Hypothermic Neuroprotection Is Associated With Recovery of Spectral Edge Frequency After Asphyxia in Preterm Fetal Sheep

Guido Wassink, MSc*; Robert D. Barrett, PhD*; Joanne O. Davidson, PhD; Laura Bennet, PhD; Robert Galinsky, PhD; Michael Dragunow, PhD; Alistair J. Gunn, MBChB, PhD

Background and Purpose—Electroencephalographic recovery is predictive of outcome after perinatal hypoxia–ischemia, but it is unknown whether early changes in electroencephalographic 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 minutes, followed by sham hypothermia or whole-body cooling started either 30 minutes 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 electroencephalographic amplitude ($P<0.05$).

Conclusions—Recovery of higher electroencephalographic frequencies may be a biomarker of effective hypothermic neuroprotection in the preterm-equivalent brain. **(*Stroke*. 2015;46:585-587. DOI: 10.1161/STROKEAHA.114.008484.)**

Key Words: EEG ■ hypothermia

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 with hypothermia delayed for 5 hours, just before the typical onset of postasphyxial 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 to 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 to 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 minutes, followed by sham hypothermia (occlusion-normothermia, n=12), or whole-body cooling started from 30 minutes (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, blood gases, EEG activity, and temperature were recorded throughout the experiment. EEG power and spectral

Received December 15, 2014; final revision received December 15, 2014; accepted December 17, 2014.

From the Department of Physiology (G.W., R.D.B., J.O.D., L.B., R.G., A.J.G.), and Department of Anatomy with Radiology (M.D.), University of Auckland, Auckland, New Zealand.

*G. Wassink and Dr Barrett are joint first authors.

The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.114.008484/-/DCI.

Correspondence to Alistair J. Gunn, MBChB, PhD, Deptartment of Physiology, School of Medical Sciences, Faculty of Medical and Health Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand. E-mail aj.gunn@auckland.ac.nz

edge frequency (SEF) were calculated. Electroencephalographic seizures were identified. Data are 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, IL) 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 $PaCO_2$ compared with occlusion-normothermia (Table I in the online-only Data Supplement) and no effect on mean arterial pressure (Table II in the online-only Data Supplement). 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 ($P<0.05$; versus sham-normothermia; Figure 1). Compared with occlusion-normothermia, EEG power was reduced from 1 to 12 hours with early hypothermia, and 6 to 12 hours with delayed hypothermia ($P<0.05$). Delayed hypothermia was associated with suppressed EEG power compared with early hypothermia from 24 to 30 hours ($P<0.05$).

SEF was significantly suppressed with occlusion-normothermia until 72 hours after occlusion compared with 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 to 72 and 78 to 162 hours ($P<0.05$). Examples of continuous EEG are shown in Figure I in the online-only Data Supplement.

Relationship Between Extradural Temperature and EEG Amplitude

All groups showed a significant relationship between extradural temperature and EEG amplitude (Figure II in the online-only Data Supplement). Delayed hypothermia was associated with a greater effect on EEG amplitude (2.16±0.79 μV/°C, $r=0.64$, $P<0.001$) than early hypothermia (0.53±0.08 μV/°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 ($P<0.05$; Table III in the online-only Data Supplement).

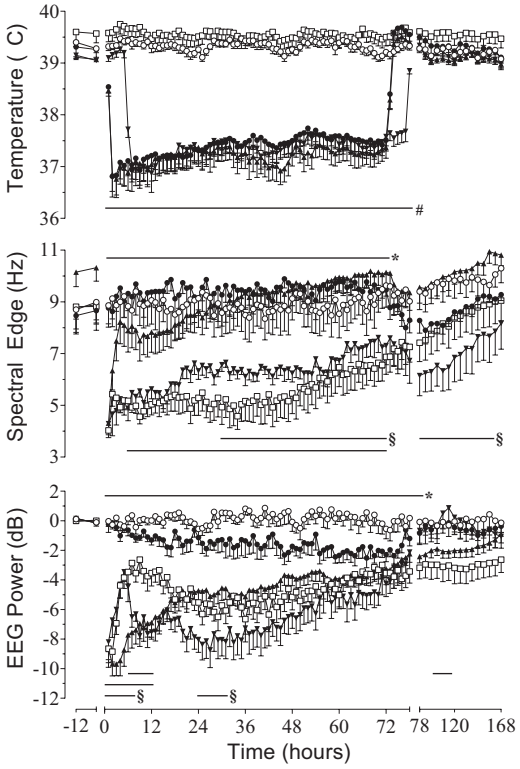

Figure 1. Changes in extradural temperature (°C), spectral edge frequency (Hz), and EEG power (dB) in the sham-normothermia (open circles), sham-hypothermia (closed circles), occlusion-normothermia (open squares), occlusion-early hypothermia (triangles), and occlusion-delayed hypothermia (inverted triangles) groups. Data are mean±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.

