Protecting the Newborn Brain: New Avenues

Nicola J Robertson
FRCPCH, PhD
Professor of Perinatal Neuroscience & Honorary Consultant Neonatologist
Intrapartum-related hypoxic events

- Major cause of global mortality
  - ¼ of world’s 3 million neonatal deaths
  - In survivors cause of one of the highest numbers of DALYs

- 85% in SE Asia & SS Africa

- Incidence
  - 15/1000 (SS Africa)
  - 1.5/1000 high income countries

Lee et al., Pediatr Res 2013
Where you are born affects outcome
Lawn et al., Pediatr Res 2013

- **High Income**: 11m
- **Middle Income**: 34m
- **Low Income**: Home birth (50m) vs. Facility birth (40m)
HYPOXIA-ISCHAEMIA
Phases of Brain Injury

- **Primary phase (minutes)**
  - Cerebral hypoxia-ischaemia of sufficient severity to deplete tissue energy reserves

- **Latent phase (hours)**
  - Reperfusion and reoxygenation and restoration of glucose use and high energy phosphates (latent phase)

- **Secondary phase (days)**
  - Decrease in high energy phosphate in parallel with cell injury

- **Tertiary phase (weeks, years)**
  - Long term cell regeneration and repair
Primary phase

- overflow of glutamate activates NMDA receptors
- intracellular accumulation of Ca2+
- substantial production of NO

Johnston et al., Lancet Neurology 2011
Latent phase (from 1-6h)

- Recovery of oxidative metabolism vs residual mitochondrial injury

- programmed cell death

- inflammation

- receptor hyperactivity

Johnston et al., Lancet Neurology 2011
Secondary phase (hours to days)

- Deteriorating mitochondrial function
- Seizures
- Hyperperfusion
- Cytotoxic oedema
- Cell death
Tertiary phase: astrogliosis, increased EAA, continuing inflammation and epigenetic changes

Fleiss & Gressens, Lancet Neurology 2012
$^{31}$P & $^1$H MRS primary and secondary energy failure
Therapeutic hypothermia: caveats
Therapeutic hypothermia reduces adverse outcome in intensive care settings

• Therapeutic hypothermia (cooling to 33.5°C for 72 h within 6 h) reduces adverse outcome at 18 months (typical RR 0.75% CI 0.68-0.83) and at school age¹

• NNT 6-7

• Despite treatment 40% babies have adverse outcome

1. Jacobs et al., Cochrane Rev 2013
RCT of Optimizing (Longer, Deeper) Cooling for Neonatal Hypoxic-Ischaemic Encephalopathy
(Shankaran et al., E-PAS2014:2823.8)

• Term infants moderate to severe NE randomised:
  – 33.5°C for 72h or 120h
  – 32.0°C for 72h or 120h

• 364 of planned 726 infants enrolled
  – Trial closed for safety and futility following recommendation from the Independent Data and Safety Monitoring Committee on 27/11/13
RCT of Optimizing (Longer, Deeper) Cooling for Neonatal Hypoxic-Ischaemic Encephalopathy
(Shankaran et al., E-PAS2014:2823.8)

<table>
<thead>
<tr>
<th>In Hospital Mortality</th>
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<td>Duration of cooling</td>
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<td>72h</td>
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Hypothermia in the presence of infection

- Therapeutic hypothermia ineffective or harmful in the presence of infection/inflammation (Mourvillier, JAMA 2013)
- In a neonatal rodents cooling not protective in inflammation-sensitized HI (Osredkar et al., 2013)
- Hypothermia can alter immune responsiveness (Jenkins et al., 2013)
Adjunct Therapies for Neonatal Encephalopathy
12 neuroscientists across the world

Which Neuroprotective Agents are Ready for Bench to Bedside Translation in the Newborn Infant?

