

Sphingosin-1-Phosphate-receptor modulation ameliorates neonatal white matter damage and improves long-term cognitive development

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Target audience: Hyperoxic models of perinatal brain injury, neuroprotection, diffusion tensor imaging.

Introduction: Additional oxygen is widely used in resuscitation and in treatment of neonatal lung disease. Furthermore, premature infants are subjected much earlier to relative hyperoxia, because of a dramatic rise of oxygen tissue tension compared with intrauterine conditions. There is increasing evidence that hyperoxia may negatively influence brain development and maturation [1]. It has been shown that hyperoxia induces immature oligodendroglial cell death, triggering transient hypomyelination which is associated with ultrastructural changes in the developing white matter and with motor-cognitive deficits in adolescent and young adult rodents [2]. To date, there is no causal therapy to treat white matter injury especially in preterm born infants. Thus, new treatment strategies potentially aiming at the attenuation of cell death and the enhancement of hypomyelination are urgently required. The sphingosine-1-phosphate (S1P) receptor modulating substance Fingolimod (FTY720) is the first clinically approved S1P receptor modulating substance for the treatment of the most common chronic inflammatory and demyelinating disease of the central nervous system, relapsing-remitting multiple sclerosis [3]. Therefore, we investigated the effect of FTY720 in a model of premature birth, *i.e.* hyperoxia-induced brain injury, focusing on white matter by assessing cellular and structural changes with diffusion tensor imaging at 9.4T, histology and protein analysis.

Materials and Methods: 6-days after birth (P6), rats were subjected to hyperoxia (80% O₂ for 24h) or normoxia (21% O₂ for 24h). With beginning of the exposure animals received a single *i.p.* injection with 1 mg/kg FTY720, diluted in PBS volume 0.1ml/10 g bodyweight. Control animals received an injection with vehicle (PBS). At P7, P11 and P125, rats were sacrificed and brains were formalin-fixed for subsequent histology and *ex-vivo* MRI, resulting in 4 groups: Hyperoxia+vehicle (HO), normoxia+vehicle (NO), Hyperoxia+FTY720 (HO+FTY720) and normoxia+FTY720 (NO+FTY720). To evaluate mature oligodendrocytes APC-CC1 and Olig2 co-staining were performed in P7 rats. Myelination was assessed at P11 with the primary mouse anti-Myelin Basic Protein (MBP) antibody. At P125, *ex-vivo* brains were scanned on an actively-shielded horizontal 9.4T/31cm magnet (Agilent/Magnex) equipped with 12-cm gradient coils (400mT/m, 120µs) with a transceive 35-mm birdcage RF coil. A Spin-Echo sequence was used with diffusion gradients applied along six spatial directions [4] with intensity, duration and diffusion time set to 22 G/cm, 3 ms and 20 ms, respectively (*b*-value of 1185 s.mm⁻²). A FOV of 26×26 mm² was sampled on a 128×64 cartesian grid. 12 slices of 0.8 mm thickness were acquired in the axial plane with 10 averages and TE/TR = 30/2000 ms. Using in house Matlab script (Mathworks, Natick, MA), diffusivity values (Mean: MD, axial: D_∥ and radial: D_⊥) as well as fractional anisotropy (FA) were derived from the tensor. Two different white matter regions of the brain were analyzed (Fig. 1A): the corpus callosum (CC) and the external capsule (EC). Significant differences of diffusivity and FA values between the groups were assessed by a Mann-Whitney test.

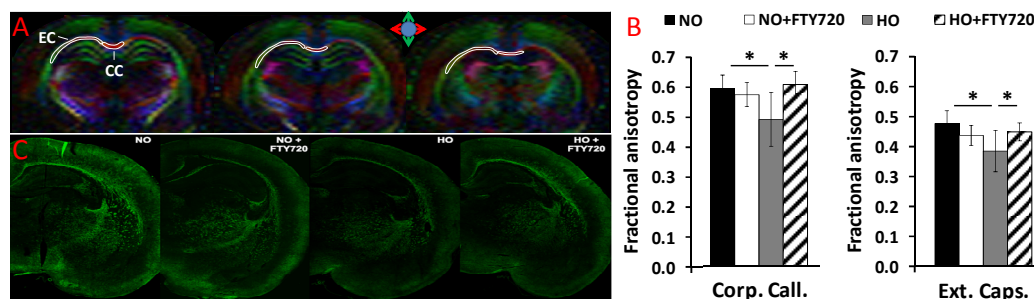


Fig. 1: A, typical color maps of a P125 NO rat. ROIs (CC and EC) are displayed on the maps. B, histograms of the FA values in the CC (Corp. Call.) and EC (Ext. Caps.). (Mean values \pm SD. *: $P < 0.05$). Myelin basic protein expression (C) at P11, *i.e.* 4 days post hyperoxia: hypomyelination in HO rats restored by FTY720 is obvious.

Results and Discussion: At P7, we detected a significant increase in oligodendrocyte death in the developing white matter 24 hours after hyperoxia, which was abolished in FTY720 treated hyperoxic animals: NO = 3.9 ± 1.2 , HO = 6.6 ± 1.9 , HO+FTY720 = 4.0 ± 1.8 . At P11, a significant decrease of MBP protein expression was detected in hyperoxia exposed animals as compared to normoxic controls in immunohistochemistry (Fig. 1C, mean intensity: NO = 663.4 ± 101.7 vs. HO = 464.4 ± 97.1). Hyperoxia-induced hypomyelination was partially reversed by FTY720 treatment leading to significantly increased levels of MBP expression for the combined treatment of FTY720 and hyperoxia as compared to hyperoxia only (Fig. 1C, mean intensity HO+FTY720 = 670.9 ± 64.7). In the white matter (corpus callosum and external capsule) at P125, FA values were significantly lower in the HO group than in the NO group. Indeed, FA values in the HO group were also lower than in the HO+FTY720 group whereas no difference was observed between NO, HO+FTY720 and NO+FTY720. Mostly, this FA decrease was related to a significant increase of D_⊥ in the white matter of HO rats. At long term, FA values of HO rats treated with FTY720 were restored and comparable to sham (NO) rats (Fig. 1B). These results provide strong evidence for a protective effect of FTY720 on oligodendrocyte degeneration and differentiation in hyperoxia-induced white matter damage coinciding with microstructural ameliorations detected by DTI in later life. This study might pave the way for further pre-clinical testing in order to verify FTY720 as a potential therapeutic agent

References: [1] Volpe JJ. Lancet Neurol 2009; [2] Brehmer F PlosOne 2012; [3] Brinkmann V Nature Reviews Drug Discov 2010; [4] Basser PJ. Magn Res Med 1998.

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