, suggesting that autophagy may reduce brain SNCA and attenuate its secretion into the blood and/or facilitate clearance of SNCA in peripheral organs, providing a double-edged strategy for autophagic degradation of SNCA. Additionally, direct lentiviral injection into the SN and loss of TH^+ neurons is a better recapitulation of PD pathology, involving loss of SN neurons, but Nilotinib clears SNCA and reverses loss of TH^+ neurons and motor performance. Taken together, Nilotinib is a strong therapeutic candidate for human α-synucleinopathies.

Nilotinib is a nonspecific tyrosine kinase inhibitor, which reduces the level of other tyrosine phosphorylated proteins. Nilotinib is FDA approved at 50–1200 mg/day and is well tolerated in human leukemia patients (300–400 mg/kg daily), with mild reported side effects. The use of low Nilotinib concentrations, including 1 mg/kg and 5 mg/kg suggests that possible nonspecific pleotropic effects are likely to be reduced without interference with Nilotinib ability to clear SNCA. Nilotinib treatment leads to autophagic clearance of SNCA, decreases ABL1 activity, and prevents loss of TH^+ neurons. Therefore, clinical use of ABL1 inhibition may have dose-limiting toxicity, but it may be used at low doses due to the slow and progressive nature of neurodegenerative diseases. Although Nilotinib is washed out of the brain within several hours, its effects do not seem to be restricted by its presence in the brain, suggesting that turning autophagy on/off over a protracted period of SNCA accumulation may be a useful strategy to clear toxic proteins without pushing neurons to self-cannibalization.

Nilotinib clearance of p-MAPT suggests that free unbound p-MAPT can be cleared via autophagy, sparing intact MAPT that is bound to microtubules, further indicating that tyrosine kinase inhibition may be a useful strategy for p-MAPT clearance in dementia and Parkinsonism. Furthermore, despite the lack of a genetic link between SNCA and PARK2/parkin, which mediates autophagy, both familial and sporadic PD involve death of SN neurons, suggesting that Nilotinib may activate PARK2 to facilitate autophagic clearance of SNCA, in agreement with published reports that ABL1 activation inhibits PARK2 function.

In summary, Nilotinib stimulates simultaneous autophagic clearance of SNCA and p-MAPT. Therefore, the next step will be to conduct phase II clinical trials to evaluate Nilotinib effects in PD and other α-synucleinopathies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.