Correlation of 31P MRS Metabolite Ratios and Near-Infrared Spectroscopy Measurements of the Redox State of Cytochrome Oxidase During and After Hypoxia-Ischemia in the Piglet

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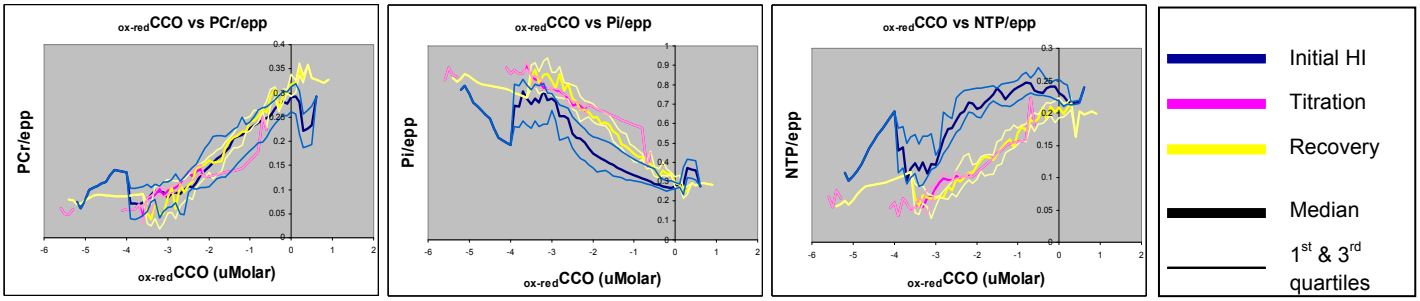
Introduction: Neonatal encephalopathy (NE) subsequent to perinatal hypoxia-ischemia (HI) is associated with high mortality and morbidity rates worldwide. Transient hypoxia-ischemia (HI) in the piglet is an established pre-clinical NE model**[1, 2]**. Phosphorus (^{31}P) magnetic resonance spectroscopy (MRS) can be acquired sequentially in our piglet model and metabolite levels during transient HI have been used previously to quantify acute-HI severity **[1]** and as outcome biomarkers **[2]**. To investigate brain haemodynamic and metabolic changes during transient HI and recovery we integrated broadband near-infrared spectroscopy (NIRS) with ^{31}P MRS. This dual modality can characterise the time courses of brain oxygenation, haemodynamic and metabolic changes continuously and non-invasively offering insights into brain pathophysiology. In addition, to HbO, Hb, and CBV, broadband NIRS can be used to measure the redox state of cytochrome-c-oxidase ($_{ox\text{-}red}$CCO). CCO is the terminal electron acceptor of the mitochondrial electron transfer chain which catalyses over 95% of oxygen metabolism, thereby driving aerobic adenosine triphosphate (ATP) synthesis and playing a central role in the maintenance of mitochondrial function. The aim of these experiments was to correlate $_{ox\text{-}red}$CCO with ^{31}P MRS metabolite ratios during and after transient HI in the piglet.

