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CPL-500-036 – NOVEL AND HIGHLY BIOAVAILABLE PDE10A INHIBITOR ACTIVATES CYCLIC NUCLEOTIDES DEPENDING SIGNALING IN RAT STRIATUM

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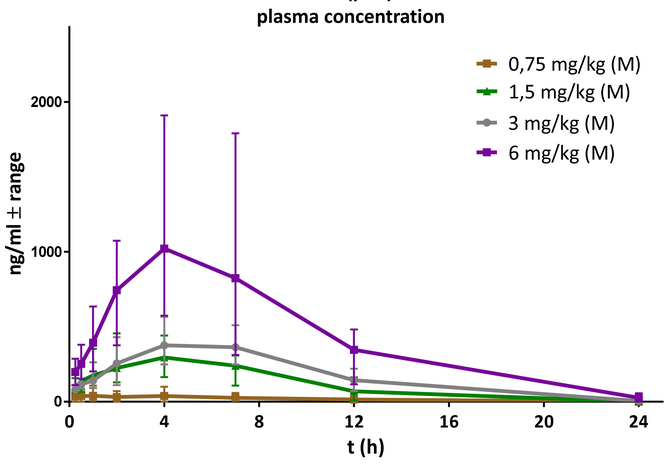
INTRODUCTION

Phosphodieserase 10A (PDE10) is specifically expressed in medium spiny neurons (MSNs) in the striatum and hydrolizes both, cAMP and cGMP – cyclic nucleotides that are secondary messengers in many types of cells. Striatal neurons are part of neuronal loops system and connect frontal cortex, thalamus and projecting back to cortex. MSNs regulate brain activity by modulating input signals from the cortex via activation of D1 pathway and D2 pathway. Inhibition of PDE10A in the striatopallidal circuit leads to diminishing of the D2 pathway activation signalling in contrast to the direct circuit, where stratonigral inhibition of the PDE10A result in activation of the D1 pathway regulated signalling. Balance between these two circuits plays essential function in controlling motor and executive functions. It is postulated that modulation of both circuits by inhibition of PDE10A may lead not only to treating the positive symptoms, but also to improving negative or cognitive dysfunctions in schizophrenia.

In the present study we assessed the pharmacokinetic and pharmacodynamic properties of a novel PDE10A inhibitor - CPL-500-036 in comparison to reference inhibitors.

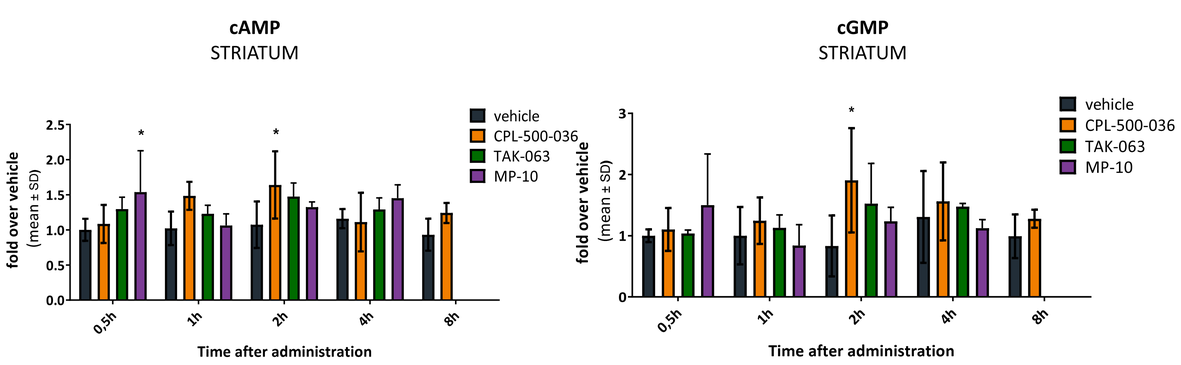
RESULTS

PHARMACOKINETIC PARAMETERS OF CPL-500-036 IN RATS



Species		Rat	Rat	Rat	Rat
Strain		Wistar	Wistar	Wistar	Wistar
Animal number/ gender		5M	5M	5M	5M
Dose		0,75 mg/kg	1,5 mg/kg	3 mg/kg	6 mg/kg
Route		Oral (solution)	Oral (solution	Oral (solution)	Oral (solution)
Parameter	Unit	Value			
AUC(0-t)	µg*h/l	505	2413	3519	10363
AUC(0-inf)	µg*h/l	1444	2866	5007	17505
Kel	1/h	0,16	0,26	0,18	0,17
T1/2kel	h	37	3,17	6,36	15
Cmax	ng/ml	66	371	462	1228
Tmax	h	2,2	4,80	5,20	5,20

