Erythropoietin (EPO) as Treatment of Mild Traumatic Brain Injury: A Multi Modal MRI Study

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Introduction: It is estimated that 1.7 million people suffer from mild traumatic brain injury (mTBI). MTBI results in physical, cognitive, emotional, and sleep-related disorders. As of now there is no treatment for mTBI. Recent studies in experimental mTBI suggest that Erythropoietin (Epo) may improve the outcome in mTBI. The neuroprotective effect of Epo is mediated by anti-inflammatory, anti-apoptotic, and vascular mechanisms [1-3].

Material and Methods: A total of 72 Long Evans rats were included in this study, divided into three groups: sham, saline-treated, and Epo-treated. The sham group consisted on eight animals, which were scanned 4 weeks after the sham procedure. The groups of saline- and Epo-treated animals were subdivided into four groups. Each of these subdivided groups (n=8) were scanned 1, 2, 3, or 4 weeks after injury; the studies were not longitudinal. The animals were anesthetized during the scans with 1.5 to 2.5% Isoflurane in a 70:30 mixture of air and oxygen supplied through a mask. The heads were fixed with mouth and ear bars. The breathing rate, pulse rate, rectal temperature and blood oxygen level were constantly monitored. The body temperature was maintained at 36±0.5˚C with a feedback controlled warm air system. The animals were sacrificed after the scans and processed histologically. The result of the histology was not available at the time this abstract was written. The injury model of mTBI was a controlled cortical impact (35psi, impact velocity 3mm/s) through a 10mm-diameter craniectomy. Further details can be found elsewhere [4, 5].

Multi modal MRI that included, anatomical, DTI, MRS, pre- and post-contrast MRI was performed on a 7T Bruker Biospec (USR 70/30 Paravision 5.1 software). 3D Modified Driven-Equilibrium Fourier Transformation (MDEFT) images were acquired for visualizing the anatomy. The TR/TE/TI was 4000ms/5ms/1300ms and the pulse angle was 15^0. The spatial resolution was 0.137mm x 0.137mm x 0.5mm. 50 slices were acquired within 27min. Dual echo 2D RARE (Rapid Acquisition and Relaxation Enhancement) images were acquired to measure the contusion volume (TR/TE1/TE2 = 5000ms/23ms/70ms, RARE factor 4, 2 averages, 20 slices, spatial resolution 0.137mm x 137mm x 1mm, scan time 11min). 2D DTI was acquired to study the micro structure of the brain (TR/TE= 10,000ms/27ms, b value 800s/m^2, 9 b_0, 42 gradient directions with bipolar icosahedral encoding scheme, 2 acquisitions per direction, resolution 0.27mm x 0.27mm x 0.5mm, 40 slices, and a scan time of 1hr). We used navigator echoes, double sampling, and saturation bands to enhance the DTI data quality. A Point Resolved Spectroscopy Sequence (PRESS) was used to acquire three volumes per animal (2.5mm^3, location injury site, ipsilateral hippocampus, contralateral hippocampus) with a TR/TE=2500ms/ 20ms, 256 acquisitions, and a scan time of 11min. Finally, T1 weighted Spin Echo images were acquired before and 5 minutes after application of 0.1mmol Gadodiamide (Omniscan) per kg/bw via the tail vein. The TR/TE were 500ms/10ms and the scan time was 2min. The resolution was 0.137mm x 0.135mm x 1mm.

Results: Contrast enhanced T1-weighted MRI did not reveal any compromised blood brain barrier at any time point. The spectroscopically determined ratios of N-Acetyl aspartate/Creatine (NAA/Cr) or Choline/Creatine did not have any significant differences between ipsi- and contralateral regions at four weeks post impact. However, at three weeks post impact, there was a transient and significant drop of the NAA/CR ratio in saline treated animals compared to Epo-treated animals at this time point. The contusion volumes of the three groups were not significantly different from each other at four weeks. However, the average contusion volume of the saline treated animals was at four weeks significantly larger than that measured in the same group at two weeks (not shown). Only the DTI metrics showed at four weeks significant differences and these were observed exclusively in the ipsilateral hemisphere: The FA of the fimbria of saline treated animals was significantly smaller than that of sham operated animals (Fig.1 left). The mean diffusivity (MD) of the cortex was significantly larger in the saline treated animals than in the Epo-treated animals (Fig.1 middle). The radial diffusivities (RD) of the external capsule (ex caps) and cortex were significantly larger in the saline treated animals than in the sham operated animals. Also the RD of the cortex was significantly larger in saline- than in Epo-treated animals.

