Optimal therapeutic hypothermia temperature following perinatal asphyxia: a magnetic resonance spectroscopy biomarker and immunohistochemistry study in the newborn piglet.

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Background: Therapeutic hypothermia for neonatal encephalopathy improves survival with normal neurological outcome[1]. Despite treatment, however, 50% of treated infants still have adverse outcome; tailoring cooling with more precision may thus provide further benefit. The specific brain temperature providing optimal neuroprotection is unknown although a target whole-body temperature of 33-34°C is currently used. We have previously shown in the piglet that optimal neuroprotection by delayed cooling occurs at different temperatures in the cortical (33°C) and deep grey (35°C) matter[2].

Aim: To assess the optimal temperature for regional neuroprotection using ^{1}H MRS biomarkers[3] supported by immunohistochemistry to quantify cell death.

Methods: Twenty-eight Large White male piglets (< 24 h of age) were randomized (all groups n=7), with intervention from 2-26 h, to: (i) normothermia (38.5°C); (ii) hypothermia (35°C); (iii) hypothermia (33.5°C); (iv) hypothermia (30°C). Serial MRS was acquired before, during and up to 48 h after transient cerebral hypoxia-ischaemia (fig.1). Areas Under the Curve (AUC) for the ^{1}H-MRS lactate/creatine (Lac/Cr) peak-area ratio in ventromedial forebrain (vmFB; predominantly grey matter) and dorsal sub-cortical (dsc; predominantly white matter) voxels were calculated (fig.1). Cell death at 48h was quantified with TUNEL staining on paraffin-embedded tissue in corresponding regions.

Results: Compared with normothermia, cooling to 35° and 33.5°C produced consistent 50-75% reduction in density of TUNEL+ cells with significant decreases in insular cortex, hippocampus, subcortical WM, thalamus and putamen (p<5% in ANOVA and post-hoc TUKEY). Cooling to 30°C did not further reduce TUNEL+ cell density compared to 33.5° and 35°C in dCTX; more cell death was seen in the deep grey matter (thalamus and putamen) at 30°C (fig.2, right). MRS biomarker analysis (fig.2, left) revealed some elevation of Lac/Cr AUC in the vmFB voxel at 30°C compared with 33.5° and 35°C (p=0.07). Raised Lac/Cr and increased cell death at 30°C was not observed in the dsc voxel or in the dorsal parietal cortex (dCTX) (fig 2).

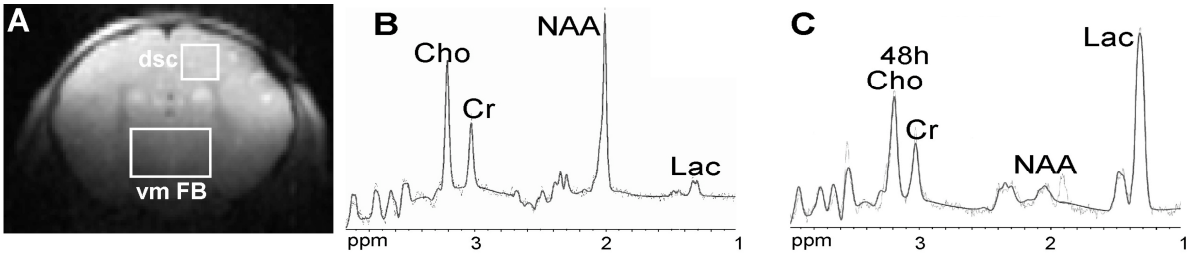

Fig 1. MRS voxel positions (A) and preinsult (B) and 48 h (C) dsc voxel spectra from a normothermic piglet. NAA - N-acetylaspartate; Cho - choline.

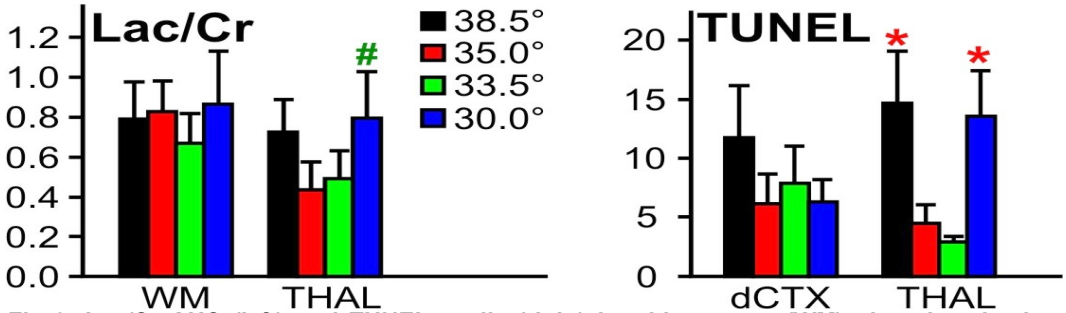

Fig 2. Lac/Cr AUC (left) and TUNEL+ cells (right) in white matter (WM), dorsal parietal cortex (dCTX) and thalamus (THAL). #p<10%. * p<5% compared to 35°&33.5°C groups (ANOVA and posthoc Tukey).

Conclusion: Cooling to 30°C enhanced hypoxic-ischaemic injury in the thalamus and basal ganglia compared to cooling to 33.5°C and 35°C. We demonstrate a higher threshold optimum temperature for neuroprotection in the deep grey matter than in WM/dCTX based on immunohistochemical markers of cell death. Systemic effects of cooling to 30°C may have exacerbated these detrimental effects on deep grey matter cell death. These data support previous work in the developing brain[2] and are very relevant to clinical practice for optimal therapeutic hypothermia in newborn infants with birth asphyxia.

References: 1. Edwards AD et al., BMJ 2010; 340;c363; **2.** Iwata 0 et al., Ann Neurol 2005;58:75-87; **3.** Thayyil et al., Pediatrics 2010;125:E 382-95