Striatal Neurons and Microglia

Occlusion was associated with significant loss of neurons in the caudate and putamen ($P<0.05$; versus sham-normothermia; Figure 2 and Figure III in the online-only Data Supplement). 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 ($P<0.05$; versus sham-normothermia; Figure 2 and Figure III in the online-only Data Supplement). 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

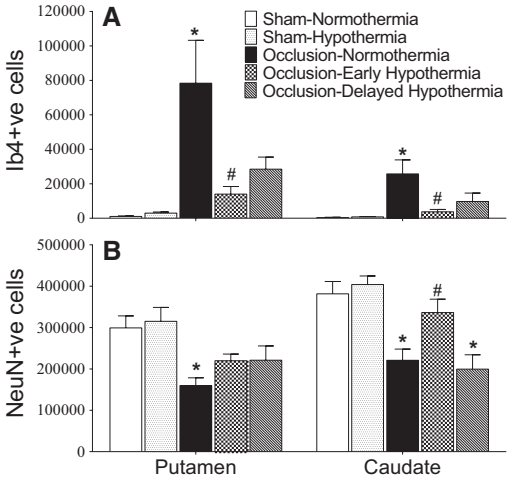

Figure 2. Numbers of IB4-positive microglia cells (**A**) and NeuN-positive neurons (**B**) in the caudate and putamen 7 days after (sham) occlusion in preterm fetal sheep. Data are mean±SEM. **P*<0.05, vs sham-normothermia; #*P*<0.05, occlusion-hypothermia groups vs occlusion-normothermia.

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 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. Because 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.

Sources of Funding

This study was funded by the Health Research Council of New Zealand and Gravida. Mr Guido Wassink was supported by the University of Auckland Health Research Doctoral Scholarship. Dr Barrett was supported by a scholarship from the William Georgetti Charitable Trust.

Disclosures

None.

References

1. Edwards AD, Brocklehurst P, Gunn AJ, Halliday H, Juszczak E, Levene M, et al. Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data. *BMJ*. 2010;340:c363.
2. Azzopardi D; TOBY study group. Predictive value of the amplitude integrated EEG in infants with hypoxic ischaemic encephalopathy: data from a randomised trial of therapeutic hypothermia. *Arch Dis Child Fetal Neonatal Ed*. 2014;99:F80–F82. doi: 10.1136/archdischild-2013-303710.
3. Kochs E. Electrophysiological monitoring and mild hypothermia. *J Neurosurg Anesthesiol*. 1995;7:222–228.
4. Clark RS, Kochanek PM, Marion DW, Schiding JK, White M, Palmer AM, et al. Mild posttraumatic hypothermia reduces mortality after severe controlled cortical impact in rats. *J Cereb Blood Flow Metab*. 1996;16:253–261. doi: 10.1097/00004647-199603000-00010.
5. Abend NS, Topjian A, Ichord R, Herman ST, Helfaer M, Donnelly M, et al. Electroencephalographic monitoring during hypothermia after pediatric cardiac arrest. *Neurology*. 2009;72:1931–1940. doi: 10.1212/WNL.0b013e3181a82687.
6. Drury PP, Davidson JO, Mathai S, van den Heuij LG, Ji H, Bennet L, et al. nNOS inhibition during profound asphyxia reduces seizure burden and improves survival of striatal phenotypic neurons in preterm fetal sheep. *Neuropharmacology*. 2014;83:62–70. doi: 10.1016/j.neuropharm.2014.03.017.
7. Barlow RM. The foetal sheep: morphogenesis of the nervous system and histochemical aspects of myelination. *J Comp Neurol*. 1969;135:249–262. doi: 10.1002/cne.901350302.
8. George S, Gunn AJ, Westgate JA, Brabyn C, Guan J, Bennet L. Fetal heart rate variability and brain stem injury after asphyxia in preterm fetal sheep. *Am J Physiol Regul Integr Comp Physiol*. 2004;287:R925–R933. doi: 10.1152/ajpregu.00263.2004.
9. Inder TE, Buckland L, Williams CE, Spencer C, Gunning MI, Darlow BA, et al. Lowered electroencephalographic spectral edge frequency predicts the presence of cerebral white matter injury in premature infants. *Pediatrics*. 2003;111:27–33.
10. Bennet L, Roelfsema V, George S, Dean JM, Emerald BS, Gunn AJ. The effect of cerebral hypothermia on white and grey matter injury induced by severe hypoxia in preterm fetal sheep. *J Physiol*. 2007;578(pt 2):491–506. doi: 10.1113/jphysiol.2006.119602.