Relation between 1H and 31P MRS biomarkers and immunohistochemical markers of cell death and inflammation in a perinatal asphyxia piglet model

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Background: Perinatal asphyxia affects 2-3/1000 term births in the developed world and is associated with high morbidity and mortality rates. A recent meta-analysis demonstrated that the cerebral ^{1}H-MRS lactate/N acetyl aspartate (NAA) peak area ratio acquired between 5-14 days after birth is the most sensitive and specific MR biomarker of long term neurodevelopmental outcome in infants following perinatal asphxyia[1]; lactate/NAA is already used as a bridging biomarker in pre-clinical studies and as a surrogate endpoint in phase II neuroprotection trials in perinatal asphyxia. The reciprocal changes in lactate and NAA (increase and decrease respectively) following hypoxia-ischaemia enhance sensitivity to detect neural injury in the sub-acute phase after hypoxia-ischaemia.
Aim: To assess the relation between ^{1}H and ^{31}P MRS biomarkers and immunohistochemical markers of cell death (quantified using TUNEL + nuclei) and neuroinflammation (microglial de-ramification reflecting phagocytocis of neural debris)[2] following global hypoxia-ischaemia.
Methods: Twenty-eight Large White male piglets (<24 h of age) underwent transient global hypoxia-ischaemia and serial 1H (white matter (WM) and deep grey matter (DGM)) and 31P MRS (whole brain) data acquisitions up to 48h after injury. At 48h brains were perfusion fixed, post-fixed and paraffin embedded. Adjacent sections were stained for nuclear DNA fragmentation with TUNEL and IBA1 immunoreactivity. TUNEL+ nuclei were counted in 3 fields (at 40x magnification, area of 0.76mm^2) and the average converted into counts per mm^2. IBA1+ microglial cell bodies and branch density were determined at 40x magnification using a 0.24x0.24mm square grid, placed in 3 fields for each brain region and counting the number of cell bodies inside the grid (CBD) and the average number of branches (B) crossing the 3 horizontal (top, middle and bottom) and the 3 vertical (left, middle, and right) 0.24mm long gridlines. This determined the microglial ramification index (B^2 / CBD). Correlation of metabolites detected by MRS biomarker AUCs (area under the curve, X-axis) with the average density of TUNEL+ cells (Y-axis) and microglial ramification across the forebrain were assessed.
Results: Of all MRS-based biomarkers, Lac/NAA showed a particularly strong positive correlation with TUNEL+ nuclei averaged across the forebrain 48h and NTP/EPP a strong correlation with microglial ramification (both $p<0.01$) after transient global hypoxia-ischaemia. The highest R^2 values were seen with WM Lac/NAA (R^2=0.57) and Pi/EPP (R^2= 0.52), decreasing gradually for Lac/Cr (R^2=0.50), NAA/Cr (R^2=0.49), NTP/EPP (R^2=0.36) and PCr/EPP (0.32) with comparatively low levels for brain pHi (R^2 = 0.14). Cho/Cr AUCs showed no correlation. Microglial ramification was particularly correlated with NTP/EPP (R^2=0.40) and less well correlated with Lac/NAA (R^2= 0.24).

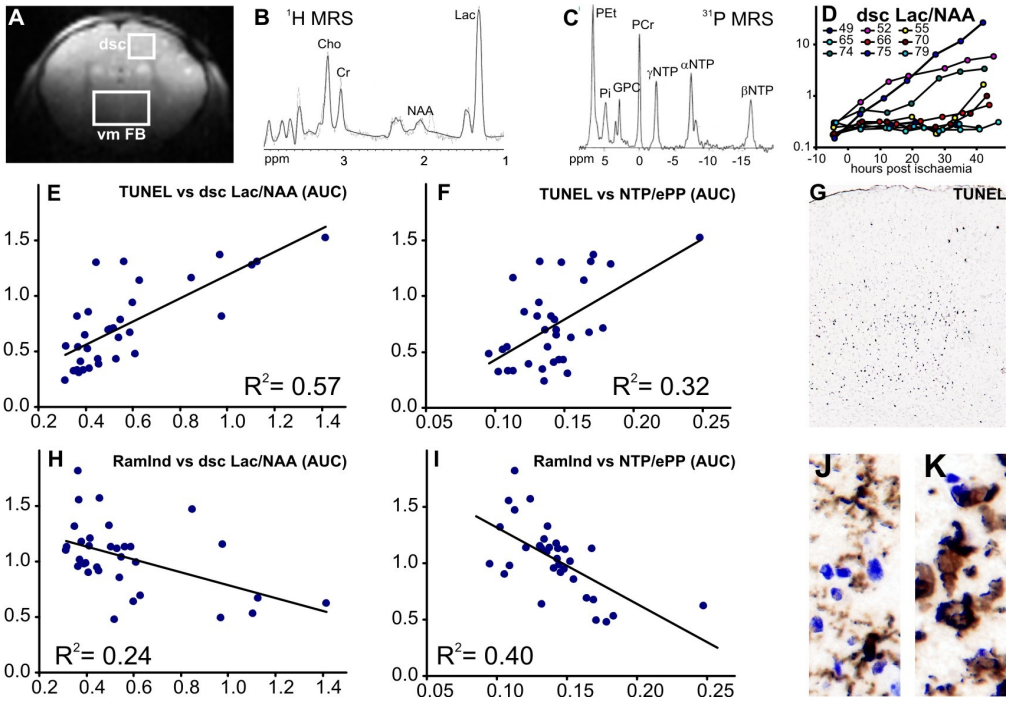

Fig. Scout MR image (A) showing voxel positions for the dsc (dorsal subcortical white matter) and vmFB (ventromedial forebrain) and representative (B) 1H and (C) 31P MR spectra from an injured (B) and uninjured (C) animal. (D) Lac/NAA data plots following global hypoxia-ischaemia (HI) for untreated animals. Correlation of MRS biomarker AUCs (area under the curve, X-axis) for (E) Lac/NAA and (F) NTP/EPP in the 48h after global HI with average density of TUNEL+ cells (G) across the forebrain (Y-axis). Each data point represents a single animal. (I) Microglial ramification was most closely correlated with NTP/EPP and less with (H) Lac/NAA. Representative photomicrograph of IBA-1 staining of a naive brain with maximal microglial ramification (left) and almost total loss of microglial ramification with severe HI and no intervention (right).

Conclusion: Lac/NAA showed a particularly strong positive correlation with TUNEL+ nuclei averaged across the forebrain 48h after transient global hypoxia-ischaemia. This may reflect the gradual inability of neurons to metabolize lactate even before NAA and energy rich phosphates are depleted. Whole brain NTP/EPP showed a strong correlation with microglial ramification. Injury-associated activation and phagocytosis of neural debris (due to necrotic cell death) are associated with rapid microglial branch loss. Microglial de-ramification may thus reflect non-apoptotic forms of cell death.

References: 1. Thayyil et al., Pediatrics 2010;125:E 382-95 2. Bohatschek et al., J Neurosci Res 2001;5