The study of molecular changes induced by CPL500-036, a potential novel anti-dyskinetic drug, in 6-OHDA rat model of PD

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INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurotransmission, mainly in the striatum [1]. Dopamine replacement therapy, e.g. with levodopa (L-DOPA), remains the gold standard of therapy. Unfortunately, following years of exposure, levodopa causes motor complications such as levodopa-induced dyskinesias (LIDs). Phosphodiesterase 10A regulates cAMP/cGMP downstream signaling (e.g. cAMP/PKA/DARPP-32) thus having a key role in the regulation of dopaminergic signaling in the direct and indirect striatal pathways [2]. Studies in rodent models of PD have shown changes in cAMP/cGMP levels associated with the development of LIDs [3,4].

CPL500-036 is a novel PDE10A inhibitor developed in laboratories of Celon Pharma S.A. [5]. It is characterized by high *in vitro* and *in vivo* potency, it is highly selective and has good oral bioavailability (F > 70%) and BBB penetration (B/P = 0,4) in rats. Several behavioural studies have confirmed its antipsychotic and procognitive action in rats (MED > 0,03 mg/kg). CPL500-036 is currently investigated in clinical trials. In October 2019 CelonPharma S.A. completed a phase I clinical trial for CPL500-036 [6].

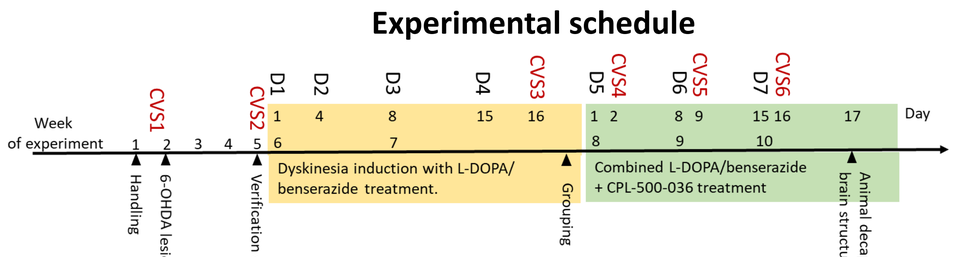
In this study, we aimed to verify the molecular response to the novel PDE10A inhibitor, CPL500-036, in the unilateral model of Parkinson's disease in rats. CPL500-036 significantly reduces the level of LIDs in 6-OHDA rat model already at a dose of 0,03 mg/kg after chronic administration. Based on theses results, dopamine level in substantia nigra and concentration of cyclic nucleotides and phosphorylation of proteins in striatum has been investigated.

MATERIALS AND METHODS

In vivo rat studies

1. Ctr (solvent+solvent) po
2. L-DOPA 6 mg/kg/benserazide 6 mg/kg plus solvent po
3. L-DOPA 6 mg/kg/benserazide 6 mg/kg plus CPL-500-036 0.03 mg/kg po
4. L-DOPA 6 mg/kg/benserazide 6 mg/kg plus CPL-500-036 0.1 mg/kg po
5. L-DOPA 6 mg/kg/benserazide 6 mg/kg plus CPL-500-036 0.3 mg/kg po

Experimental schedule



Behavioral tests:
C – cylinder test
V – vibrissae test
S – stepping test
D – dyskinesia

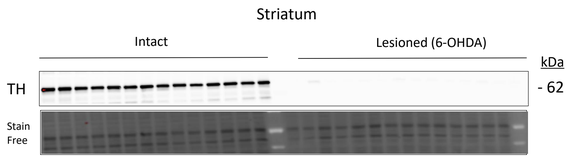
Experimental milestones:
1. Handling, VSC tests – pretest (behavioral baselines)
2. 6-OHDA lesions
3. Verification of lesion degree –CVS2 tests (behavior after toxin-induced damage)
4. Dyskinesia induction –L-DOPA/benserazide administrations:
   D1-4 tests at 1, 4, 8, 15-day of administration;
   CVS3 at 16 day of L-DOPA administration (behavioral state before introducing CPL).
5. Homogenous group creation based on animal dysinesia level.
6. L-DOPA/benserazide + CPL combined treatment: D5-7 tests at 1, 8 and 15 day (anti-dyskinetic actions of CPL); CVS at 2, 9 and 16 day (effect of CPL on L-DOPA antiparkinsonian action – ON phase)
7. Animal decapitation, brain structures dissection for further biochemical analyses

Molecular pathway:

