Antenatal Therapy to Improve Neurological Outcome
A Role for Allopurinol, Indomethacin and Mg?

Neonatologie und Padiatrische Intensivmedizin
13-14 November, Freiburg, D
Antenatal Neuroprotection

- Very/extremely preterm newborn
- Term newborn
Incidence Diffuse White Matter Damage or (Non-Cystic) PeriVentricular Leukomalacia (NC-PVL)

- Up to 50% according to imaging studies

- Probably most important reason for:
  - The high incidence of adverse cognitive dev. (25-50%)
  - The high incidence of cerebral paresis (5-10%)

Volpe JJ, Lancet 2009
Etiology of NC-PVL

Three (interrelated) causative factors important:

- Maternal / Fetal/Perinatal Inflammation
- Fetal Hypoxia-Ischemia /Reperfusion
- (Hyperoxia?)
Mechanisms of White matter Damage (I)

Activation of NMDA-receptor
Mechanisms of White matter Damage (II)

(Fetal) Hypoxia-Ischemia/inflammation/(hyperoxia?)

1. $\text{O}_2^\cdot$, $\text{H}_2\text{O}_2$

2. $\text{OH}^\cdot$

3. nNOS & iNOS activation

NOO$^-$/OH$^\cdot$

(Apotototic) Pre-OD death

Arrested "abnormal pre-OLs"

Neuronal-axonal diseasecc

Pro-inflammatory cytokines

Glial Cell activation
Neuroprotection with Magnesium

Inhibition of NMDA-receptor
Meta-analysis RCTs with Magnesium During Imminent Preterm Birth

**Figure 2**
Effect of magnesium sulfate on cerebral palsy

<table>
<thead>
<tr>
<th>Study</th>
<th>Relative risk (fixed) (95% CI)</th>
<th>Magnesium</th>
<th>Control</th>
<th>Weight (%)</th>
<th>Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mittendorf et al</td>
<td>6.77 (0.37-125.7)</td>
<td>3/30</td>
<td>0/29</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Crowther et al</td>
<td>0.13 (0.01-2.51)</td>
<td>0/55</td>
<td>3/51</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Magpie</td>
<td>0.85 (0.56-1.31)</td>
<td>36/629</td>
<td>42/626</td>
<td>27.7</td>
<td></td>
</tr>
<tr>
<td>Marret et al</td>
<td>0.66 (0.11-3.94)</td>
<td>2/404</td>
<td>3/401</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Rouse et al</td>
<td>0.70 (0.41-1.19)</td>
<td>22/352</td>
<td>30/336</td>
<td>20.2</td>
<td></td>
</tr>
<tr>
<td>Rouse et al</td>
<td>0.59 (0.40-0.85)</td>
<td>41/1188</td>
<td>74/1256</td>
<td>47.4</td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td>0.69 (0.55-0.88)</td>
<td>104/2658</td>
<td>152/2699</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Mechanisms of White matter Damage (III)

(Fetal) Hypoxia-Ischemia/inflammation/(hyperoxia?)

1. 

- $O_2^\cdot$, $H_2O_2$
- $OH^\cdot$
- Indomethacin

2. 

- nNOS & iNOS activation
- $\text{NOO}^-/OH^\cdot$

3. 

- Pro-inflammatory cytokines
- Glial Cell activation

(Apotototic) Pre-OD death

Arrested “abnormal pre-OLs

Neuronal-axonal disease

Indomethacin
Tocolytic indomethacin: effects on neonatal haemodynamics and cerebral autoregulation in the preterm newborn
However, no evidence for improved (or adverse) neuro-developmental outcome after tocolysis with Indomethacin for imminent preterm birth*

*Klauser CK et al J Matern Fetal neonatal Med 2012
*Ehsanipoor et al, Am J Perinatol 2011
“Pending” Therapies

- Melatonin
- Erythropoietin (postnatal)
- Caffeine (postnatal)
- (Mesenchymal) Stem Cells (postnatal)
Prevention/Reduction of severe IVH after Early Indomethacin Treatment

It has been assumed that Indomethacin decreases Germinal perfusion lowering the risk for severe IVH

Ballab P, Perinatol 2014
### Table II. Neonatal characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Time of indomethacin prophylaxis</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(&lt;6\ h (n = 730))</td>
<td>( &gt;6\ h (n = 138))</td>
</tr>
<tr>
<td>GA, weeks, mean ± SEM</td>
<td>26.3 ± 1.9</td>
<td>26.7 ± 2.3</td>
</tr>
<tr>
<td>Birth weight, g, mean ± SEM</td>
<td>861 ± 208</td>
<td>885 ± 225</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>363 (50)</td>
<td>68 (49)</td>
</tr>
<tr>
<td>White race, n (%)</td>
<td>623 (86)</td>
<td>120 (88)</td>
</tr>
<tr>
<td>Outborn, n (%)</td>
<td>25 (3)</td>
<td>28 (20)</td>
</tr>
<tr>
<td>5-minute Apgar score &lt;5, n (%)</td>
<td>102 (14)</td>
<td>21 (16)</td>
</tr>
<tr>
<td>Resuscitation in labor and delivery room, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intubation</td>
<td>527 (72)</td>
<td>105 (77)</td>
</tr>
<tr>
<td>Chest compressions</td>
<td>38 (5)</td>
<td>12 (9)</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>11 (2)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Respiratory support in the first 24 hours, n (%)</td>
<td>716 (98)</td>
<td>132 (96)</td>
</tr>
<tr>
<td>Surfactant use, n (%)</td>
<td>590 (81)</td>
<td>117 (85)</td>
</tr>
<tr>
<td>Pneumothorax, n (%)</td>
<td>39 (5)</td>
<td>14 (10)</td>
</tr>
<tr>
<td>Neonatal seizure, n (%)</td>
<td>30 (4)</td>
<td>8 (6)</td>
</tr>
</tbody>
</table>
Indomethacin <6 h reduces severe IVH incidence (II)

