P.3.c. Psychotic disorders and treatment – Treatment (basic)

P.3.c.001 The prolyl oligopeptidase inhibitor IPR19 improves cognitive deficits in schizophrenia-like mouse models

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Cognitive decline associated with schizophrenia is considered a key feature of the disease since might precede the onset of the disease and continues after psychosis, being a predictor of the disorder evolution. Current antipsychotic drugs for positive or negative symptoms have little or no effect on cognitive deficits. Furthermore, an exacerbation of cognitive deficits by some antipsychotic drugs is plausible.

Prolyl oligopeptidase (POP) is an 81-kDa monomeric serine protease that is expressed in brain and other tissues. Experimental data showed that POP inhibitors have neuroprotective, anti-amnesic and cognition-enhancing properties. In the present study, the pharmacological activity of IPR19 [(S)-1-((2S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl) pyrrolodine-2-carbonitrile], a new potent and selective POP inhibitor, was evaluated in three schizophrenia-like mouse models based on pharmacological subchronic phencyclidine (PCP) and acute dizocilpine (MK801) administration, and offspring of mothers with immune reaction induced by polyinosinic:polycytidylic acid (poly(I:C)) administration during pregnancy. Behavioral responses in novel object recognition (NOR) test, T-maze, eight-arm radial maze, Morris water maze and prepulse inhibition (PPI) were tested under basal conditions and after acute IPR19 (5 mg/kg ip) administration in C57/BL6 male mice. Data were analyzed using unpaired Student's t-test, and one-way ANOVA test followed by Dunnett's post hoc test at $P < 0.05$. The number of animals was previously estimated by sample size calculations and ranged from 6 to 23 animals per group.

In NOR test, MK801-treated animals and offspring of poly(I:C)-treated mothers showed a lower ratio of time devoted to the novel object than controls. IPR19 reversed the effect, restoring basal values. In T-maze test at 10 s and/or 40 s delay, PCP-, MK801 and poly(I:C) models displayed fewer correct choices than controls. The effect was reversed by acute IPR19 in PCP and poly(I:C) models but not in MK801 model. In eight-arm radial maze test, PCP, MK801 and poly(I:C) models made less correct choices than the vehicle-treated counterparts. Acute IPR19 attenuated the percentage of errors in PCP and poly(I:C) models whereas was ineffective in MK801 model. In Morris water maze test, mice under PCP and MK801 treatments, and poly(I:C) offspring spent less time in the target quadrant where the platform was located than control mice. IPR19 had the capacity to reverse this decrease in the three models. A strong decrease in the PPI at 81, 85, and 90 db was observed after acute MK801 administration and with less intensity in offspring of poly(I:C) mothers. In contrast, subchronic PCP treatment did not induce effects on PPI. IPR19 reversed the effects of acute MK801 across the different intensities to values that did not differ from those in controls whereas IPR19 did not modified the impaired PPI response in the poly(I:C) model.

In conclusion, the POP inhibitor IPR19 has the capacity to enhance cognitive performance in different phenotype animal models of schizophrenia, suggesting that the compound may have therapeutic potential in the cognitive deficits associated to this psychiatric disorder. The finding becomes particularly valuable as a new option of pharmacological mechanism for the treatment of cognitive dysfunctions which represent the most treatment-elusive symptoms of schizophrenia.

Disclosure statement: This study was supported by MINECO-FEDER funds (RTC-2014–1645–1 SAF-2013–48586-R BIO2013–40716R INC-TU-2011–1442 PTQ-11–04688 INC-FPGS-2011–1708 INC-TU-2012–2312) by CDTI funds (IDI-20120546) the Basque Government (IT616–13) the Generalitat de Catalunya (XRB and 2014SGR-521).

P.3.c.002 Selective prolyl oligopeptidase inhibition by IPR19 as a new pharmacological treatment of the cognitive impairment associated with schizophrenia

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Prolyl oligopeptidase (POP) is an 81-kDa monomeric serine protease expressed in brain and other mammal tissues. Recent advances suggest that this enzyme participates in protein-protein interactions that control brain functions and cognition processes. POP inhibition shows neuroprotective, anti-amnesic and cognition-enhancing properties in some rodent models. IPR19, [(S)-1-((2S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl) pyrrolodine-2-carbonitrile], is a potent and selective POP inhibitor that displays outstanding cognition-enhancing effects in three validated schizophrenia-like mouse models (poly(I:C), MK801 and PCP). These observations thus show the potential of this compound for the treatment of the cognitive impairment associated with schizophrenia. The present study evaluated the activity and selectivity of the mechanism of action, pharmacokinetics, and toxicity of IPR19. This compound was designed and tuned to have a peptidomimetic scaffold with an optimal balance between inhibitory potency, selectivity, and ADME properties.

