Normobaric Hyperoximia Increases Cerebral Injury Caused by Perinatal Hypoxia: DTI Study in Rats

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Introduction: Perinatal hypoxia has an adverse effect on the normal neurological development of infants. Common treatment is inhalation of oxygen enriched air (hyperoximia). Today it is not clear whether hyperoximic treatment is a suitable therapy for the injured brain (1). The P7 animal model -in which rats with ischemic injury are treated with hypoxia (HI) - is commonly used to study the effects of perinatal ischemia. The main objective of this study is to investigate the in vivo the changes in lesion size and gray and white matter integrity in the P7 model treated with HI or HI followed by hyperoximia (HHI) using high resolution structural MRI and diffusion tensor imaging (DTI) at different time points after the insult.

Materials and Methods: 43 rat pups were divided into three groups: normal, HI, and HHI. At day 7 after birth (P7) the animals of the HI and HHI group were anesthetized with isoflurane and the left common carotid artery was ligated and transected. The animals were subjected to hypoxia (40 minutes, 8% O_2, rest N_2) in a humidified incubation chamber (37°C). The animals of the HHI group were additionally subjected to >95% oxygen (rest N_2) for 40 minutes. They were scanned on a 7T Bruker Biospec 30cm horizontal bore scanner equipped with a gradient insert (inner diameter 116 mm). A 72mm inner diameter volume coil was used for signal stimulation. A custom designed surface coil was used for signal detection. Animals were anesthetized during the scan with 2% isoflurane in an air/oxygen mixture. The respiration rate, surface body temperature, blood oxygen level, and heart rate were continuously monitored. Multi-slice, contiguous coronal MR images were acquired with dual echo RARE (FSE). The lesion volumes were determined in the long echo RARE images. Diffusion-weighted EPI data (DWI) was acquired with a rotationally-invariant icosahedral encoding scheme (N = 42). The maps of the DTI parameters MD, axial diffusivity λl, radial diffusivity λt, and FA were generated from the preprocessed DTI data sets using DtiStudio (Johns Hopkins University, Baltimore, MD). The measurements were focussed on four gray matter structures (hippocampus, caudate putamen, cortical plate, and cortical mantle) and seven white matter structures (body (bcc), genu (gcc), and splenium (scc) of the corpus callosum, external (ec) and internal capsule, fimbria, and cingulum).

Results: Figure 1 displays a normal (left), HI (middle), and HHI animal (right) three weeks PI. The top row presents anatomical RARE images, the bottom row color coded FA maps. Most significant differences of the DTI data were found in the corpus callosum three weeks PI: 43% gcc, 24% bcc, and 19% in the scc (summarizing over all treatments at all time points). The radial diffusivity λt of bcc and gcc was significantly different between normals versus HI, normals versus HHI, and HI versus HHI 3w PI. In scc and ec significant differences of λt were present between normals and HII. The same structures of the corpus callosum showed signicantly decreased FA values between normal and HII animals (not shown). The corresponding FA maps (bottom row) show severe damages of several brain structures in the ipsilateral hemisphere in HI and even more severe in HHI animals. In HHI (bottom right) almost no anisotropic structures are present, including gray matter. Also, the structures of the contralateral hemisphere appear fuzzy in the HI and the HHI animal compared to the normal rat (bottom left). The ipsilateral hemisphere in both, HI and HHI, is much smaller than in the normal animal. Figure 2: The brain of normal animals (654 ± 186 mm^3) is significantly larger ($p<0.05$) than that of HHI (430 ± 71 mm^3) animals (HI: 577 ± 109 mm^3). The total volume of lesions (edema and hematoma) in HI treated animals was 72 ± 96 mm^3, while 360 ± 255 mm^3 in HHI treated animals ($p<0.04$, unpaired t-test).