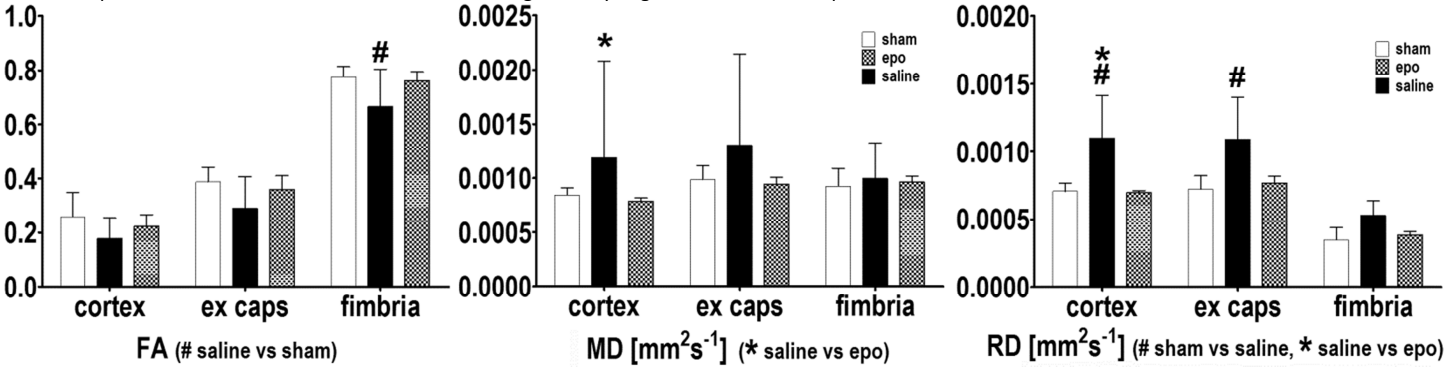

Figure 1: Displayed are bar graphs of significant differences in **FA** (left), **MD** (middle), and **RD** (right). The analysis was performed with two way ANOVA and a Bonferroni post-test ($P < 0.05$). The analysis included also contralateral structures, internal capsule (ipsi- and contralateral), splenium, and genu. These results are not displayed because their differences were not significant. The ipsilateral structures which had significant differences between the three groups at four weeks were the fimbria (**FA**), cortex (**MD and RD**), and external capsule (**RD**).

Conclusion and Discussion: At four weeks, only the DTI metrics of the ipsilateral hemisphere showed significant differences. Significant differences were found in the cortex, external capsule and fimbria. These structures are closest to the injury site. Decreased FA values indicate in general damage of tissue. Elevated MD and RD indicate enlarged intercellular space, caused possibly by edema and/or loss of tissue. Epo has been described as neuroprotective and that it suppresses intracellular inflammatory processes and apoptosis. It promotes neurogenesis and angiogenisis [1-3]. Due to these characteristics, Epo-treatment appears effective in maintaining the integrity of neural tissue. The absence of BBB leakage confirms the mild character of the injury. DTI proves to be a valuable tool for the analysis of mTBI.

References: [1] Lu et al (2005), J Neurotrauma 22, 1011. [2] Siren et al (2001), Proc Natl Acad Sci USA 98, 4044. [3] Villa et al (2003), J Exp Med 198, 971. [4] Robertson et al (2012), J Neurotrauma 29, 1–10. [5] Robertson et al (2012), J Neurotrauma 29, 1156-1166.