IPR19 inhibited recombinant hPOP in vitro, showing an IC50 of 80±6 nM. Furthermore, the compound was cell-permeable, since it inhibited endogenous POP in living SH-SY5Y cells (IC50 of 131±2.3 nM). In cell extracts, the IC50 was 41±1.7 nM. The activity of IPR19 over other proteases of the S9 family, such as the Fibroblast activation protein (FAP) and the Dipeptidyl peptidase (DPP-IV), was very low. IPR19 selectivity was also explored through a broad panel (>50) of human receptors, ion channels, and transporters. At 10 microM, IPR19 did not show significant binding to the alternative targets explored, thus revealing the exquisite selectivity of this compound for POP. In addition to potency and selectivity, the IPR19 showed high proteolytic stability; its half-life in CD-1 mouse blood was above 120 min, while it registered 45 min in liver mouse microsomes, and over 24 h in

human serum. Regarding the in vitro permeability of blood-brain barrier (BBB) models, IPR19 showed a logP of 2.2, 13.6±0.2% of transport in the BBB-PAMPA assay, and 25±2% in the Caco-2 permeability model. For the latter, the (A-B)/(B-A) ratio was 0.72, which indicates that IPR19 is not a P glycoprotein substrate. In vivo data showed that after acute IPR19 i.p. administration (5 mg/kg) in mice, the AUCblood/AUCbrain ratio was 0.21. This value indicates a notable permeability of the compound across the BBB. Once in the brain tissue, IPR19 inhibited POP activity. Thus, a single i.p. dose of IPR19 (5 mg/kg) decreased ex-vivo POP activity to 45±7%, which was obtained at 6 h after administration of compound. POP activity returned to basal values 12 h after the injection (93±14%). This long inhibition period is related to the intrinsic nature of IPR19, which has a nitrile group that reacts with the hydroxyl moiety of Ser554 of POP to make an imidate. Neither cytotoxicity (MTT assays) nor genotoxicity (AMES and zebra fish assays) was observed for IPR19.

In conclusion, IPR19 is a potent, selective, BBB-permeable and non-toxic POP inhibitor that shows potential for the treatment of the cognitive impairment associated with schizophrenia. The mode of action of IPR19 differs greatly from other classical mechanisms, thus opening a new door for an unmet clinical need.

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P.3.c.003 AUT6, a novel Kv3 channel modulator, reverses cognitive and neurobiological dysfunction in a rat model of relevance to schizophrenia symptomatology

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Mainly located on parvalbumin (PV) GABAergic interneurons, the voltage gated potassium channel Kv3.1 is closely involved in brain circuitry thought to be affected in schizophrenia. Previous findings from our laboratory demonstrated the efficacy of an acute treatment with AUT6, a novel Kv3.1 modulator, to restore cognitive and social deficits in the sub-chronic phencyclidine (PCP) rat model of schizophrenia [1,2]. Kv3 channel modulators may thus provide treatment of these unmet clinical needs in schizophrenia. Our aim here was to assess efficacy of chronic treatment with AUT6 to reverse cognitive and neurobiological deficits in our carefully validated PCP model.

Adult female hooded-Lister rats received PCP (2 mg/kg; n = 30) or vehicle (n = 10) intra-peritoneally for 7 days. After 6-weeks washout, PCP-treated rats received AUT6 (60 mg/kg; per os; "AUT6"; n = 10) or vehicle ("VEH", n = 10 and "PCP", n = 10) for 21 days or 21 days plus 7 days washout ("AUT6wo", n = 10). Cognitive function was assessed in the novel object recognition test on days 1, 7, 14 and 21, and also on days 22 and 28 for the AUT6wo group. On the last day of testing, rats were sacrificed and PV and Kv3.1b positive-cell densities were quantified using immunohistochemistry. Analysis of densities were processed in the prefrontal cortex and hippocampus, brain regions of particular relevance to the pathology of schizophrenia.

Behavioural analysis showed a significant novel object recognition deficit in the PCP group that was reversed by concomitant AUT6 treatment on days 1, 7, 14 and 21 ($P < 0.01$) but not after 1 day of washout in AUT6wo group. The PCP-induced novel object recognition deficit was associated with significantly reduced number of neurons with PV expression in the hippocampus ($P < 0.01$), notably in the dentate gyrus ($P < 0.01$) and CA3 area ($P < 0.05$), and in the infralimbic part of the prefrontal cortex ($P < 0.05$). Chronic treatment with AUT6 significantly reversed the parvalbumin neuronal deficit in the hippocampus and the infralimbic cortex ($P < 0.05$ and $P < 0.01$, respectively), an effect that was no longer observed after 7 days of washout. Kv3.1 channel-positive cell density was significantly reduced in the prefrontal cortex ($P < 0.05$), notably in the prelimbic ($P < 0.05$) and infralimbic ($P < 0.05$) cortices, in the AUT6 group only. Using immunofluorescence analysis, we also verified that the Kv3.1b channels were co-localised on PV interneurons in rat brain control tissues. Percentages reaching 80 to 90% of colocalisation were thus measured in the hippocampus and prefrontal cortex.

Chronic treatment with AUT6 provided sustained improvement of cognitive and neuropathological deficits in a validated animal model of schizophrenia symptomatology. The efficacy of AUT6 to restore cognitive function was associated with reversal of the PV interneuron deficit observed in the PCP rats. The modulation of Kv3 channels on PV neurons could thus be an important novel approach for improving cognitive deficits in schizophrenia and could potentially restore neuronal function.

References

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Disclosure statement: Sources of financial sponsorship: the work is supported by Innovate UK and Autifony Therapeutics Limited. Declaration of interest from: JCN has received expenses to attend conferences and fees for lecturing and consultancy work (including attending advisory boards) from the manufacturers of various antipsychotic drugs

P.3.c.005 Impact of antipsychotic treatment on levels of glucose, cholesterol and triacylglycerol in serum of rats perinatally treated with phencyclidine

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Schizophrenia a severe neuropsychiatric disorder, which affects 1% of world's population. Patients with schizophrenia have 20% shorter life span than general population. [1] Antipsychotic (AP) treatment is considered as therapy of choice. Typical APs predominantly block D2 dopamine receptors, while atypical APs