- **cAMP/cGMP levels –** the striatal tissue was prepared according to manufacturer instruction of ELISA kit (Cayman Chemicals, Item NO. 581001) and analyzed by using UPLC methods.
- **Dopamine level –** dopamine from substantia nigra were extracted, derivatized using ethyl chloroformate and was quantified in its stable derivative form in the presence of internal standard 3,4- dihydroxybenzylamine (DHBA) using highly sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS, column ZORBAX Eclipse Plus C18: 3.0 x 150 mm; 3.5 – micron).
- **Protein levels/phosphorylation -** striatum tissue was homogenized in trichloroacetic acid (TCA). The protein pellets were resuspended in Laemmli buffer, shaked overnight at 4°C until the pellet dissolves and next denaturaded at 95°C. The total and phosphoprotein levels antibodies against: GluR1, GluR1 pSer845, PDE10A (both from Merck Millipore), DARPP-32, DARPP-32 pThr34, MSK1, MSK1 pSer376, ERK1/2, ERK1/2 pThr202/Tyr204 (all from Cell Signaling Technology) were used. To validate changes in the level of protein interest, the Stain-Free technology (Bio-Rad) was used to normalize total protein on the blot. The changes of phosphorylation of protein were normalized to total protein and the data between different blot was normalized to one sample, which was run on every blot. All. antibodies were used according to manufacturer's protocol.
- Dopaminergic neuronal cell death was measured by tyrosine hydroxylase (TH) expression (Merck Millipore).

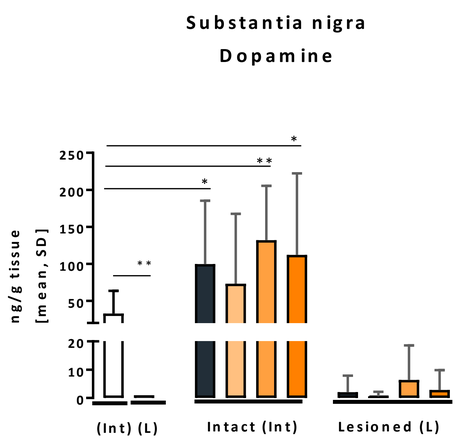
RESULTS

The 6-OHDA lesion produced a complete loss of striatal TH-positive neurons



Representative Western blot of striatal TH in the intact and lesioned site (n =14 rats per group)

L-DOPA increased significantly dopamine levels in substantia nigra in intact side

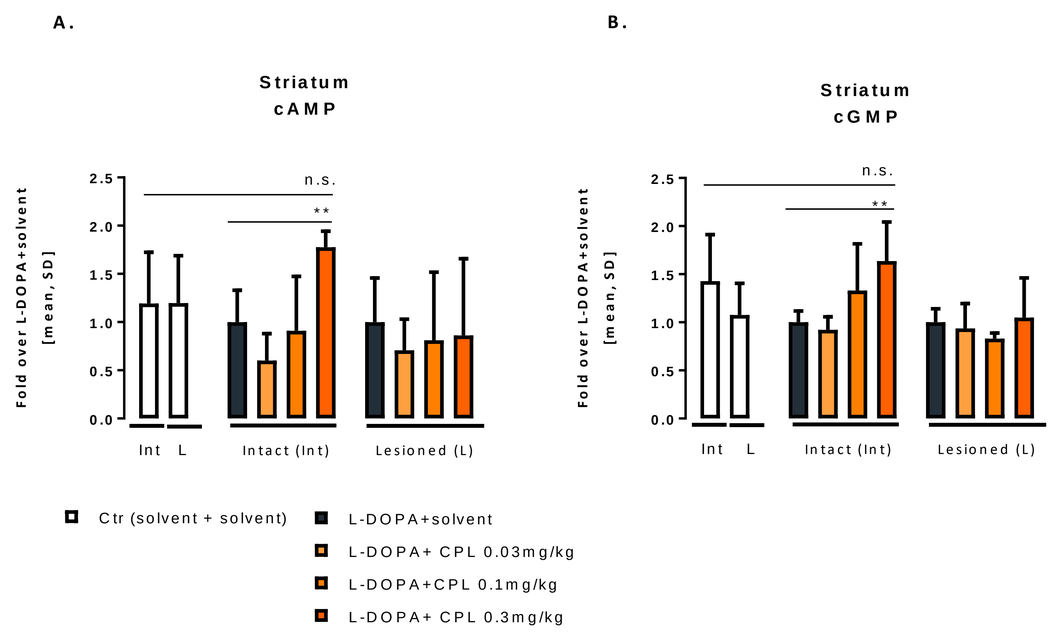


Data are presented as the mean ±SD (N=7-9 rats per group)
*p≤0,05, **p≤0,01, n.s. – non significant vs. intact site CTR (solvent + solvent); Dunette's for multiple comparisons.