Mirza et al, J Pediatr 2013
Indomethacin reduces severe IVH incidence (II)

% Severe IVH

Severe IVH Risk

- Without Prophylactic Indomethacin
- With Prophylactic Indomethacin
### Short-term Outcome: PDA, IVH

#### Study or Subgroup

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Treatment Events</th>
<th>Control Events</th>
<th>Total</th>
<th>Weight</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mahony 1985</td>
<td>13</td>
<td>13</td>
<td>54</td>
<td>9.7%</td>
<td>1.04 [0.53, 2.03]</td>
<td>1985</td>
</tr>
<tr>
<td>Puckett 1985</td>
<td>3</td>
<td>3</td>
<td>16</td>
<td>2.3%</td>
<td>1.00 [0.24, 4.23]</td>
<td>1985</td>
</tr>
<tr>
<td>Ment 1985</td>
<td>1</td>
<td>4</td>
<td>24</td>
<td>3.1%</td>
<td>0.25 [0.03, 2.08]</td>
<td>1985</td>
</tr>
<tr>
<td>Rennie 1986a</td>
<td>5</td>
<td>8</td>
<td>24</td>
<td>5.9%</td>
<td>0.68 [0.26, 1.79]</td>
<td>1986</td>
</tr>
<tr>
<td>Krueger 1987</td>
<td>2</td>
<td>4</td>
<td>15</td>
<td>2.9%</td>
<td>0.57 [0.12, 2.67]</td>
<td>1987</td>
</tr>
<tr>
<td>Vincen 1987</td>
<td>3</td>
<td>2</td>
<td>15</td>
<td>1.5%</td>
<td>1.50 [0.29, 7.73]</td>
<td>1987</td>
</tr>
<tr>
<td>Gutierrez 1987</td>
<td>9</td>
<td>13</td>
<td>30</td>
<td>10.1%</td>
<td>0.67 [0.34, 1.32]</td>
<td>1987</td>
</tr>
<tr>
<td>Ment 1988</td>
<td>0</td>
<td>1</td>
<td>18</td>
<td>1.2%</td>
<td>0.30 [0.01, 6.91]</td>
<td>1988</td>
</tr>
<tr>
<td>Hanigan 1988</td>
<td>13</td>
<td>8</td>
<td>55</td>
<td>6.2%</td>
<td>1.60 [0.72, 3.55]</td>
<td>1988</td>
</tr>
<tr>
<td>Bandstra 1988</td>
<td>14</td>
<td>13</td>
<td>99</td>
<td>9.9%</td>
<td>1.09 [0.54, 2.19]</td>
<td>1988</td>
</tr>
<tr>
<td>Bada 1989</td>
<td>9</td>
<td>12</td>
<td>71</td>
<td>9.2%</td>
<td>0.74 [0.33, 1.64]</td>
<td>1989</td>
</tr>
<tr>
<td>Morales-Suarez 1994</td>
<td>8</td>
<td>14</td>
<td>40</td>
<td>10.7%</td>
<td>0.57 [0.27, 1.21]</td>
<td>1994</td>
</tr>
<tr>
<td>Ment 1994a</td>
<td>7</td>
<td>5</td>
<td>27</td>
<td>3.4%</td>
<td>1.76 [0.63, 4.94]</td>
<td>1994</td>
</tr>
<tr>
<td>Ment 1994b</td>
<td>16</td>
<td>29</td>
<td>209</td>
<td>21.4%</td>
<td>0.59 [0.33, 1.05]</td>
<td>1994</td>
</tr>
<tr>
<td>Couser 1996</td>
<td>1</td>
<td>1</td>
<td>43</td>
<td>0.7%</td>
<td>1.09 [0.07, 16.94]</td>
<td>1996</td>
</tr>
<tr>
<td>Yaseen 1997</td>
<td>1</td>
<td>1</td>
<td>13</td>
<td>0.8%</td>
<td>0.93 [0.06, 13.37]</td>
<td>1997</td>
</tr>
<tr>
<td>Supapannachart 1999</td>
<td>0</td>
<td>1</td>
<td>15</td>
<td>1.1%</td>
<td>0.33 [0.01, 7.58]</td>
<td>1999</td>
</tr>
</tbody>
</table>

