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Short Erythropoietin treatment following Hypoxia-Ischemia in the immature rat brain: macro-, micro-structural and metabolic assessment using multimodal MR

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Introduction:

The 3-day old rat (P3) shares some similarities in terms of cortical neuronal, glial and oligodendroglial development to the very preterm infant around 24-28 weeks of gestation. Animal models of preterm brain injury can be achieved by Hypoxia-Ischemia (HI). Erythropoietin (EPO) has been shown to be neuroprotective in different models of preterm brain injury [1] but has not been tested in a very immature model of HI injury. Here we investigated the neuroprotective effect of short EPO treatment in a model of neonatal HI injury in the P3 rat brain using high-field multimodal NMR techniques: T_2W imaging, Magnetic Resonance Spectroscopy (MRS) and Diffusion Tensor Imaging (DTI).

Materials and Methods:

P3 male Wistar pups underwent moderate HI injury. Right carotid artery cauterization was performed followed by hypoxia for 30 minutes at 6% O_2. All MR experiments were performed on an actively-shielded 9.4T/31cm magnet (Varian/Magnex) equipped with 12-cm gradient coils (400mT/m, 120µs) with a quadrature transceive 20-mm surface RF coil. 5h following HI, Fast Spin Echo T_2W images were performed to detect presence of injury and to quantify the volume of the lesion. Injured pups were randomized into NACL group (n=6): injected intraperitoneal with NaCl 0.9% and EPO group (n=6): injected intraperitoneal with EPO 10U/g body weight/day during the first week after HI. A control group (Ctl, n=6) which did not underwent any surgery and hypoxia was also investigated.

To study the effects of this acute treatment, at P25, Fast Spin Echo T_2W images were acquired for volumetric measurements and to position the MRS Volume Of Interest (VOI). After automatic FASTMAP [2] shiming, spectra acquisition on a VOI of 1.5×1.5×2.5 mm^3 within the cortical lesion (ipsilateral for EPO and NACL groups) or the corresponding cortical area (Control group) was performed using an ultra-short echo time (TE/TR = 2.7/4000 ms) SPECIAL spectroscopy method [3]. 30 series of FIDs (16 averages each) were acquired, individually corrected for frequency drift, summed together and corrected for residual eddy current effects using the reference water signal. Proton spectra were analyzed with LCModel [4]. DTI experiments were also performed using a semi-adiabatic double spin-echo sequence [5] with the following parameters: Icosahedral 21 directions diffusion gradient sampling scheme (b = 1000 $s.mm^{-2}$), FOV = 23 × 15 mm^2, matrix size = 128 × 64 zero-filled to 256 × 168, 8 slices of 0.8 mm thickness in the axial plane, 8 averages with TE/TR = 42/2000 ms. Diffusivity values (ADC, $D_{//}$ and $D_{\perp}$) as well as fractional anisotropy (FA) were derived from the tensor and averaged in ROIs manually delimited in the external capsule (EC) and the sensorimotor cortex (SCx).

Volumetric measurements were performed using BrainVisa/Anatomist [6]: percentage of injured cortex and percentage of cortical loss were calculated at P3 and P25, respectively. A Mann-Whitney test was used to compare statistically values between the different groups: Ctl vs. EPO, Ctl vs. NACL as well as EPO vs. NACL.

Results:

Volumes: There was no significant difference between EPO and NACL groups regarding the percentage of injured cortex at P3 as well as the percentage of cortical loss at P25 (Fig. 1 - P3: 18.5±8.6% vs. 18.2±6.0%, P25: 14.0±9.4% vs. 14.3±7.1% for EPO and NACL, respectively).