EFFECT OF PDE10A INHIBITORS ON CYCLIC NUCLEOTIDE LEVELS IN RAT STRIATUM



MATERIALS & METHODS

Pharmacokinetic (PK)
The pharmacokinetic parameters of CPL-500-036 were evaluated in male Wistar rats (n=5 per group) following single oral administration of the compound at a dose of 0,75; 1,5; 3, 6 mg/kg.
Animals were sacrificed (under isofluran anasthesia) at 15 min, 30 min, 1 h , 2 h, 4 h, 7 h, 12 h and 24 h. Blood samples were collected and plasma was obtained; brain tissues of each specimen were homogenized. The plasma and brain concentrations of CPL-500-036 were evaluated with LC/MS/MS method.

Target Occupancy
In vivo affinity study of the CPL-500-036 with the plasma concentration-time-occupancy relationship analysis were comissioned to Invicro Ltd. The animals were treated with the CPL-500-036 (0,1-0,5 mg/kg) and [11C] IMA107 and scanned at various time-points using PET method.

Pharmacodynamic (PD)
Pharmacodynamic analysis of PDE10A inhibitors was performed *ex vivo* in male Spargue-Dawley rats. Animals was orally administrated with CPL-500-036 (3mg/kg), TAK-063 (3mg/kg) and MP-10 (10mg/kg) and sacrified using microwave irradation after 0,5; 1; 2; 4; 8 hours after compound administration. Tissue samples (striatum, hippocampus and prefrontal cortex) were further used for cyclic nucleotide, protein phosphorylation and trancriptomic analysis.

Cyclic AMP and GMP examination
Cyclic nucleotides measurements in striata tissue from one hemisphere were performed by ELISA (Cayman Chemicals) according to the manufacturer's protocol.

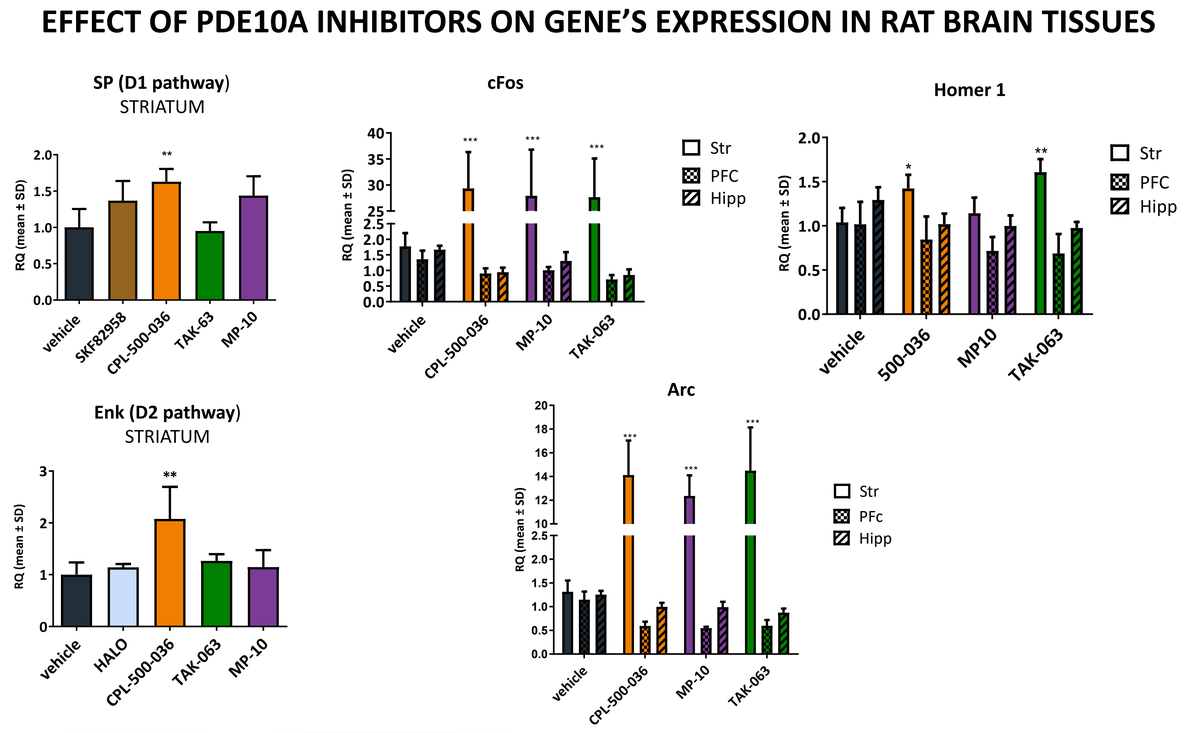
Phosphorylation analysis:
Dissected striata from all rats were homogenized in RIPA buffer containing inhibitor coctail or trichloroacetic acid and sonicated. Lysates were used to Western blot analysis of total and phosphoprotein levels using antibodies against: GluR1, GluR1 pSer845 (both from Merck Millipore), DARPP-32, DARPP-32 pThr34, CREB, CREB pSer133, MSK1, MSK1 pSer376, ERK1/2 , ERK1/2 pThr202/Tyr204, histone H3, histone H3 pSer10 (all from Cell Signaling Technology), all normalized to β-tubulin (Merck Millipore) levels. All antibodies were used according to manufacturer's protocol.

