[N-71] Rewarming from therapeutic hypothermia induces cerebral cortical neuron apoptosis in a swine model of neonatal hypoxic-ischemic encephalopathy

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Introduction: Despite the use of therapeutic hypothermia, neurologic morbidity remains high in neonatal hypoxic-ischemic encephalopathy (HIE). While experimental models of HIE have shown that hypothermia decreases neuronal death from necrosis, hypothermia itself may be deleterious and rewarming may induce apoptosis. In a neonatal swine model of hypoxic-asphyxic (HA) cardiac arrest that results in HIE, we tested whether 1) piglets that were rewarmed after arrest and hypothermia would have more apoptosis than piglets that remained hypothermic, 2) rapid rewarming would induce more apoptosis than slow rewarming after arrest, and 3) caspase inhibition would prevent cell death during rewarming.

Methods: Neonatal piglets were randomized to undergo HA cardiac arrest or sham surgery. Piglets were then divided into 4 temperature groups: 1) normothermia; 2) whole-body hypothermia; 3) hypothermia + rapid rewarming (4°C/h); or 4) hypothermia + slow rewarming (0.5°C/h). The induction of hypothermia was delayed for 2 h and maintained overnight. Separate groups of piglets underwent arrest + delayed hypothermia + rapid rewarming with subdural administration of a caspase-3 inhibitor or artificial cerebral spinal fluid (aCSF). All piglets were euthanized at 29 h after resuscitation or sham surgery. Brains samples were prepared for histological quanitification of apoptotic profiles in motor and piriform cortex by hematoxylin and eosin stain using morphological criteria for apoptosis. Age-matched naïve pigs that did not receive anesthesia or surgery were prepared as an additional control group. Parametric data were analyzed by ANOVA with post-hoc Holm-Sidak test or t-test. Non-parametric data were analyzed by Kruskal-Wallis One-Way Analysis of Variance on Ranks with post-hoc Dunn’s Method or Mann-Whitney rank sum test.

Results: Piglets that were rewarmed (n=16) had significantly more apoptotic profiles than piglets that remained hypothermic (n=8) after arrest in motor ($p<0.05$) and piriform ($p<0.05$) cortex. Piglets that were rapidly rewarmed after arrest (n=8) had significantly more apoptotic profiles in motor cortex than hypothermic arrest (n=8), normothermic arrest (n=6), normothermic sham (n=8), and naïve piglets (n=7; $p<0.05$ for all comparisons). The number of apoptotic profiles in motor or piriform cortex did not differ between piglets that were slowly or rapidly rewarmed after arrest. Naïve, normothermic sham, normothermic arrest, and hypothermic arrest groups had similar amounts of apoptosis in motor and piriform cortex. Piglets that received the subdural caspase-3 inhibitor after arrest and rapid rewarming (n=6) had fewer apoptotic profiles than piglets that received aCSF (n=6) in motor ($p = 0.002$) and piriform cortex ($p = 0.003$).

Conclusions: Rewarming produced more cortical neuron apoptosis than sustained hypothermia or normothermia in a swine model of HIE. This deleterious effect appears independent of rewarming rate. The promotion of apoptosis by rewarming was inhibited by a caspase-3 inhibitor, which supports the conclusion that these cortical neurons are dying through caspase-dependent mechanisms. Caspase inhibition could become an adjuvant therapy to mitigate secondary brain injury during rewarming.