MRS: Very good spectral quality was achieved in the current study, as judged from water linewidth, obtained with FASTMAP ranging from 8 Hz to 10Hz (Fig. 2). Due to very thin cortical structure in the rat pup brain, MRS was performed on a very small volume of 12 µl placed on the cortex. On the overall study SNR was equal in average to 24 ± 2. Such consistent data was subjected to spectral analysis and absolute quantification by LCModel provided the concentration of 17 metabolites defined as the "neurochemical profile". Comparing ipsilateral and control cortices (Fig. 3), for the NACL group as well as for the EPO group significant decrease of [NAA] was observed in the lesion area. For the EPO group, [Mac] and [Lac] were found significantly lower in the injured than in the control cortical area. Only for the NACL group, [Asp], [Ins] and [Glu]+[Gln] were significantly decreased in the injured cortex.

DTI: For only the two first image-planes analyzed (Figs. 4 and 5) FA values were found significantly lower in the external capsule of the NACL as well as of the EPO group than in the corresponding region of the Control group. No difference was found between EPO and NACL groups.

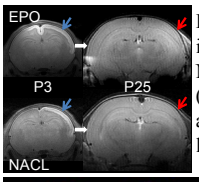

Figure 1: Typical T_2W image of an EPO and a NACL rat pup from P3 (hypersignal-blue arrows) to P25 (cortical loss – red arrows)

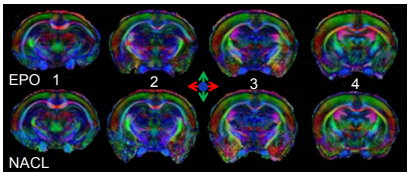

Figure 4: Typical DEC map of an EPO and a NACL P25 rat pup: white matter injuries in external capsule are obvious. Slices (1 to 4) correspond to the image-planes analyzed.

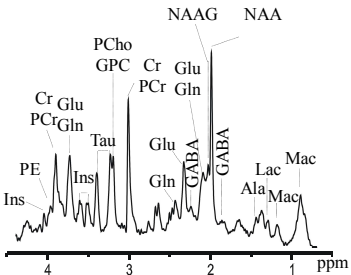

Figure 2: Typical spectrum in the ipsilateral cortex of an EPO P25 rat pup at 9.4T.

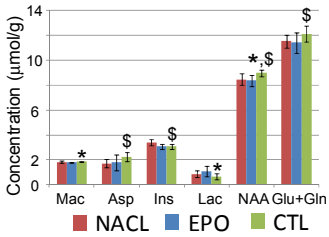

Figure 3: Histogram of the concentrations ± SD for the significant metabolites of the NACL, EPO and Control groups. *,$:p<0.05 NACL vs. Ctl and EPO vs. Ctl, respectively.

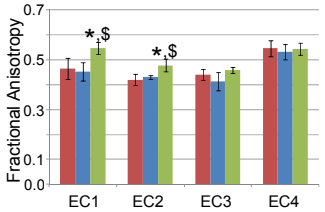

Figure 5: Histogram of the FA±SD for the NACL, EPO and Control groups at the 4 different image-planes analyzed. *,$:p<0.05 NACL vs. Ctl and EPO vs. Ctl, respectively.

Discussion and conclusion:

This study shows a full characterization of the P3 HI model 22 days following insult by using multimodal NMR techniques: MRI, MRS and DTI. Indeed, using multimodal NMR is very useful to characterize such a model with a compromised metabolism, cortical loss as well as white matter damage 22-d following HI on the P3 pup rat brain. As a result, these techniques allow an accurate assessment of the effects of a neuroprotective treatment such as EPO. Nevertheless, on the P3 HI model, acute treatment of EPO appears to not have any effect neither on tissue loss nor on white matter injuries or altered metabolism. In a previous study [7], we have shown a functional recovery despite absence of anatomical loss recovery in response to a chronic EPO treatment. The reasons for an absence of neuroprotective effect in our model of HI injury in the very immature brain are not known. Others have shown neuroprotection in similar models of injury but at an older age [8]. Age, dose and timing of administration need further investigation to better understand this absence of neuroprotection by EPO. This result can be of high interest in the clinical field of preterm's neuroprotection.

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