# Neuroprotective effect of AZP2006 on dopaminergic neurons after injury

*Cecilia Estrella[1], Nicolas Salomé[2], Régis Bordet[3], Noelle Callizot[1] and Philippe Verwaerde[1]*
*[1]AlzProtect SAS, 85C rue Nelson Mandela, Parc Eurasanté-Le Galénis 59120, Loos, France*
*[2] Faculté de Médecine, Université de Lille- 59000 Lille, France*
*[3]INSERM U1171, Faculté de Médecine, Université de Lille- 59000 Lille, France*

AlzProtect
Therapeutic Solutions for Neurodegeneration

## INTRODUCTION

**Parkinson's disease is an age-related neurodegenerative disorder characterized by progressive and selective loss of dopaminergic neurons (DA) in the substantia nigra that contributes to the cardinal motor symptoms of the disease: bradykinesia, postural deficits and resting tremor.**
**AZP2006 is a disease-modifying small molecule that readily crosses the blood-brain barrier. It displays neuroprotective properties promoting neuron survival, neurite outgrowth and synaptogenesis *in vitro*.**
**AZP2006 increases the levels of the multi-functional neurotrophic factor Progranulin (PGRN). PGRN is a secreted glycoprotein primarily expressed in mature neurons and microglia. PGRN acts as an autocrine neurotrophic factor promoting neuronal survival and enhancing neurite outgrowth. It may also act as anti-inflammatory factor in neuroinflammation.**
**Considering the properties of AZP2006, the aim of this study was to investigate the neuroprotective effects of AZP2006 on both dopaminergic neurons and motor functions in Parkinson's disease (PD) models.**

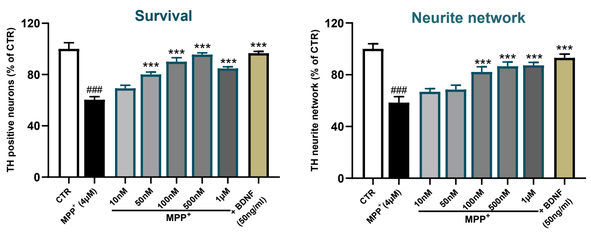
**AZP2006 is currently developed for the treatment of Progressive Supranuclear Palsy (PSP). Orphan drug designation has been granted to AZP2006 by the EMA and the FDA.**

## RESULTS

### *AZP2006 promotes DA neurons survival*

#### *1) DA toxicity induced by MPP+*

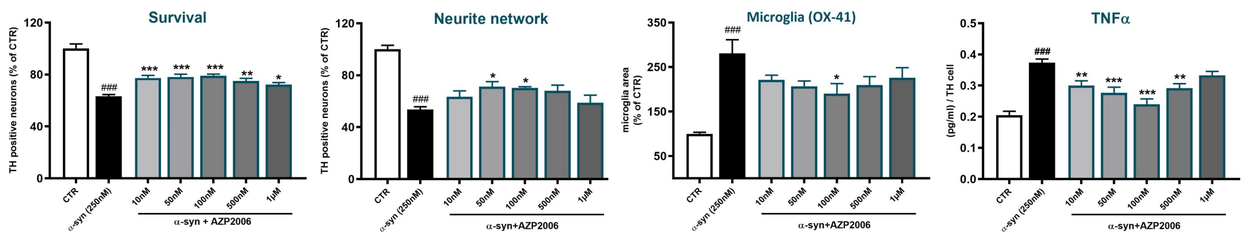
Rat primary mesencephalic neurons cultured with microglia were injured with the dopaminergic neurotoxin 1-methyl-4-phenylpyridinium ($MPP^+$, 4µM) and co-incubated with AZP2006 for 48h. BDNF (50ng/ml) was used as positive test control.
$MPP^+$ induced a severe loss of DA (TH-positive) neurons and a shrinkage of the neurite network. AZP2006 exerted neuroprotective effects on both neuronal survival and neurite network.




*Data expressed as percentage of control show the mean ± SEM. One-way ANOVA followed by Dunnett's test, n=6. ### or *** indicate p<0.001 vs CTR (#) or vs MPP+ condition (*)*

#### *2) DA toxicity induced by α-synuclein*

The α-syn peptide preparation (250 nM) was applied on rat primary mesencephalic neurons cultured with microglia and co-incubated with AZP2006 for 48h.
α-syn induced a loss of DA (TH-positive) neurons, reducing the neurite network and increasing the active microglia (OX-41) and the levels of TNFα. AZP2006 significantly protected neurons and their neurite network reducing microglia activation and TNFα release.

