A Spontaneously Reversible Diffusion Weighted Signal Intensity Changes Following Neonatal Hypoxia-Ischemia

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INTRODUCTION: Perinatal hypoxia-ischemia (H/I) is the leading cause of morbidity and mortality in infants and children. The FDA has not yet approved any treatment following perinatal H/I because pharmacological treatments shown to significantly reduce infarction volume in animal models usually fail to provide the same benefits when applied to humans [1]. The conclusion of typical animal trials, based on statistical evidence, is that the treated group has a smaller infarct volume than the control group, without assessment of brain volume that is undergoing H/I changes prior to the application of a therapeutic agent. In this study the volume of hyperintense signal on DW-images was assessed prior to the administration of therapeutic agent and then compared to the 7-days post H/I increased signal volume T2-weighted images. This procedure allows the study of reversible DW signal intensity changes and determination of whether this is a spontaneously occurring process during the 7-day recovery period from H/I, or it is a consequence of the drug administration. We tested the hypothesis that repetitive administration of aminoguanidine [2] (inducible nitric oxide synthase inhibitor) or minocycline [3] (p38 kinase inhibitor, Ca^{2+} chelator, caspase 1 and 3 and iNOS inhibitor) will provide significant reduction of the hyperintense DW signal volume following neonatal H/I.

METHODS: Male and female 7-day old Wistar rats were anesthetized with halothane (4% induction, 1.5% maintenance in 30% O_2/balance N_2) and the right carotid artery was double ligated. To induce hypoxia, each animal was exposed to a gas mixture of 8% O_2/balance N_2 for 2 h, in 37^{o}C water bath. MR imaging was performed on a 3.0 T MRI spectrometer (Medspec S300, Bruker Instruments, Ettlingen, Germany) with a human head gradient coil (50 mT/m gradient strength). A group of six 7-day old pups was imaged at the same time using a multiple animal probe [4]. All animals were imaged 30 min following H/I using diffusion-weighted imaging sequence (TR/TE=1500/68.8 ms, Δ=35.22, δ=20 ms, b-value=730, 128X128 matrix, 625X625 μm resolution, 1 mm slice thickness, 0.5 mm slice separation, NEX=1, 3 min 12 s total imaging time) and isoflurane anesthesia (2%). All injection volumes were 10 μl/g of vehicle or drug dissolved in vehicle. Thirteen animals were injected with minocycline (45 mg/kg) dissolved in phosphate buffered saline (PBS), eighteen animals received PBS and sixteen animals received saline at 1h 30 min following H/I, and then every 24 h for 72 h. Rectal temperature was measured 1 h 30 min post injection. Ten animals received aminoguanidine (100 mg/kg) dissolved in saline and ten animals received saline at 4h following H/I and then every 12 h for 72 h. The initial infarct size was assessed from 4 coronal slices using semi-automated routine and the CCHIPS/IDL software [5] by an investigator blinded to the treatment groups. Infarct volumes were corrected for brain edema as described previously [6] and calculated as percent of the contralateral hemisphere. Seven days following H/I each animal was imaged individually using a T2-weighted spin echo sequence (TR/TE=3000/10.13-151.95 ms, 256 X 128 matrix size, field of view 2 x 2 cm^2, N=15 echoes, NEX=2, 10 slices, 0.5 mm slice thickness in 13 min) in a 2 cm slotted tube resonator and small aperture gradient coil (9.5 cm aperture and 1.1 T/m gradient strength). The final infarct volume was determined from 8 slices using the CCHIPS/IDL software [5]. A paired t-test was used to assess the difference in the initial vs final hyperintense signal volumes. The difference in the initial vs. final infarct volume was calculated for each animal. We used analysis of variance to test the null hypothesis that the mean differences between initial and final infarct volume were equal among different treatment groups.

RESULTS: Pups injected with minocycline and aminoguanidine did not have significantly different rectal temperatures at 1 h 30 min following the injections from the pups injected with saline or PBS. Interestingly, there was a significant reduction of the volume of initially observed elevated DW signal when compared to the volume of elevated T2 signal intensity at 7 days following H/I for all treatment groups ($p<0.0005$ for the minocycline and aminoguanidine treated group, $p<0.0001$ for the saline and PBS treated groups). Pups that received minocycline treatment had (mean±standard deviation) 30±13.7% reduction in the infarct volume compared to 26±15.8% with saline, 25±12.7% with PBS and 25±13.1% with aminoguanidine. The mean differences between initial and final infarct volume reduction were not significantly different among different treatment groups. When the initial infarction volumes were separated into the two sub-groups: severe (50% or more of the ipsilateral hemisphere has abnormal signal intensity on DW-images) vs. moderate-mild (less than 50% of the ipsilateral hemisphere has abnormal DW-images signal), there was no statistical significance between the treatment groups either. Interestingly, pups that had mild-moderate initial infarct volume had no statistically different reduction in the final infarct volume than the pups with severe initial infarction. This was independent of treatment.

DISCUSSION: DW imaging, highly sensitive to cytotoxic edema, was used to evaluate the increased signal intensity volume before attempting drug intervention. This allows us to study reversibility of initial early DW signal intensity changes in the treated and control animals. Imaging six animals at the same time allows animals from the same litter to be studied minimizing the differences in the genetic background on the outcome and the recovery from H/I, and further reduces variations due to the different environmental conditions during H/I and recovery. Since we found that control animals have the same spontaneous recovery as drug treated animals, it makes harder to discern any protective effect of the administered drug. Both minocycline and aminoguanidine failed to show any neuroprotective effect different than the control saline or PBS injected animals. It appears that the mechanism of recovery is independent of drug action. Some of the animals in each treatment group exhibited remarkable reduction of the initially increased signal intensity on diffusion weighted images (Fig. 1 A and B) and had normal T2-weighted signals at 7 days post H/I (Fig. 1 C and D). Further elucidation of the mechanism behind the reversible diffusion weighted signal changes may provide clinically beneficial therapy following neonatal H/I.

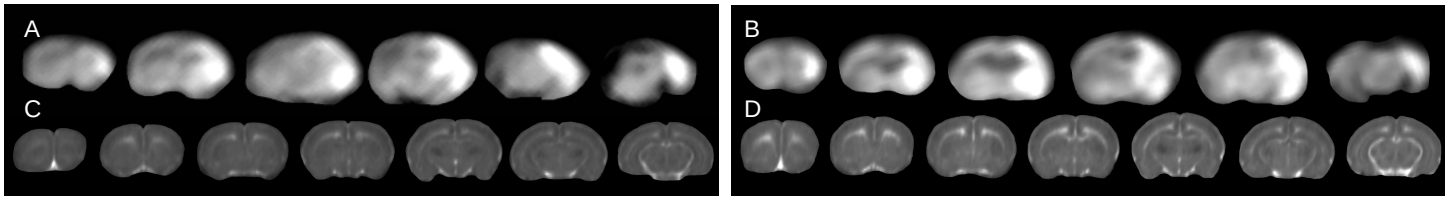

Figure 1. DW-images of minocycline treated (A) and PBS treated (B) animals that had remarkable recovery and ~40% reduction in the infarct volumes following hypoxia-ischemia. Both animals were from the same litter and imaged at the same time (30 min following H/I), using the multi animal probe. T2-weighted images of the same animals 7-days following hypoxia-ischemia, (C) minocycline treated, (D) PBS treated.

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