Nicola J. Robertson, MB ChB, PhD¹, Sidhartha Tan, MD², Floris Groenendaal, MD, PhD³, Frank van Bel, MD, PhD³, Sandra E. Juul, MD, PhD⁴, Laura Bennet, PhD⁵, Matthew Derrick, MD², Stephen A. Back, MD, PhD⁶, Raul Chavez Valdez, MD⁷, Frances Northington, MD⁷, Alistair Jan Gunn, MB ChB, PhD⁵, and Carina Mallard, PhD⁸

Robertson et al., J Pediatrics 2012
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<td>Rank (%)</td>
<td>1 (90%)</td>
<td>2 (86%)</td>
<td>3 (80%)</td>
<td>4 (72%)</td>
<td>5 (68%)</td>
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Noble Gas Neuroprotection

**Xenon**
- Phase II neuroprotection trials
- €20-30 per liter
- Needs recirculating ventilator
- Anaesthetic

**Argon**
- Most abundant inert gas
- Low price (9c / litre)
- 100-200 times cheaper than xenon
- No anaesthetic properties
- Neuroprotective effects similar to xenon
MELATONIN
Aaron Lerner (1920-2007): Discovery & Chemical Characterization of Melatonin

- 1958 - discovery/characterization of melatonin by Aaron Lerner, Yale

- Melatonin
  - Extracted from bovine pineal glands
  - Remarkable effects on pigmentation in amphibians (not mammals)
  - Reawakened interest in the pineal gland (seat of the soul)

- Discovery of huge importance for medicine
Biologic Roles of Melatonin

• Many biologic functions
  – Waxing and waning of seasonal reproduction (1965)¹
  – Regulation of circadian rhythm²
  – Sleep promotion
  – Immune-enhancement/anti-inflammatory³
  – Oncostasis⁴
  – Antioxidant⁵

• Categorized by the FDA as a dietary supplement and not regulated as a pharmaceutical drug

¹ Hoffman and Reiter, 1965
² Reiter, 1991
³ Carillo-Vico, 2005
⁴ Shiu, 2007
⁵ Tan et al., 1993
Melatonin: molecule of darkness

Brzezinski, NEJM 1997
Diurnal variations in melatonin levels

Grivas and Savvidou, Scoliosis 2007
Age variations in melatonin levels

Melatonin peaks in early childhood. Melatonin continues to decline in middle age. Older people produce negligible amounts of melatonin.

Puberty occurs as melatonin declines. Newborns produce minimal melatonin.

Grivas and Savvidou, Scoliosis 2007
Neuroprotective efficacy of melatonin in experimental stroke

- Systematic review & meta-analysis
  - 400 animals, 28 studies
  - Effect size or improvement in outcome with melatonin given after cerebral HI versus control group calculated

MacLeod et al., J Pineal Res 2005;38:35-41
Neuroprotective efficacy of melatonin in experimental stroke

- Solid vertical line – treatment and control are equal
- Point estimates and 95% CI for studies
  - Ranked for the effect size of melatonin

MacLeod et al., J Pineal Res 2005;38:35-41
Neuroprotective efficacy of melatonin in experimental stroke

- Global estimate of the overall neuroprotective effect of melatonin

- Highly significant improvement in outcome (point estimate 42.8%)

- Maximum efficacy with
  - > 5mg/kg (substantially higher than those used for jet lag (0.05mg/kg))
  - 15% increase in effect size with repeated doses
Aim of this study

• To determine whether iv melatonin given 10 mins after resuscitation augments hypothermic neuroprotection in our piglet perinatal asphyxia model

1. Pilot Safety Study (dose de-escalation)

2. Efficacy study - Defined Primary Outcome Measures
   – $^1$H MRS (lactate/N-acetyl aspartate)
   – TUNEL positive cell counts
One aspect of our model is that we can quantify and standardise the severity of the HI insult in bore of 9.4T MR system.
- Neonatal intensive care for 48 h after HI
- Physiological and biochemical data
- Metabolic / temperature homeostasis
- EEG, NIRS
- Bridging 1H MRS biomarker (Lactate/NAA)
Pilot Safety Study: effect on mean arterial blood pressure of melatonin with cooling
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Experimental Plan (n=17, 8 hypo, 9 hypo + mel)

Hypothermia vs Melatonin-Augmented Hypothermia

Blood samples for pharmacokinetics

5mg/kg/h (30MG/KG/24h) MELATONIN iv at 10 min after HI in 2.5% ethanol. Repeated at 24h

Baseline

HI & resus

MELATONIN & COOLING

33.5°C

Vehicle (ethanol 2.5% and saline)

