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

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Background: Therapeutic hypothermia for neonatal encephalopathy improves survival with normal neurological outcome1. 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) matter2.

Aim: To assess the optimal temperature for regional neuroprotection using 1H MRS biomarkers supported by immunohistochecmistry 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 1H-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 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).

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 brain2 and are very relevant to clinical practice for optimal therapeutic hypothermia in newborn infants with birth asphyxia.


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).