Rewarming from therapeutic hypothermia induces cortical apoptosis in a swine model of neonatal hypoxic-ischemic encephalopathy

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Abstract

Introduction: When treating neonatal hypoxic-ischemic encephalopathy (HIE), adverse effects of rewarming could reduce neuroprotection from therapeutic hypothermia. We tested whether rewarming induces apoptosis in a swine model of HIE.

Methods: Piglets underwent hypoxic-asphyxic cardiac arrest or sham surgery followed by 1) normothermia; 2) overnight hypothermia; 3) hypothermia + slow rewarming; or 4) hypothermia + rapid rewarming. Some piglets received arrest + rapid rewarming with a subdural caspase-3 inhibitor or artificial cerebral spinal fluid (aCSF). Apoptotic and TUNEL-positive profiles were quantified in motor and piriform cortex. Activated caspase-3 was measured by western blot.

Results: Rewarmed piglets had more apoptosis in motor cortex than those that remained hypothermic after arrest (n = 8; p < 0.05). The number of apoptotic cells did not differ by rewarming rate. Caspase-3 activation was greater in rapidly rewarmed piglets in comparison to those that were slowly rewarmed or that remained hypothermic after arrest (n = 4; p < 0.05). Piglets that received the caspase-3 inhibitor had less apoptosis than piglets that received aCSF (n=6) in motor (p = 0.002) and piriform cortex (p = 0.004).

Discussion: Rewarming produced more apoptosis than sustained hypothermia, and rapid rewarming activated caspase-3 more than slow rewarming. Caspase-3 inhibition prevented apoptosis from rewarming, suggesting a potential role as an adjuvant therapy.

Background

• Despite therapeutic hypothermia, ~35% of neonates with hypoxic-ischemic encephalopathy develop moderate to severe disabilities 1
• Although hypothermia decreases neuronal necrosis in experimental models of hypoxic brain injury, rewarming may induce apoptosis

Hypotheses

• Piglets that are rewarmed after hypoxic-asphyxic brain injury will have more apoptosis than those that remain hypothermic
• Rapid rewarming will induce more apoptosis than slow rewarming
• Subdural administration of a caspase-3 inhibitor will prevent cell death during rewarming

Methods

• Naïve and sham-operated controls
• Hypoxic-asphyxic cardiac arrest: 45 min of FiO2 0.10 + 7 min asphyxia
• Four temperature groups: 1) Normothermia
• Delayed overnight hypothermia (2 h delay in inducing hypothermia)
• Delayed hypothermia + slow rewarming (0.5°C/h)
• Delayed hypothermia + rapid rewarming (4°C/h)
• Some piglets received arrest + rapid rewarming + either subdural caspase-3 inhibitor or artificial CSF (aCSF)
• Apoptotic profiles were quantified using morphologic criteria with H&E stain and verified with TUNEL in motor cortex (layers 2 and 3) and piriform cortex (layer 2)
• Western blots for activated caspase-3 in sensorimotor and piriform cortex
• The anesthetic regimen with the least apoptosis was identified and used throughout the 29 h experiments: fentanyl + N2O + pancuronium
• Data analysis: Kruskal-Wallis one way analysis of variance on ranks with post-hoc Dunn’s method, or Mann Whitney rank sum tests

Results

In comparison to piglets that remained hypothermic (HypOT) after arrest, piglets that were slowly or rapidly rewarmed had more (A) apoptotic and (B) TUNEL+ profiles in motor cortex (*p < 0.05). (NormoT, normothermia)

• The quantities of apoptotic and TUNEL+ profiles in piriform cortex were similar between piglets that were rewarmed and those that remained hypothermic after arrest

Conclusions

• Rewarming after hypoxic-asphyxic brain injury may increase cortical apoptosis through a caspase-3 dependent pathway
• Rapid rewarming may be more deleterious than slow rewarming
• Caspase inhibition has potential as an adjuvant therapy to reduce secondary brain injury from rewarming

References