The Importance of Reperfusion Injury in Antenatal Hypoxia-Ischemia: Novel Fetal MRI Diagnostic Parameters and Novel Antioxidant Therapy

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Introduction
We have previously shown the ability of diffusion weighted MR-derived index, using the nadir of ADC during hypoxia-ischemia (H-I) to predict postnatal motor deficits in survivors. The contribution of immediate reperfusion injury to motor deficits has not been fully understood. We used a novel lipophilic Mn porphyrin antioxidant in addition to ascorbate + trolox to test our hypothesis. Mn porphyrin-based SOD mimics and peroxynitrite scavengers have been successfully used in treating stroke, spinal cord injury, amyotrophic lateral sclerosis, and Parkinson's disease. The lipophilic Mn(III) meso-tetrakis(N-n-hexylpyridinium-2-yl) porphyrin, MnTnHex-2-PyP, is a more promising strategy than its hydrophylic analogues as it shows better ability to cross the blood brain barrier.

Objective.
To test whether reperfusion brain injury after fetal H-I can be ameliorated by maternal administration of exogenous antioxidants and test efficacy of MnTnHex-2-PyP as a neuroprotective agent.

Methods
40 min in utero H-I was induced on E25 (77% gestation) dams as described previously [1]. The antioxidants used were Ascorbate + Trolox or MnTnHex-2-PyP, both given after onset of uterine ischemia (post-treatment). Fetuses were imaged serially in utero on 3T magnet using diffusion-weighted sequence. Apparent diffusion coefficient (ADC) was calculated. Newborn kits were delivered by C-section at E31 and postnatal kits underwent neurobehavior battery at E32 [1]. Surviving kits were stratified into 2 groups depending on whether ADC at the end of hypoxia period further declines in reperfusion phase (“reperfusion injury”) or recovery towards the baseline (“no reperfusion injury”). Time-dependent accumulation of MnTnHex-2-PyP in fetal tissues was obtained by time course of T1 maps in 2 control E25 dams and using T1-relaxivity values of standards in tissue homogenates. Another subset of rabbit dams received MnTnHex-2-PyP starting at 30 min before onset of uterine ischemia and continuing after uterine ischemia (pre+post).

Results
In control saline-administered dams, the incidence of hypertonia in survivors (n=49) was 57% and the odds ratio of hypertonia to occur with a reperfusion drop in ADC was 8.8. Ascorbate + Trolox post-treatment decreased the incidence of hypertonia to 30% (n=30 survivors). MnTnHex-2-PyP post-treatment resulted in 63% hypertonia (n=22 survivors) and more mortality (48% compared to 23% for controls). Analysis of MnTnHex-2-PyP concentration time course revealed that the drug only reached concentration of ~2 µM in fetal brains by 30 min after onset of infusion, possibly explaining the lack of effect on eventual outcome by missing the immediate reperfusion period. Additional studies showed that MnTnHex-2-PyP pre+post-treatment resulted in 31% hypertonia in survivors (n=16) but mortality was 31%. With all treatments, the odds ratio of hypertonia to occur with reperfusion drop in ADC decreases to 2.3.

Conclusions
The diagnosis of reperfusion injury can be made by MRI using a further drop in ADC as a marker. The presence of reperfusion injury markedly increases the chances of having postnatal hypertonia and other motor deficits. The pathogenetic consequences of oxidative burst during reperfusion can be investigated by the use of antioxidants. As a therapy, antioxidants are viable candidates for neuroprotection when given after onset of hypoxia-ischemia but protective effect depends upon rapid passage to the fetal brain. Maternal administration of MnTnHex-2-PyP is a promising preventive therapy and passage to fetal brain can be tracked by MRI but the therapeutic window and toxicity needs to be further defined.