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Hypothermic neuroprotection is associated with recovery of spectral edge frequency after asphyxia in preterm fetal sheep

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Abstract

Background and Purpose - EEG recovery is predictive of outcome after perinatal hypoxia-ischemia, but it is unknown whether early changes in EEG can predict the response to therapeutic hypothermia in the preterm brain.

Methods - 0.7 gestation fetal sheep received umbilical cord occlusion or sham occlusion for 25 min, followed by sham hypothermia or whole-body cooling started either 30 min or 5 hours after occlusion, and continued for 72 hours.

Results - Early but not delayed hypothermia reduced neuronal loss and microglial induction in the striatum, with faster recovery of spectral edge frequency, reduced seizure burden and less suppression of EEG amplitude ($p < 0.05$).

Conclusions - Recovery of higher EEG frequencies may be a biomarker of effective hypothermic neuroprotection in the preterm-equivalent brain.

Introduction

Mild induced hypothermia improves intact recovery in term infants with moderate to severe hypoxic-ischemic encephalopathy but has not been tested in preterm infants.¹ Early electroencephalographic (EEG) monitoring is predictive of long-term outcome after perinatal hypoxia-ischemia at term,² but the effect of mild hypothermia on EEG recovery in preterm infants is unknown. In normal humans, mild hypothermia (to ~33.5°C) is associated with a small reduction in EEG amplitude, with a minor shift in frequencies to theta and beta activity.³ In contrast, mild hypothermia after traumatic brain injury in rats increased the proportion of isoelectric EEG,⁴ and after pediatric cardiac arrest, worsening of EEG background abnormalities during hypothermia was associated with adverse outcomes.⁵ Thus, the severity of brain injury may modulate the effects of hypothermia on brain activity.

In the present study, we examined the hypothesis that early induction of hypothermia after asphyxia in preterm (0.7 gestation) fetal sheep would be associated with improved striatal neuroprotection and EEG recovery compared to hypothermia delayed for 5 hours, just before the typical onset of post-asphyxial seizures.⁶ At this age, brain development is broadly consistent with 28 to 32 weeks gestation in humans.⁷

Methods

Detailed methods are provided in the online-only Data Supplement.

Surgical procedures

All procedures were approved by the Animal Ethics Committee of The University of Auckland. Fetal sheep at 97-99 days gestation (term=147 days) were instrumented with brachial artery catheters and extradural EEG electrodes. Thermistors were placed over the parasagittal dura and in the esophagus. An occluder was fitted around the umbilical cord and a cooling coil was tied over the fetal back.

Experimental proceedings

At 103-104 days gestation, fetuses were randomly assigned to sham occlusion followed by normothermia (sham-normothermia, n=8) or whole-body cooling for 72 hours (sham-hypothermia, n=8) or umbilical cord occlusion for 25 min, followed by sham hypothermia (occlusion-normothermia, n=12) or whole-body cooling started from 30 min (occlusion-early hypothermia, n=10) or 5 hours (occlusion-delayed hypothermia, n=7) after occlusion and continued for 72 hours. Mild whole-body cooling was induced by circulating cold water through the cooling coil. Seven days after occlusion the ewes and fetuses were killed.

Mean arterial pressure (MAP), blood gases, EEG activity, and temperature were recorded throughout the experiment. EEG power and spectral edge frequency (SEF) were calculated. Electroencephalographic seizures were identified. Data are mean \pm standard error of the mean (SEM).

Immunohistochemistry

Brain sections were stained for NeuN (neuronal survival) and IB4 (activated microglia) in the caudate nucleus and putamen, and positive cells were counted stereologically.

Statistical analysis

Data were evaluated by repeated measures ANCOVA (SPSS v22, SPSS Inc., Ill., USA), and Sidak post-hoc analysis. The within subjects' correlation was assessed between EEG amplitude (μV) and extradural temperature. Statistical significance was accepted at $p < 0.05$.

Results

Blood composition, arterial pressure, and extradural temperature

Umbilical cord occlusion was associated with profound hypoxia, mixed metabolic and respiratory acidosis, and hypotension, with rapid recovery after release of occlusion. Hypothermia was associated with a small increase in pH and glucose, and a lower PaCO₂ compared with occlusion-normothermia (Data Supplement Table I), and no effect on MAP (Data Supplement Table II). Fetal extradural temperatures are shown in Figure 1.

EEG power and SEF

Occlusion was associated with suppressed EEG power until 84 hours after asphyxia in the occlusion-normothermia group, and 72 hours in both hypothermia groups ($p < 0.05$; vs. sham-normothermia, Figure 1). Compared to occlusion-normothermia, EEG power was reduced from 1–12 hours with early hypothermia, and 6–12 hours with delayed hypothermia ($p < 0.05$). Delayed hypothermia was associated with suppressed EEG power compared to early hypothermia from 24–30 hours ($p < 0.05$).