The expression of PDE10A was decreased in lesioned site

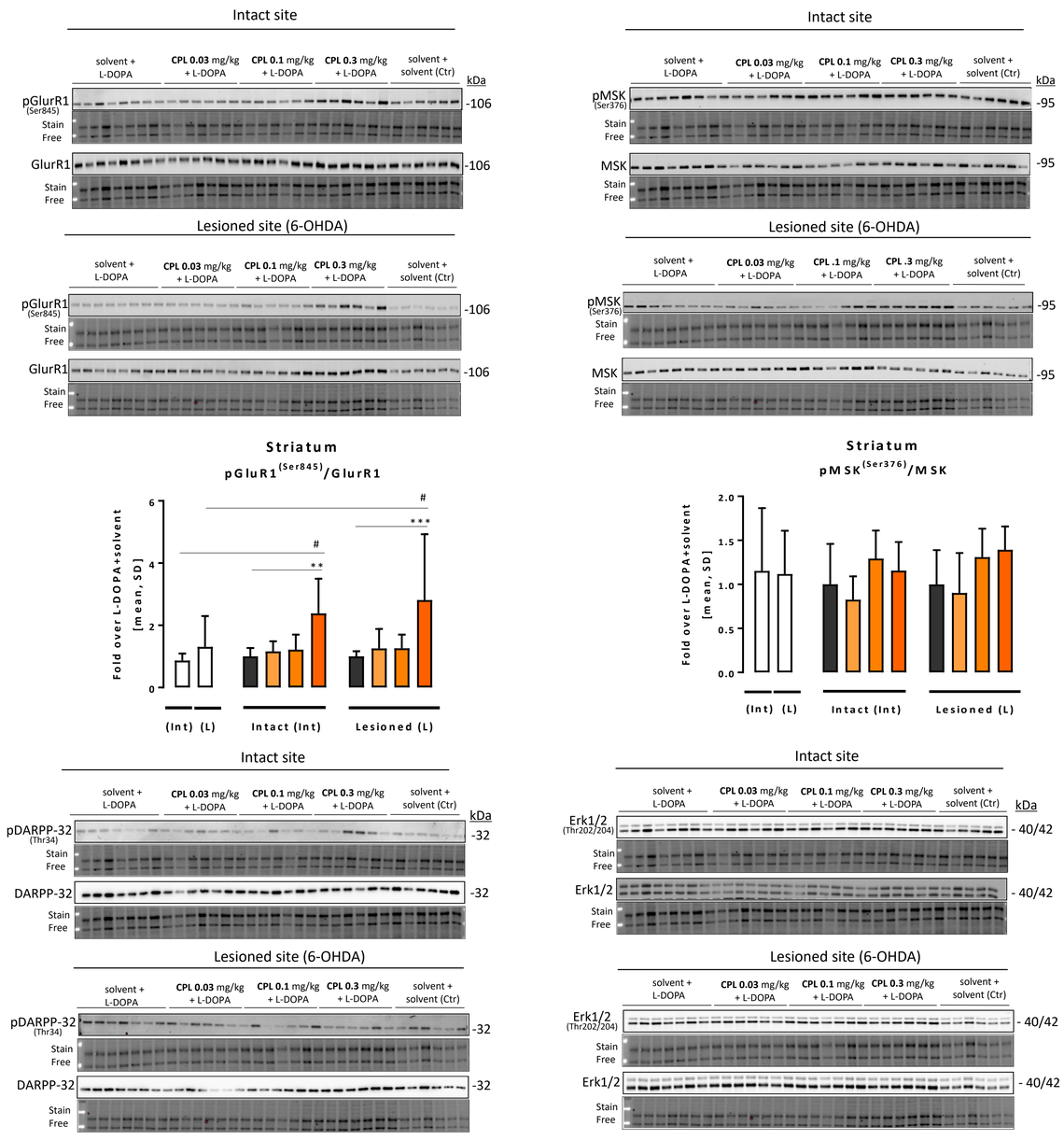
Representative Western blot of striatal PDE10A expression in the intact and lesioned hemispheres (n =5-6 rats per group).
*p≤0,05 vs. solvent+ solvent treated group, Bonferroni for multiple comparisons

The level of cAMP and cGMP increased significantly with the highest dose of CPL500-036 in the intact site



Data are presented as the mean ±SD (N=7-9). *p≤0,05, **p≤0,01 vs. L-DOPA + solvent, n.s. – non significant vs. Ctr (solvent+solvent); Dunette's for multiple comparisons

The highest dose of CPL500-036 significantly increased GluR1(Ser 845) in striatum



Data are presented as the mean ±SD (N=9-10 rats per group)
*p < 0.05, **p < 0.01, ***p<0,001 vs. L-DOPA + solvent treated group, Bonferroni for multiple comparisons

SUMMARY AND CONCLUSIONS

- The 6-OHDA lesion produced a complete loss of striatal TH-positive neurons (DA depletion).
- The expression of PDE10A was decreased in lesioned site.
- The levels of dopamine increased after L-DOPA treatment in substantia nigra (SN) in intact site to reach statistical significance compared solvent + solvent treated groups. Combining L-DOPA with CPL500-036 in doses of 0,1 and 0,3 mg/kg increased the level of dopamine in the intact site of SN compared solvent + solvent treated group significantly. No effect was observed in lesioned site.
- The level of cAMP and cGMP increased significantly in the intact site of striata of animals treated with the highest dose of CPL500-036 (0,3 mg/kg) compared to L-DOPA + solvent treated groups. Levels of cAMP/cGMP remained unchanged in the lesioned site.
- Significant changes of phosphorylation of GluR1(Ser 845) were observed in striatum of both hemispheres (intact and lesioned of the animals treated with a dose of 0.3 mg/kg of CPL500-036). No significant changes were observed in protein phosphorylation of DARPP-32(Thr34), MSK(Ser376) and pERK(Thr202/Tyr204).

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