The antioxidant N-acetyl-L-cysteine exerts strong neuroprotective effects in both *in-vitro* and *in-vivo* models of Parkinson's disease

NEUROFIT PRECLINICAL RESEARCH

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Introduction

Parkinson's disease (PD) is a devastating neurodegenerative disorder for which there is no cure. It is caused by the loss of dopaminergic (DA) neurons in the striatum, responsible for disabling motor symptoms. The neurotoxin 6-hydroxydopamine (6-OHDA) is used for the modeling of Parkinson's disease in both in-vitro and in-vivo experiments. In lab animals, 6-OHDA produces striatal dopamine depletion along with sensorimotor deficits. In cell cultures, 6-OHDA induces death of dopaminergic neurons in dose-dependent manner.

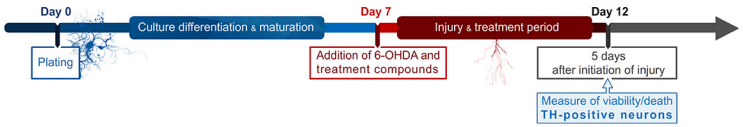
Objectives

The neuroprotective potential of 4 different treatment mechanisms including the inhibition of oxidative stress by the antioxidant N-Acetylcysteine (NAC) is evaluated in 6-OHDA-intoxicated dopaminergic neurons in culture. The most efficient treatment is then assessed 6-OHDA hemiparkinsonian rats for its ability to prevent the development of motor symptoms and dopamine depletion.

Assessment of neurotoxicity and neuroprotection in neuronal cultures

Culture of neurons from the mesencephalic brain of rat embryos (day 15 of gestation).

Measure of death of dopaminergic neurons by immunostaining of Tyrosine hydroxylase - positive neurons.



Assessment of sensorimotor motor deficit in 6-OHDA hemiparkinsonian rats

Stereotactic injection 6-OHDA in the medial forebrain bundle of male wistar rats.

Measure of behavioral motor performance of rats.

Quantification of striatal dopamine.

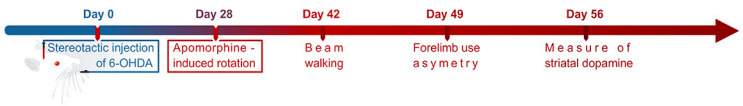
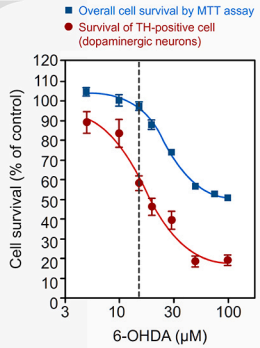
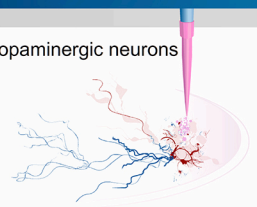


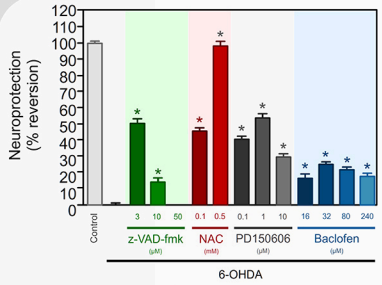
Figure 1: 6-OHDA-induced death of dopaminergic neurons





15 µM was chosen as concentration of **6-OHDA** to specifically assess the death of dopaminergic neurons since at this concentration marked death of TH-positive neurons was observed while the overall status of the cell cultures in the MTT assay was not affected.

Figure 2: Neuroprotective potential of treatment mechanisms



Baclofen (GABAB receptor agonist),
Z-vad-fmk (caspases inhibitor) and
PD150606 (calpain inhibitor)
partially prevented the death of dopaminergic neurons induced by 6-OHDA intoxication.
The antioxidant NAC fully prevented the neurotoxic action of 6-OHDA on dopaminergic neurons.

Figure 3: Measure of the striatal level of Dopamine and its metabolites (HVA and DOPAC)

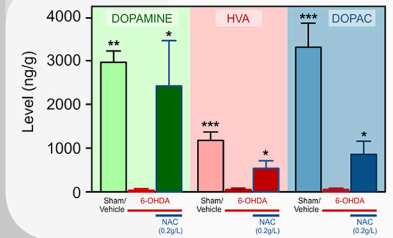
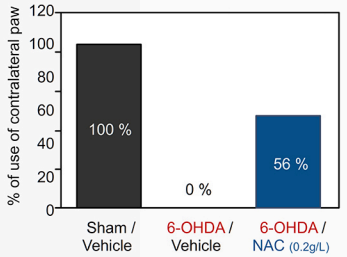


Figure 4 : Cylinder test (Limb asymmetry use)

Forelimb contacts with the wall of an open-top, clear plastic cylinder while rearing are counted during 2 min period.
The percentage of use of the affected forelimb (contralateral) is calculated as index of motor function in this test.



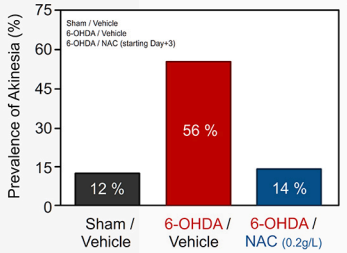
6-OHDA hemiparkinsonian rats showed dramatic motor deficit in the contralateral forelimb.

NAC treatment markedly improved the contralateral forelimb motor deficit observed in 6-OHDA hemiparkinsonian rats.

Figure 5 : Beam walking test

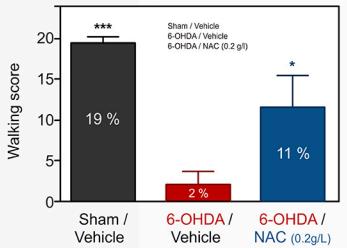
Rats walk across an elevated 2.5 m long beam graduated every 50 cm.
Successful walk every 50 cm is scored with 1 point.
Misplacement is scored with -1.

■ Delayed motor initiative



Rats with delayed motor initiative are those that do not move within the 120 s after the initiation of the test and thus are considered having limb akinesia.

■ Walking performance



The prevalence of Akinesia was markedly high in 6-OHDA hemiparkinsonian rats. In addition, 6-OHDA hemi parkinsonian rats showed poor beam walking performance. NAC treatment markedly improved the motor deficit observed in 6-OHDA hemi parkinsonian rats.