SEF was significantly suppressed with occlusion-normothermia until 72 hours after occlusion compared to sham-normothermia ($p < 0.05$; Figure 1). In contrast, early hypothermia showed rapid recovery of SEF to sham-normothermia values within 4 hours, with higher SEF than delayed hypothermia from 30–72 and 78–162 hours ($p < 0.05$). Examples of continuous EEG are shown in Data Supplement Figure I.

Relationship between extradural temperature and EEG amplitude

All groups showed a significant relationship between extradural temperature and EEG amplitude (Data Supplement Figure II). Delayed hypothermia was associated with a

greater effect on EEG amplitude ($2.16 \pm 0.79 \mu\text{V}/^\circ\text{C}$, $r=0.64$, $p<0.001$) than early hypothermia ($0.53 \pm 0.08 \mu\text{V}/^\circ\text{C}$, $r=0.50$, $p<0.001$).

Seizures

Numbers of seizures, seizure burden (total min of seizure activity) and mean seizure duration during recovery were reduced after early but not delayed hypothermia ($p<0.05$; Data Supplement Table III).

Striatal neurons and microglia

Occlusion was associated with significant loss of neurons in the caudate and putamen ($p<0.05$; vs. sham-normothermia, Figure 2 and Data Supplement Figure III). Neuronal survival was increased after early but not delayed hypothermia in the caudate nucleus. Both occlusion-hypothermia groups showed intermediate neuronal survival in the putamen between sham-normothermia and occlusion-normothermia.

Occlusion was associated with intense induction of microglia in the caudate and putamen ($p<0.05$; vs. sham-normothermia, Figure 2 and Data Supplement Figure III). This was markedly reduced after early hypothermia ($p<0.05$), but only partially suppressed after delayed hypothermia.

Discussion

This study demonstrates that mild whole-body cooling in preterm fetal sheep, started within 30 minutes after severe asphyxia, was associated with faster recovery of SEF to sham control values in the first 4 hours, and subsequently markedly reduced seizure burden and less suppression of EEG power and amplitude, than hypothermia delayed until 5 hours after asphyxia. Consistent with this, early but not delayed cooling was associated with improved striatal neuronal survival and suppression of microglia induction after 7 days recovery. These findings raise the possibility that delayed recovery of SEF could help identify infants who benefit less from hypothermia.

Prolonged umbilical cord occlusion was associated with moderate to severe subcortical neural injury but sparing of the cortex after 3 days recovery,⁸ and with marked prolonged suppression of EEG power and SEF, consistent with clinical evidence in preterm infants that reduced SEF is associated with adverse outcomes.⁹ Continuous EEG recordings showed epileptiform transient activity from 3 to 8 hours after occlusion-normothermia. Early but not delayed body cooling was associated with suppression of these abnormal events and partial restoration of low-amplitude but higher-frequency EEG activity. Since synchronous EEG activity reflects cortico-thalamo and cortico-cortical feedback between neurons, we speculate that recovery of higher frequency EEG activity with early hypothermia may reflect better preservation of intracortical synaptic connectivity or thalamic neurons.¹⁰

The finding that early cooling was associated with reduced seizures but less suppression of overall EEG amplitude/power than delayed cooling strongly suggests an indirect effect, mediated by neuronal protection. We speculate that early recovery of SEF and reduced suppression of EEG amplitude during cooling may be biomarkers of clinical response to therapeutic hypothermia.

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Disclosures

None.

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Figure Legends

Figure 1. Changes in extradural temperature ($^{\circ}\text{C}$), spectral edge frequency (SEF, Hz), and EEG power (dB) in the sham-normothermia (open circles), sham-hypothermia (closed circles), occlusion-normothermia (open squares), occlusion-early hypothermia (triangles), and occlusion-delayed hypothermia (inverted triangles) groups. Data are mean \pm SEM; 6 hourly averages during baseline and after 78 hours, and hourly averages until 78 hours. # $p < 0.05$; normothermia *vs.* hypothermia, * $p < 0.05$; sham-normothermia *vs.* occlusion-normothermia, † $p < 0.05$; occlusion-early hypothermia *vs.* occlusion-normothermia, ‡ $p < 0.05$; delayed hypothermia *vs.* occlusion-normothermia, § $p < 0.05$; occlusion-early hypothermia *vs.* delayed hypothermia.

Figure 2. Numbers of IB4 positive microglia cells (panel A) and NeuN positive neurons (panel B) in the caudate and putamen 7 days after (sham) occlusion in preterm fetal sheep. Data are mean \pm SEM. * $p < 0.05$; *vs.* sham-normothermia, # $p < 0.05$; occlusion-hypothermia groups *vs.* occlusion-normothermia.