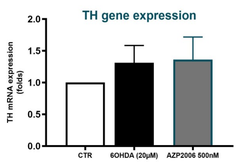


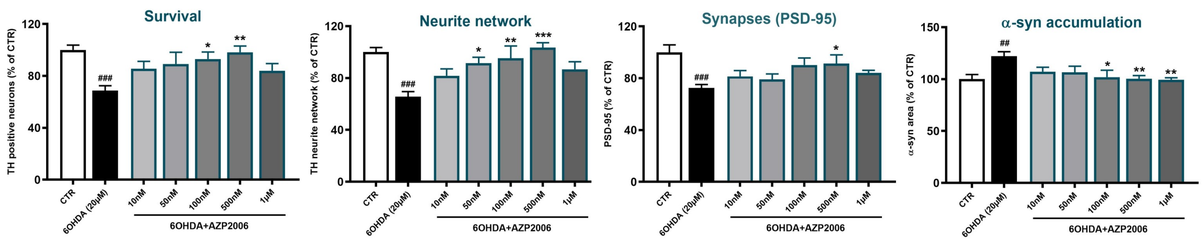

*Data expressed as percentage of control show the mean ± SEM. One-way ANOVA followed by Dunnett's test, n=6. *, ** or *** indicate p<0.05; p<0.01 or p<0.001 vs α-syn or ### vs CTR condition*

### *AZP2006 promotes neuronal survival via PGRN*

#### *3) DA toxicity induced by 6-OHDA*

Rat primary mesencephalic neurons cultured with microglia were injured with 6-hydroxydopamine (6-OHDA, 20µM) and co-incubated with AZP2006 for 48h.
In presence of 6-OHDA, a large loss of DA (TH-positive) neurons and number of synapses (PSD-95) was observed as well as a decrease of the neurite network. 6-OHDA also induced a large and significant α-syn aggregation inside TH- neuron cytoplasm.
AZP2006 exerted neuroprotective effects on neuronal survival, synapses and the neurite network without increasing the TH gene expression. AZP2006 added concomitantly with 6-OHDA was able to significantly protect neurons from α-syn accumulation.



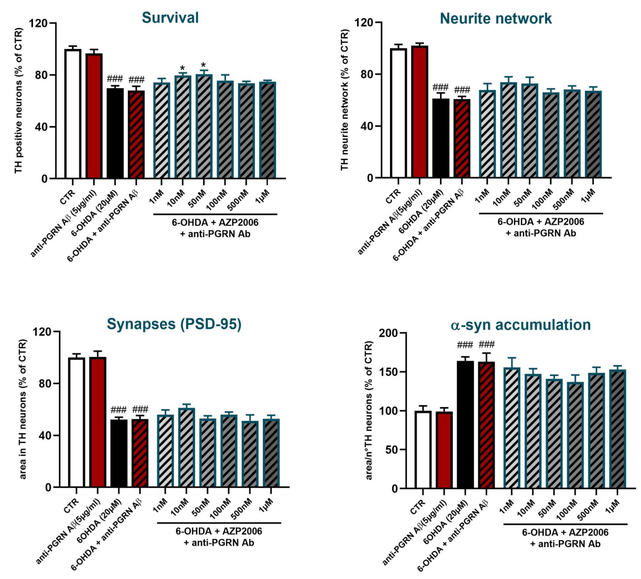




*Data expressed as percentage of control show the mean ± SEM. One-way ANOVA followed by Dunnett's test, n=6. *, ** or *** indicate p<0.05; p<0.01 or p<0.001 vs 6-OHDA and ## or ### vs CTR condition*

Mouse primary mesencephalic neurons cultured with microglia were injured with 6-OHDA (20µM) and co-incubated with AZP2006 in presence of anti-PGRN Ab (5µg/ml) for 48h.

***Blocking Ab Anti PGRN***

The neuroprotective effect of AZP2006 on neurite network, synapses and α-syn accumulation was abolished in presence of the anti-PGRN Ab. However, a slight effect on neuron survival was still evidenced.

