Targeting the P2Y-like receptor GPR17 to foster recovery after brain ischemia

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Stroke is the 2nd most common cause of death in Europe and a leading cause of disability (Wittchen et al., 2011 for which there are currently no effective therapies. The discovery that quiescent multipotent neural precursor cells are dispersed throughout the entire adult cerebral parenchyma has raised the hypothesis that approaches aimed at implementing endogenous neurogenesis and gliogenesis may be beneficial in stroke (Li et al., 2010; Zhang et al., 2013). In particular, NG2 glia, also known as Oligodendrocyte Precursor Cells (OPCs), are activated in the peripheral area of the ischemic 'core' and can spontaneously differentiate into mature oligodendrocytes, the myelin-repairing cells, thus helping re-establishing cell-to-cell communication within the lesioned area and favoring functional recovery (Zhang et al., 2011). Unfortunately, this process is 'per se' normally insufficient to allow complete recovery, thus, the possibility to implement remyelination has emerged as a relatively unexploited new therapeutic approach in cerebral ischemia. In this respect, our own data and current literature have highlighted the membrane GPR17 receptor as a key regulator of NG2 glia reactivity. GPR17 is a P2Y-like receptor, specifically responding to uracil nucleotides and cysteinyl-leukotrienes (Ciana et al., 2006), which plays a pivotal role in orchestrating NG2 glia differentiation and maturation (Coppi et al., 2013; Fumagalli et al., 2011; Lecca et al., 2008). Our previous in vitro data demonstrated that GPR17 activation by its endogenous ligands (UDP-glucose or LTD4) promotes NG2 glia maturation to mature oligodendrocytes, while its inhibition by either receptor antagonists (e.g cangrelor) or specific silencing RNAs markedly impairs their differentiation (Fumagalli et al., 2011). Of note, in the well-established permanent middle cerebral artery occlusion (MCAo) model, GPR17 was found up-regulated in NG2 glia around lesion sites (Lecca et al., 2008) suggesting that this receptor may actively contribute to reparative mechanisms after ischemia and, for this reason, be exploited for new pharmacological approaches to foster functional recovery. Since none of the endogenous ligands are selective for GPR17, through an in silico screening, new GPR17 ligands have been identified and among these, Asinex 1 (Asn1) (Eberini et al., 2011)

On this basis, here we aimed at i) analyzing the effects of the new synthetic GPR17 ligand Asinex 1 (Asn1) on OPC maturation and ii) characterizing GPR17 changes after MCAo in an inducible reporter GPR17-iCreERT2xCAG-GFP mouse line developed in collaboration with the University of Munich. The latter is quite important to define the best temporal window for pharmacological intervention with Asn1 in brain ischemia.

Immunocytochemistry and western-blot analysis of markers of mature oligodendrocytes (e.g. CNPase and myelin basic protein MBP) revealed that 48h exposure to Asn1 promotes NG2 glia differentiation. Moreover, we also observed that a 12-days exposure to Asn1 favors myelin deposition in an in vitro model of NG2 glia co-cultured with dorsal root ganglia neurons.

Changes of GPR17 were analyzed by confocal analysis during the post-ischemic period in the GPR17-iCreERT2xCAG-GFP mice. In these mice, upon tamoxifen administration, cells expressing GPR17 and their progeny can be visualized in vivo by GFP fluorescence (Viganò et al., submitted). In detail, animals were sacrificed at different times (72 hours, 1, 2, 4, 6 and 8 weeks) after MCAo and immunohistochemistry was performed. Our data show that the absolute number of recombined cells (GFP+ cells/mm2) significantly increased in the ipsilateral side starting from from 72 hours after permanent MCAo compared to the contralateral side, suggesting that the GPR17-positive pool of NG2 cells is extremely reactive to ischemic damage and directly involved in tissue remodelling.

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