Real-time PCR:
For Real time PCR analysis rats were sacrified by microwave irradation after 4h of compounds administration and haloperidol (1mg/kg) SKF82958 (2mg/kg) were used as a D1 and D2 pathway activators, respectively. The total RNA from striatum, hippocampus and prefrontal cortex was isolated using RNeasy Lipid Tissue kit (Qiagen) according to the manufacturer protocol. The cDNA template for Real-time PCR reaction were prepared using PrimeScript RT Reagent Kit (Takara). Real time PCR with the TaqMan hydrolysis probes (Thermo Fisher Scientific) were used for transcriptomic analysis of following genes: Enk, SP, Homer1, cFos, Arc

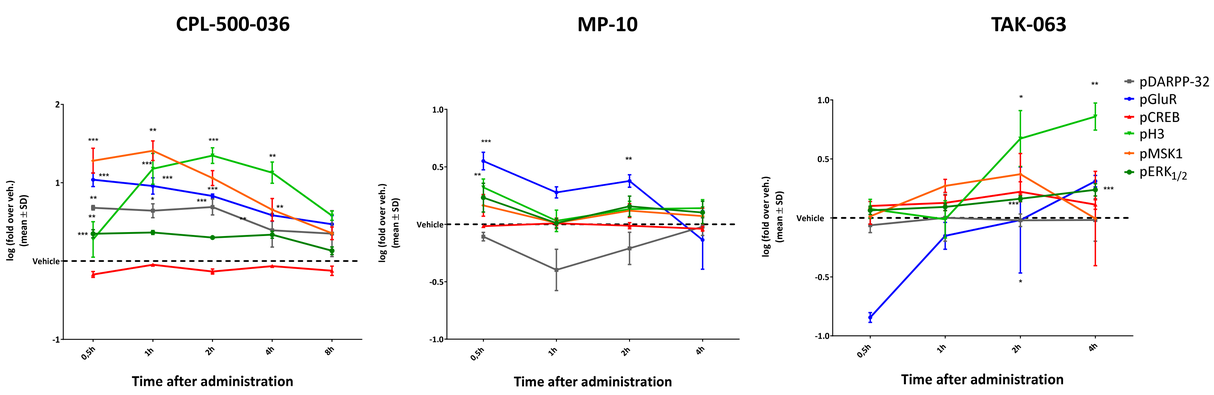
Statistic analysis:
Statistical significance was determined using two-way ANOVA with the Bonfferoni post-hoc test for the Western blot and cAMP/cGMP measurements. One-way ANOVA with the Bonfferoni post-hoc test was used for qPCR results. P values ≤0.05 were considered significant.