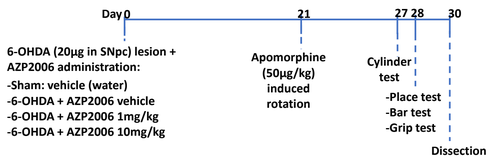



*Data expressed as percentage of control show the mean ± SEM. One-way ANOVA followed by Dunnett's test, n=6. ### indicate p<0.001 vs CTR or anti-PGRN-Ab and * p<0.05 vs 6-OHDA+anti-PGRN-Ab condition*

### *AZP2006 prevents motor disfunctions*

#### *4) Nigro-striatal lesion induced by infusion of 6-OHDA in the SNpc*

The experimental model of nigro-striatal lesion induced by 6-OHDA was used to assess the ability of AZP2006 to restore motor and sensorimotor functions in the rat.
Two-month Wistar male rats (n=10 /group) were unilaterally lesioned (20µg 6-OHDA) /rat into the right **Substantia Nigra pars compacta** (SNpc). Since the intra-SNpc injection will yield a complete and immediate lesion, a short therapeutic window is left to administer AZP2006. Thus, in order to test a possible effect in neuroprotection, AZP2006 (1 or 10mg/kg) was administered p.o. (gavage) 30 min after the 6-OHDA infusion.




Rats with unilateral 6-OHDA lesions of the nigrostriatal DA pathway exhibit contralateral rotational behaviour in response to the systemic administration of the DA agonist Apomorphine.

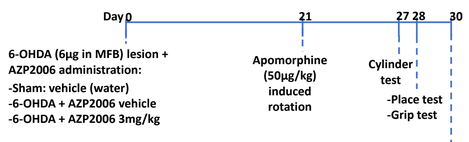
**Apomorphine-induced rotations**

| Treatment | Number of rotations |
|---|---|
| Sham | -23.20 ± 11.31 |
| 6-OHDA | 72.50 ± 24.89 |
| 6-OHDA+AZP2006 (1 mg/kg) | 100.40 ± 28.22 |
| 6-OHDA+AZP2006 (10 mg/kg) | 59.11 ± 8.20 |

*Note: Values expressed as means ± SD*

#### *5) Nigro-striatal lesion induced by infusion of 6-OHDA in the MFB*

Two-month Wistar male rats (n=14-18 /group) were unilaterally lesioned (6µg 6-OHDA) /rat into the right **Medial Forebrain Bundle** (MFB). Desipramine hydrochloride (25mg/kg i.p.) was administered 30 min prior to 6-OHDA injection to protect noradrenergic neurons. In order to test a possible effect in neuroprotection, AZP2006 (3 mg/kg) was administered p.o. (gavage) daily for 30 days starting the day before the the 6-OHDA infusion.




** Study is ongoing*

**Dissection:**
-AZP2006 levels in Brain, CSF, whole blood and plasma
-PGRN, TREM2, IL-6, IL1β levels in CSF, plasma and brain tissue (SN and striatum)
-Catecholamine (DA, DOPAC,HVA) levels in striatum
-IHC TH, DAT, ChAT in SN and striatum

**Apomorphine-induced rotations**

| Treatment | Number of rotations |
|---|---|
| Sham | -0.46 ± 3.60 |
| 6-OHDA | 309.3 ± 89.92 |
| 6-OHDA+AZP2006 (3 mg/kg) | 293.6 ± 109.8 |

*Note: Values expressed as means ± SD*

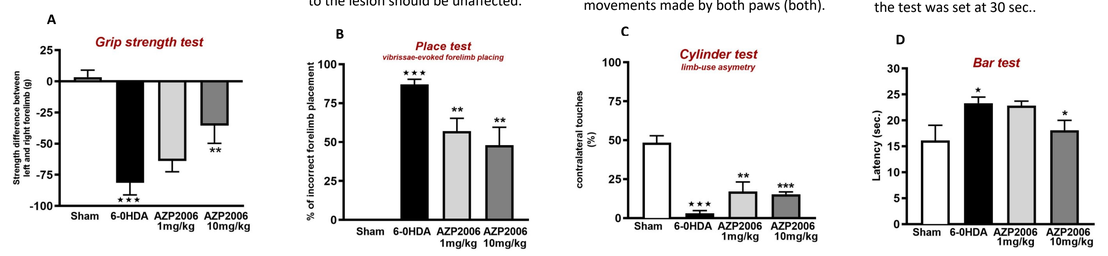
Clear spontaneous limb asymmetries were evaluated by motor evaluation tests.

