Systemic Inflammation Negates Hypothermic Neuroprotection in a Neonatal HIE-model

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Background

• 1 million new cases of neonatal encephalopathy each year\(^1\).

• Encephalopathy from HI alone is caused by sentinel events such as
  – placental abruption
  – uterine rupture
  – cord prolapse
  – shoulder dystocia\(^2\)

Fleiss et al. *Dev Med Child Neurol.* 2015
Therapeutic hypothermia (TH) is somewhat neuroprotective: poor outcome 66% → 50%.

1. Edwards et al. BMJ. 2010
Neonatal HIE is Likely To Be a Multifactorial Condition With Complex Aetiology

- **Sensitisation**
  - Antenatal factors
    - inflammation
    - maternal stress
    - hypoxia
    - malnutrition
    - genetic factors

- **Perinatal insult**
  - Perinatal factors
    - hypoxia-ischaemia
    - glutamate
    - inflammation
    - oxidative stress
    - genetic factors

- **Exacerbation**
  - Postnatal factors
    - pain
    - drugs
    - ongoing inflammation
    - genetic factors

Modified from Fleiss et al. [Dev Med Child Neurol.](https://www.nature.com/articles/d41590-015-02166-7) 2015
Fetal Exposure to Infection Contributes to the Risk of Hypoxic-Ischemic Encephalopathy.¹

Peebles et al. *BJOG*. 2002
7 day old rat pups (n=153)

Experimental Design

Intraperitoneal injections NaCl or LPS

4 hrs

Hypoxia (8% O₂ for 50 min at 36°C)

Unilateral Ligation
5 hrs NT
37°C
Veh=40, LPS=40

5 hrs HT
32°C
Veh=38, LPS=35

24 hrs survival OR 1 week survival

Western blots

Immunohistochemistry

Pathology – Area Loss (P14)
Hypothermia Did Not Offer Any Neuroprotection After LPS+HI

Increased Microglial Activation After LPS+HI

Veh

LPS

NT 37°C

HT 32°C

= Dapi, nuclei in general

= Iba1, microglia specific
Western Blots

- NeuN
- cCas3
- GFAP
- Iba1
LPS Sensitisation Increases Apoptosis

Relative cCas3 expression (P8)

- Veh-NT
- LPS-NT
- Veh-HT
- LPS-HT

N=9

*
LPS Sensitisation Exacerbates Neuronal Loss

![Graph showing relative NeuN expression (P14) with N values for each group: Veh-NT (N=9), LPS-NT (N=9), Veh-HT (N=7), LPS-HT (N=7). The LPS-HT group has the highest expression, followed by Veh-HT, LPS-NT, and Veh-NT.]
LPS Sensitisation Enhances Astrogliosis
Clinical Relevance

• Are we cooling the right cohort?

• Should we cool, when there is substantial degree of systemic inflammation?

• Further research (pre-clinical and clinical) is needed
Conclusion

In a P7 HI rat model with systemic inflammation therapeutic hypothermia is not neuroprotective neither macroscopically, nor on a cellular level.
Thanks to my group!

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- Maja Elstad
- Maria Skytioti
- Kristin Bergersen

Thoresen group, Oslo, Norway
A

% brain area loss

<table>
<thead>
<tr>
<th>Group</th>
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<th>HI</th>
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<td>Ctrl-NoHI-NT</td>
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<td>Veh-NoHI-NT</td>
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B

Veh-NT  LPS-NT  Veh-HT  LPS-HT

**Statistical Significance:**
- *: Significant difference
- NS: Not significant


3. Edwards 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

4. Peeples et al, Synergy between antenatal exposure to infection and intrapartum events in causation of perinatal brain injury at term, BJOG 2002


6. Osredkar et al, Hypothermia is not neuroprotective after infection-sensitized neonatal hypoxic-ischemic brain injury, Resuscitation, 2014

7. asdfkjad
References


Modified from Fleiss et al. *Dev Med Child Neurol*, 2015
Edwards et al. *BMJ*, 2010
Peeples et al. *BJOG*, 2002
Osredkar et al. *Dev Neurosci*, 2015

It has even been shown evidence that elective caesarean section was associated with a highly significant reduction in neonatal encephalopathy. This was in a large Australian case-control study of Badawi et al, published in BMJ in 1998.
Discussion

1. Up-regulation of the innate immune system with early initiation of inflammatory processes by hypoxia leading to glial activation and release of cytotoxic cytokines?

2. Increased NO production leading to mitochondrial dysfunction and failure of oxidative phosphorylation?

3. Endotoxin-induced hypoglycaemia impairing the metabolic response to hypoxia?

4. Exacerbation of local tissue ischaemia via activation of procoagulant molecules and release of casoactive substances such as PAF and endothelial damage?

5. Increased expression of pro-apaptotic molecules such as Fas ligand or TNF?
Hypothermia did not offer any neuroprotection after LPS+HI