RESULTS

EFFECT OF PDE10A INHIBITORS ON GENE'S EXPRESSION IN RAT BRAIN TISSUES

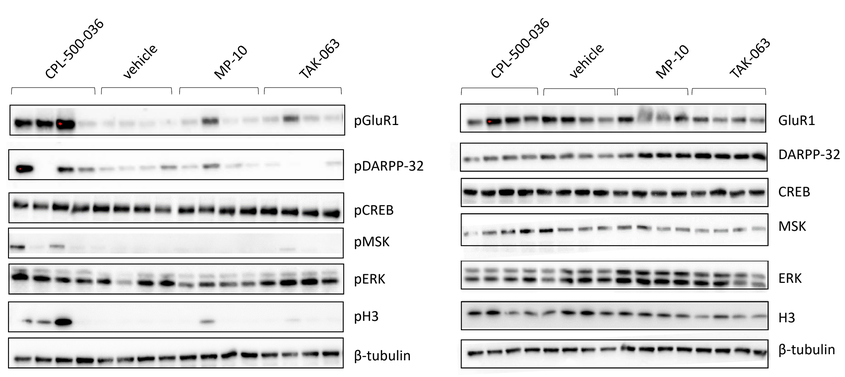


EFFECT OF PDE10A INHIBITORS ON PROTEIN PHOSPHORYLATION

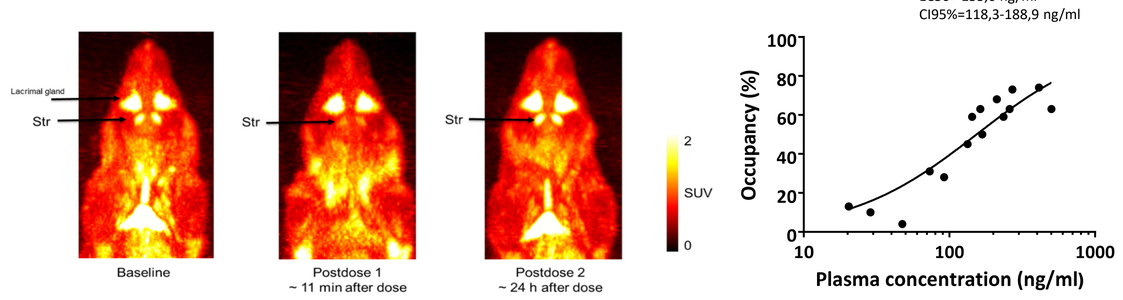


Animal No.	Compound concentration after 2h of administration [ng/ml]		
	CPL-500-036	MP-10	TAK-063
1	29,8	-	-
2	35,9	-	-
3	95,0	-	-
4	8,2	-	-
5	-	5,9	-
6	-	26,1	-
7	-	5,4	-
8	-	4	-
9	-	-	14,1
10	-	-	18,5
11	-	-	12,9
12	-	-	15,5

Representative Western blots for striatal tissue after 2h of compounds administration



TARGET OCCUPANCY OF CPL-500-036



EC50= 153,6 ng/ml
CI95%=118,3-188,9 ng/ml

CONCLUSION

- **CPL-500-036** compound has satisfactory pharmacokinetic profile with **good oral bioavailability and BBB penetration** in rats.
- **CPL-500-036** selectively binds to the PDE10A and the *in vivo* affinity (EC50) to PDE10A is estimated at 153 ng/ml of compound concentration in plasma.
- **CPL-500-036** increases the concentration of cyclic nucleotides 2h after administration to a comparable extent as the reference compounds.
- **CPL-500-036** increases the expression of D1 and D2 pathway activation markers 4 h after administration whereas MP-10 and TAK-063 has no effect on the enkephalin and substance P mRNA expression
- All of tested PDE10A inhibitors increase the mRNA expression of early response genes in rat striatum 4h after administration
- **CPL-500-036** activates the downstream signalling pathways controlled by cyclic nucleotides level and significantly increase the phosphorylation levels of striatal PKA/PKG targets such us: GluR1, CREB, DARPP-32, MSK1 and histone H3 in a time dependent manner.
- **EVALUATION OF CPL-500-036 IN PRESENTED STUDY JUSTIFIES ITS FURTHER DEVELOPMENT AS A POTENTIAL TREATMENT THERAPY FOR PSYCHOTIC DISORDERS.**