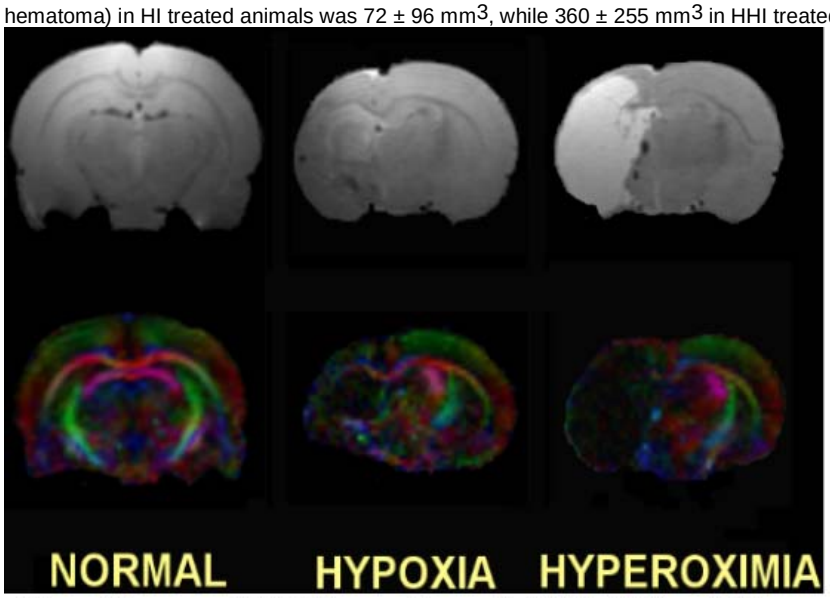

Figure 1: Top row: axial RARE images of normal (left), HI- (middle) and HHI-treated ratpups three weeks after injury. The lesions are largest in the HHI-treated rat (right). The corresponding FA maps (bottom row) show severe distortions in the ipsilateral hemisphere after hypoxia (middle) and hyperoxemia (right)

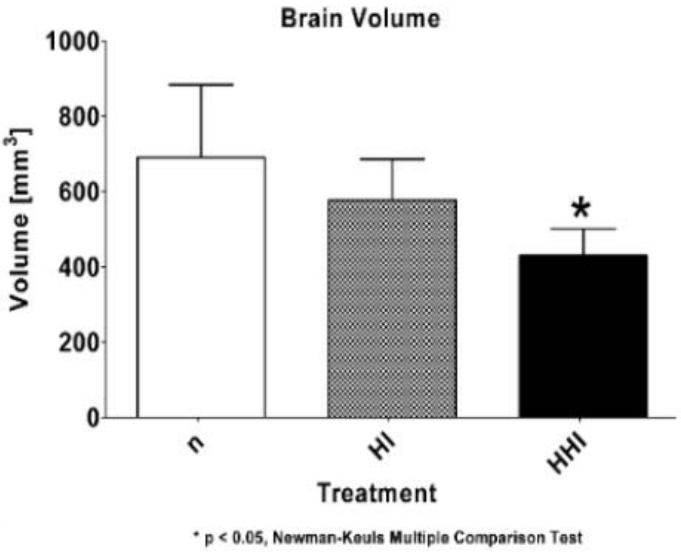

Figure 2: The brain volume of HHI-treated ratpups is significantly smaller than that of normals. The difference between HHI-treated and HI-treated ratpups is not significant

Discussion: We showed recently that the radial diffusivity λt correlates inversely with myelin density (2). Here we found significantly increased λt in gcc, bcc, scc, and ec three weeks after HHI. HI produced significant differences to normals only in gcc. Myelin staining revealed cell death in the ipsilateral and significant tissue loss in the contralateral corpus callosum 3 weeks after HHI. MRI visible lesion volumes (edema and hematoma) caused by HHI were significantly larger than those caused by HI alone. The brains of the animals, which were treated with HHI, were significantly smaller than those of normal animals. This study concludes that hyperoximic treatment of perinatal hypoxia does significantly worsen the prognosis.

References:

1. Diringer, Curr Opin Crit Care 2008; 14: 167-171
2. Bockhorst et al., J Neurosci Res 2008; 86:1520-1528