Can 1H MRS be a Surrogate for 31P MRS in Quantification of Transient Hypoxic-Ischemic Insult Severity in a Neonatal Encephalopathy Model?

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Introduction: Hypoxic-ischemic neonatal encephalopathy (NE) is associated with high mortality and morbidity rates worldwide. MRI is an effective tool for diagnosis and assessing treatment efficacy [1]. A recent meta-analysis of the prognostic accuracies of MRI methods demonstrated that the thalamic ^{1}H magnetic resonance spectroscopy (MRS) lactate (Lac)/N-acetylaspartate (Naa) peak area ratio acquired at age 5-14 days is a highly sensitive and specific biomarker of long term neurodevelopmental outcome in NE [2]. Lac/Naa and other Lac metabolite ratios constitute biomarkers which can bridge between pre-clinical and clinical studies. Transient hypoxia-ischemia (HI) in the piglet is an established pre-clinical NE model. The evolution of phosphorus (^{31}P) MRS metabolite levels during transient HI has been used previously to both quantify, and titrate in real-time, acute-HI severity [3]. In this study we correlated ^{1}H MRS Lac/Naa with ^{31}P metabolite ratios in order to assess whether ^{1}H MRS could facilitate insult titration.

Methods: Experiments were under UK Home Office guidelines. Thirteen healthy piglets (aged < 24 hr) were anaesthetised and physiologically monitored with intensive life support (2). Transient cerebral HI was induced by reducing the inspired oxygen fraction to ~ 0.12 and inflating bilateral carotid artery occluders for ~ 25 min after which the occluders were deflated and inspired O_2 was normalised. During, and up to 80m min after, transient HI ^{1}H MRS was acquired every minute in 3 piglets and ^{31}P MRS was acquired every 2 minutes in the remaining 10 piglets using a 9.4 Tesla Varian spectrometer and separate ^{1}H and ^{31}P ~60 mm diameter MRS surface coils. ^{1}H MRS used PRESS (repetition time (TR) = 5 s, echo time 288 ms, 12 averages, 2x2x2cm voxel centred, and contained entirely,within the brain). [31]P spectra were collected using single-pulse acquisition (TR = 10 s, 12 averages). Spectra were analysed using AMARES [4] as implemented in the jMRUI software [5] and the following metabolite peak area ratios were calculated: Lac/NAA from ^{1}H MRS; and inorganic phosphate (Pi)/epp, phosphocreatine (PCr)/epp, and nucleotide triphosphate (NTP)/epp from ^{31}P MRS where epp = exchangeable phosphate pool = Pi + PCr + 2γ-NTP + β-NTP. Metabolite ratio timecourses were linearly interpolated to a 1 min time resolution and median timecourses for each ratio were calculated; time t = 0 was defined as the start of transient HI. Lac/Naa was plotted against PCr/epp, Pi/epp and NTP/epp at equivalent timepoints during transient HI and recovery.

Results: Figure 1 shows the median Pi/epp and NTP/epp timecourses. The Lac/NAA timecourses for each ^{1}H MRS subject are plotted in Figure 2. In Figure 3, median Lac/Naa is plotted against (a) median PCr/epp, (b) median Pi/epp and (c) median NTP/epp. In 2 of the 1H MRS animals, the Lac/Naa rise during transient HI was reversed during recovery; in the third, Lac/Naa rose to a higher value and did not re-normalise during the recovery period. During transient HI, PCr/epp appears to decline before Lac/Naa rises and Lac/Naa begins to rise before NTP/epp declines. The rise in Lac/Naa most closely matched that of Pi/epp. At 80 min, during the recovery period, median NTP/epp stabilised lower than baseline (0.23 at baseline, 0.20 at 80 min, p<0.01, Kruskal-Wallis), median Pi/epp stabilised higher than baseline (0.26 at baseline, 0.31 at 80 min, p<0.02, Kruskal-Wallis) but median PCr/epp was similar to baseline. PCr/epp, Pi/epp and NTP/epp appeared to stabilise prior to full recovery of Lac/Naa (see curve flattening in Figure 3 at the lower recovery Lac/Naa values).

Discussion: During early HI, NTP levels are maintained via the creatine kinase reaction. This rapidly depletes PCr and PCr/epp declines. NTP synthesis also occurs via anaerobic glycolysis which generates Lac as a by-product. Pi accumulates due to PCr hydrolysis. Eventually NTP concentrations decrease and Pi rises further due to NTP hydrolysis. The correlations between the averaged time courses demonstrate PCr/epp declining initially prior to Lac/Naa increasing. Lac/Naa increase appears, in turn, to precede NTP/epp decline. The evolution of Lac/Naa during HI appears to most closely match that of Pi/epp. Further experiments with simultaneous acquisition of ^{31}P and ^{1}H MRS in the same animals are required to see whether the results are consistent with those reported here. Lac can be utilised in the brain by combustion or conversion back to glucose. Both of these processes require oxygen and the rate of Lac utilisation following HI can be influenced by factors such as the availability of glucose [6]. Two of the three [1]H-monitored animals showed evidence of Lac utilisation and recovery of Lac/Naa; in the third, the larger maximum Lac/Naa and lack of recovery to baseline suggests a more severe injury. The correlations of the median timecourses, during both HI and recovery, suggest that Lac/Naa is not a direct marker of either energy reserve (PCr) or energy status (NTP). However, ^{1}H MRS is potentially useful for quantifying transient HI insult severity. Acquisition of ^{1}H as well as ^{31}P MRS during and after HI in this model may improve understanding of the mechanisms of ischemic damage and of the great efficacy of Lac/Naa as a clinical biomarker.
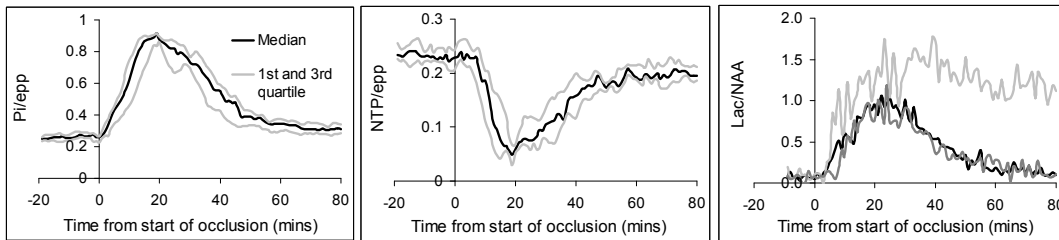

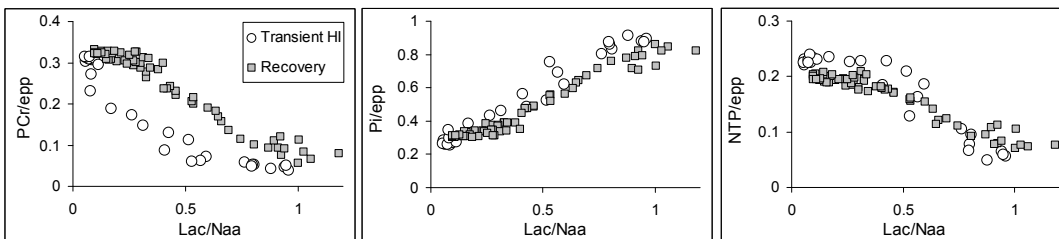**Figure 1:** (a) Median Pi/epp   (b) Median NTP/epp   **Figure 2**: Lac/NAA timecourses

Figure 3: Median Lac/NAA plotted against (a) PCr/epp, (b) Pi/epp and (c) NTP/epp. Data plotted during transient HI and during the recovery period

References:

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