The Grip test quantifies the muscular strength. Rats were allowed to grab the metal grid and were then pulled backwards in the horizontal plane. The force applied to the grid just before it loses grip was recorded as the peak tension.

Forelimb placing was measured for both forelimbs of the animal upon stimulation of the whiskers. The placement of the forelimb contralateral to the lesion is expected to be impaired upon stimulation of whiskers, while the forelimb ipsilateral to the lesion should be unaffected.

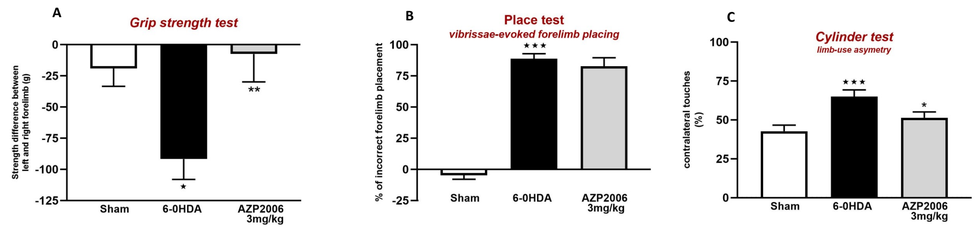
Animals were placed in a glass cylinder and recorded for 5 minutes. Forelimb asymmetry was assessed by scoring independent, weight-bearing contacts on the cylinder wall of the ipsilateral (ipsi) or contralateral (contra) paw, relative to lesioned hemisphere, as well as movements made by both paws (both).

In the Bar test, the rat is grasped around the shoulders and their forepaws are placed on a horizontal bar located 10 cm above the cage floor while the hind paws remain on the floor. The latency to remove both their paws from the block to the ground is measured. A cutoff for the test was set at 30 sec..




*Data show the mean ± SEM. One-way ANOVA followed by Dunnett's or Fisher Tests, * p<0.05; **p<0.01 and ***p<0.001 vs 6-OHDA and vs Sham; n=6-10.*

A) Grip strength test: data show the difference in the strength force between the left forelimb (contralateral) – right forelimb
B) Percentage of unsuccessful forelimb placing responses in the Place test
C) Percentage of contralateral touches relative to the number of touches (ipsi+contra) in the limb-use asymmetry cylinder test
D) Latency to remove both paws in the Bar test




*One-way ANOVA followed by Dunnett's or Fisher Tests, * p<0.05; **p<0.01 and ***p<0.001 vs 6-OHDA and ★vs Sham; n=6-10.*

### *Preclinical and clinical key features of AZP006*

- Safety pharmacology studies : AZP2006 does not show any significant effect
- Distribution: AZP2006 is rapidly absorbed following oral administration and crosses the BBB
- Metabolism : one main metabolite (M2) in dog and human
- Toxicity studies : AZP2006 orally administered once daily for up to 20 weeks (ongoing) in rats and 32 weeks dogs (ongoing) do not show any relevant finding
- Clinical studies : oral administration of AZP2006 (liquid formulations) to healthy human adults for up to 10 days was well tolerated, had a good safety profile.

## CONCLUSIONS

**AZP2006 at nanomolar concentrations showed neuroprotective effects on dopaminergic neurons injured with $MPP^+$, α-synuclein and 6-OHDA. Blocking PGRN action abolished the effect of AZP2006, indicating that this neurotrophic factor could be involved into the neuroprotective action of the compound. Similar results has been obtained in primary cortical neurons intoxicated with $A\beta_{1-42}$ peptide. Interesting, the increased neuron survival and neurite network protection were associated to the increase of released PGRN but not BDNF, evidencing the specificity of AZP2006 effect.**
**AZP2006 was able to partially inhibit the effect of 6-OHDA on motor deficits related to the depletion of dopamine. This effect could be explained by a possible protective effect of AZP2006 preventing the development of a complete neurotoxicity on the nigrostriatal pathway.**
**AZP2006 is an investigational product that is being developed for the treatment of Progressive Supranuclear Palsy (PSP). Nevertheless, these results have shown additional properties indicating a potential application for the treatment of PD.**