COOLING

33.5°C

MRI

0 h

24 h

48 h
**Insult severity**

- β-NTP 40% baseline, held for 12.5 mins
- Calculate the acute energy deficit
- Compare the insult severities between groups
Primary outcome measure: $^1$H MRS Lactate/NAA

$^1$H MRS –
depth grey & white matter

Lactate/NAA robust outcome biomarker in babies with NE
(Thayyil et al., Pediatrics)
RESULTS

• No intergroup differences
  – Body weight, age, baseline physiological and biochemical measures
  – Insult severity
  – Time to reach 33.5°C after hypoxia-ischemia
  – Blood pressure
    • Saline volume
    • Inotrope use (dopamine, dobutamine and adrenaline)
Reduction in thalamus lactate/NAA & Lac/Cr AUC in melatonin-augmented cooling
Reduction in thalamus lactate/NAA & Lac/Cr AUC in melatonin-augmented cooling
TUNEL + cells

A

- hypothermia
- +melatonin

TUNEL-Density (#/EF)

dCTX  mCTX  Pyr  Hip  pvWM  IC  Caudt  PTMN  THLM

*p < 0.05
TUNEL + cells

A

- hypothermia
- +melatonin

* \( p < 0.05 \)
TUNEL positive cell quantification

Naïve  Hypothermia  Hypothermia +Mel

TUNEL, low mag

G  H  I

1mm

TUNEL, high mag

J  K  L
Results: aEEG analysis

- Normal
- Moderately abnormal
- Severely abnormal

Graph showing aEEG scores over time:
- HypoT
- HypoT+Mel

Score range:
- 0 to 3

Time scale:
- Hours after HI insult
Melatonin modifies microglial phenotype

Gene Expression

Average of GOI/Reference Gene relative to Naive

- iNOS
- CD86
- IGF1
- ARG1
- SphK1
- SOCS3

HypoT n=7
HypoT+MEL n=7

* Indicates significant difference
Melatonin pharmacokinetics

- Half life: 21.6 +/- 3.3h
- Hypothermia: 6-15 fold higher than baseline
- Melatonin + hypothermia: 100,000 fold higher than baseline levels or peak night-time levels in man
Melatonin augments hypothermic neuroprotection in a perinatal asphyxia model

Nicola J. Robertson, Stuart Faulkner, Bobbi Fleiss, Alan Bainbridge, Csilla Andorka, David Price, Elizabeth Powell, Lucy Lecky-Thompson, Laura Thei, Manigandan Chandrasekaran, Mariya Hristova, Ernest B. Cady, Pierre Gressens, Xavier Golay and Gennadij Raivich

Robertson et al., Brain. 2013 Jan;136(Pt 1):90-105.
Anti-oxidant Effects of Melatonin

- 1993 - scavenges reactive oxygen and nitrogen species

- Strengthens enzymatic defense eg glutathione peroxidase

- Protects biological membranes against lipid peroxidation

2. Garcia et al., J Pineal Res 2014
Melatonin: influence on immune system

• Positive regulator of innate and adaptive immune response\(^1,2\)
• Immune enhancing effects

• Anti-inflammatory – silences NFKB transcription\(^3\)
• Reduces NO synthesis

1. Reiter et al., NYAcad Sci 2001
2. Wang et al., Ann Neurol 2007
3. Chuang et al., Cell Biol Int 1996
Mitochondrial protection

Melatonin

SCAVENGES ROS/RNS

PROTECTS ETC/OXPHOS Complexes

PROTECTS mtDNA

Mitochondrial DNA

Pro-apoptotic proteins

PREVENTS cytochrome c release

mPTP opening

Molecules <1.5 kDa

Cytochrome C

APOPTOSIS

Melatonin

Hassell, Reiter, Robetson. FMM Reviews 2013
Mitochondrial protection

Melatonin

SCAVENGES
ROS/RNS

ROS
RNS

PROTECTS
Electron Transport
Chain Complexes

PROTECTS
mtDNA

Mitochondrial DNA

Pro-apoptotic proteins

PREVENTS
cytochrome c
release

mPTP

Molecules
<1.5 kDA

Cytochrome C

APOPTOSIS

Hassell, Reiter, Robetson. FMM Reviews 2013
Mitochondrial protection

- Melatonin
  - SCAVENGES ROS/RNS
  - PROTECTS ETC/OXPHOS
  - PREVENTS Cytochrome c release
  - INHIBITS mPTP opening