Methods: Experiments were performed under UK Home Office guidelines. Nine healthy piglets (aged < 24 hr) were anaesthetised and physiologically monitored with intensive life support. Transient cerebral HI was induced by reducing the inspired oxygen fraction to 0.12 and inflating bilateral carotid artery occluders. During, and up to 60 min after, transient HI, whole-brain ^{31}P MRS was acquired every minute on a 9.4 Tesla Varian spectrometer using a single-pulse acquisition (TR = 10 s, 6 averages) with a ~60 mm diameter MRS surface coil. Spectra were analysed using AMARES **[3]** as implemented in the jMRUI software **[4]** and the following metabolite peak area ratios were calculated: inorganic phosphate (Pi)/epp, phosphocreatine (PCr)/epp, and nucleotide triphosphate (NTP)/epp where epp = exchangeable phosphate pool = Pi + PCr + 2γ-NTP + β-NTP. Note that most of the NTP signal derives from ATP. During transient HI the height of the β-NTP peak was monitored in real time. The period from the start of transient HI to the point at which β-NTP had fallen to 50% of its height at baseline is referred to as the **initial HI period**. At this point the inspired oxygen fraction was titrated to interactively keep the β-NTP peak height between 30% and 50% of its original height for a period of 12.5 min; this period is referred to as the **titration period**. At the end of titration the carotid arteries were de-occluded and the inspired oxygen fraction returned to normal for the remainder of the experiment; this is referred to as the **recovery period**. NIRS data were acquired continuously with 1 min time resolution. The NIRS optodes were positioned either side of the piglet's head. We used a broadband source and a multi-wavelength detection system to measure $_{ox\text{-}red}$CCO. This system allows for accurate measurements and has been used before in piglets **[5 & 6]** and human adults **[7 & 8]**. ^{31}P metabolite ratios were plotted against $_{ox\text{-}red}$CCO at equivalent timepoints during the initial HI, titration and recovery periods separately. Plots from each piglet were then linearly interpolated to 0.1 μMolar steps in $_{ox\text{-}red}$CCO and median correlations to $_{ox\text{-}red}$CCO for each metabolite ratio were calculated along with 1st and 3rd quartile ranges.

Results: Median plots for PCr/epp, Pi/epp and NTP/epp correlated with $_{ox\text{-}red}$CCO are shown in the figure. During the initial HI period both the decrease in PCr/epp and the increase in Pi/epp correlated approximately linearly with $_{ox\text{-}red}$CCO. NTP/epp was buffered initially with no apparent decline until $_{ox\text{-}red}$CCO had fallen appreciably at which point there was an approximately linear decline with $_{ox\text{-}red}$CCO. During the titration period 4 animals demonstrated increased PCr/epp and NTP/epp and decreased Pi/epp. During the recovery period 6 animals showed recovery of MRS and $_{ox\text{-}red}$CCO to near baseline values, 2 showed partial recovery and 1 showed no recovery. During both the titration and recovery periods PCr/epp, Pi/epp and NTP/epp all recovered linearly with $_{ox\text{-}red}$CCO. The relationship between NTP/epp and $_{ox\text{-}red}$CCO is clearly different during initial HI and recovery periods (seen as the apparent 'hysteresis' effect in the $_{ox\text{-}red}$CCO vs NTP/epp plot).

Discussion: During transient HI, CCO becomes increasingly reduced due to lack of molecular oxygen as an electron acceptor at the terminal end of the electron transport chain. Failure of the electron transport chain inhibits ATP manufacture via oxidative phosphorylation. ATP levels are buffered through the creatine kinase reaction leading to a decline in PCr whereas energy utilisation without simultaneous oxidative phosphorylation leads to increased Pi. When PCr reserves are depleted, ATP begins to decline. During recovery, electron transport chain activity recovers and CCO oxidises. Oxidative phosphorylation resumes, causing Pi to decline, and PCr reserves are restored via reversal of the creatine kinase. Reduced $_{ox\text{-}red}$CCO and NTP/epp during HI indicates mitochondrial dysfunction. During secondary energy failure (SEF) subsequent to reversible HI, NTP/epp has been observed to decrease linearly with $_{ox\text{-}red}$CCO **[5]** in contrast to the buffering of NTP/epp observed during HI in this work. During HI cells are responding simultaneously to a global stimulus. During SEF, different populations of cells will exhibit mitochondrial dysfunction at different timepoints and the NIRS and MRS signals represent an average over all populations. This would explain the linear relationship seen previously in SEF despite the transient buffering of ATP that is likely still occurring in individual cells as mitochondrial function declines. We speculate that during recovery from HI, different populations of cells recover at different rates and that the linear relationships observed occur as a result of this. It may be possible to use these recovery curves to estimate the extent of permanent mitochondrial damage that occurs during the initial HI and titration periods in this model and thus the proportion of tissue amenable to salvage via therapeutic intervention post HI.



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