- ATP
  - PROTECTS mtDNA
  - PREVENTS Cytochrome C release
  - APOPTOPSIS

- VDAC
  - ANT
  - CypD
  - Molecules <1.5 kDA

Hassell, Reiter, Robertson. FMM Reviews 2013
Mitochondrial protection

Hassell, Reiter, Robetson. FMM Reviews 2013
Future studies and collaborations

• Collaboration to determine
  – Safe formulation (with NO ETHANOL)
  – Further safety studies
  – Therapeutic window
  – Lowest effective dose with and without cooling
Melatonin – ready for prime time?

- Melatonin is safe even at high intravenous doses
- Melatonin (30mg/kg/over 6h) augmented hypothermic neuroprotection 10min after HI
- Melatonin’s metabolites amplify neuroprotection
- Relatively easy to administer even during transfer

- More work needed
  - Therapeutic window
  - Lowest effective dose
REMOTE ISCHAEMIC POSTCONDITIONING
Ischaemic pre-conditioning

- If the heart/brain is exposed to repeated short periods of ischaemia followed by reperfusion (typically 4 repeated cycles of 10 mins) the cells downstream of the ischaemia are protected against a subsequent prolonged period of ischaemia

- First described in 1986 by Murry
Ischaemic post conditioning

- Application of brief sublethal ischaemia in one organ after a prolonged injurious insult generates tissue protective mechanisms in the same organ\(^1,2\)

- A STIMULUS AFTER THE INSULT PROTECTS

- A STIMULUS REMOTE TO THE INJURED ORGAN PROTECTS EG LIMB

Postconditioning: A Simple, Clinically Applicable Procedure to Improve Revascularization in Acute Myocardial Infarction
Jakob Vinten-Johansen, Derek M. Yellon and Lionel H. Opie

Circulation. 2005;112:2085-2088
doi: 10.1161/CIRCULATIONAHA.105.569798
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

Prof Derek Yellon, Prof of Molecular and Cellular Cardiology, Hatter Institute, UCL
Remote ischaemic post conditioning: does it protect the brain after hypoxic-ischaemic injury?

• **Primary endpoints**
  - Brain MRS
    - lactate/N acetyl aspartate
    - NTP/ePP
  - Quantitative immunohistochemistry
Piglet model of Immediate RIPostC

Baseline   Insult

IPostC

Control

n=8

MRI

0 h  24 h  48 h
RIPostC device

- 4 cycles of 10 mins bilateral hindlimb ischaemic/reperfusion starting at resuscitation
- Device MR compatible and remotely operated
- Occlusion of both femoral arteries
  - Limb ischaemia confirmed by laser doppler, pulse oximetry and MRI
Lac/NAA white matter reduced with RIPostC

- p-value from testing the null hypothesis of no treatment difference at baseline, 24 hrs and 48 hrs
- p=0.005*
- p=0.304
- p=0.826
NTP/ePP whole brain preserved with RIPostC

- p-values from testing the null hypothesis of no treatment difference at baseline, 24 hrs and 48 hrs

- HI
- HI + RIPostC

- p = 0.481
- p = 0.039*
White matter protection with RIPostC
RIPostC reduces pro-apoptotic proteins, RO, mito Ca2+, and mito permeabilization.
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RIPostC reduces pro-apoptotic proteins, RO, mito Ca2+, and mito permeabilization.
Remote ischaemic post conditioning

- Simple intervention that harnesses/manipulates the body’s endogenous neuroprotection potential
- Low cost
- Low tech
- Potentially transferrable across all settings
- Therapeutic window up to 6h in rodent models
- Does is augment cooling?
FUTURE NEUROPROTECTION?
Future neuroprotective therapies: a cocktail & ice
Possible cocktail treatment protocol

- Melatonin
- Epo 1000U/kg

Day

0 1 2 3 4 5 6 7

cooling

? Antenatal therapies

GAS?
Acknowledgements

Gressens, Fleiss
Safe journey home!