**Total (95% CI):**

- **Treatment Events:** 771
- **Control Events:** 796
- **Total Events:** 105

**Total Weight:** 100.0%

**Risk Ratio M-H, Fixed, 95% CI:**

- **0.82 [0.65, 1.03]**

**Heterogeneity:**

- Chi² = 11.41, df = 16 (P = 0.78); I² = 0%

**Test for overall effect:** Z = 1.68 (P = 0.09)

---

*Fowlie et al, Cochrane 2010*
However, Long-term Neurodevelopmental Outcome not different between treated/non-treated preterms (I)

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Treatment</th>
<th>Control</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Total</td>
<td>8.2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Couser 1996</td>
<td>2</td>
<td>30</td>
<td>7</td>
<td>8.2%</td>
</tr>
<tr>
<td>Ment 1994b</td>
<td>13</td>
<td>166</td>
<td>14</td>
<td>18.4%</td>
</tr>
<tr>
<td>TIPP 2001</td>
<td>56</td>
<td>467</td>
<td>55</td>
<td>71.9%</td>
</tr>
<tr>
<td>Vincen 1987</td>
<td>5</td>
<td>15</td>
<td>1</td>
<td>1.5%</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>678</td>
<td>694</td>
<td>100.0%</td>
<td>1.04 [0.77, 1.48]</td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>77</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heterogeneity: Chi² = 3.76, df = 3 (P = 0.29); I² = 20%</td>
<td>Test for overall effect: Z = 0.23 (P = 0.82)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.4.2 Visual impairment

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Treatment</th>
<th>Control</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Total</td>
<td>12.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ment 1994b</td>
<td>1</td>
<td>170</td>
<td>1</td>
<td>12.7%</td>
</tr>
<tr>
<td>TIPP 2001</td>
<td>9</td>
<td>465</td>
<td>7</td>
<td>87.3%</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>635</td>
<td>639</td>
<td>100.0%</td>
<td>1.31 [0.49, 3.49]</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heterogeneity: Chi² = 0.04, df = 1 (P = 0.85); I² = 0%</td>
<td>Test for overall effect: Z = 0.50 (P = 0.62)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.4.3 Hearing impairment

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Treatment</th>
<th>Control</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Total</td>
<td>9.3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ment 1994b</td>
<td>1</td>
<td>170</td>
<td>1</td>
<td>9.3%</td>
</tr>
<tr>
<td>TIPP 2001</td>
<td>10</td>
<td>465</td>
<td>10</td>
<td>90.7%</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>626</td>
<td>633</td>
<td>100.0%</td>
<td>1.02 [0.45, 2.33]</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heterogeneity: Chi² = 0.00, df = 1 (P = 0.98); I² = 0%</td>
<td>Test for overall effect: Z = 0.04 (P = 0.97)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.4.4 Severe neurodevelopmental impairment

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Treatment</th>
<th>Control</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Total</td>
<td>10.3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bandstra 1998</td>
<td>14</td>
<td>78</td>
<td>15</td>
<td>10.3%</td>
</tr>
<tr>
<td>Ment 1994b</td>
<td>11</td>
<td>119</td>
<td>19</td>
<td>12.9%</td>
</tr>
<tr>
<td>TIPP 2001</td>
<td>118</td>
<td>444</td>
<td>117</td>
<td>76.8%</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>641</td>
<td>645</td>
<td>100.0%</td>
<td>1.04 [0.83, 1.29]</td>
</tr>
<tr>
<td></td>
<td>143</td>
<td>151</td>
<td></td>
<td>0.98 [0.79, 1.17]</td>
</tr>
<tr>
<td></td>
<td>Heterogeneity: Chi² = 2.33, df = 2 (P = 0.23); I² = 32%</td>
<td>Test for overall effect: Z = 0.40 (P = 0.69)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fowlie et al, Cochrane 2010
Antenatal Neuroprotection

- Very/extremely preterm newborn
- Term newborn
Perinatal Hypoxia-Ischemia (Birth Asphyxia):

- Clinical Outcome
  - Motor deficits (cerebral palsy)
  - Epilepsy
  - Mental retardation/ learning disabilities
  - Visual and Auditive problems
  - Behavioral problems

- Incidence: 4-9 per 1000 live births
- High mortality and morbidity (1 per 1000 live births)
Incidence of fetal hypoxia

- 10-20 fetuses per 1000 fetuses

Incidence of perinatal asphyxia

- 1-3 neonates per 1000 live births
Origin(s) of Perinatal Asphyxia

**Ante-perinatally (80%)**
- Umbilical cord compression
- Poor placental function
- Inadequate relaxation uterus/oxygenation placenta
- Early separation of placenta from uterus
- Inadequate maternal oxygenation
- Low maternal blood pressure

**Postnatally (20%)**
So, fetal hypoxia is important determinant of perinatal asphyxia and consequently of post-HI-encephalopathy.
Time profile of destructive pathways induced by fetal hypoxia-induced reoxygenation/reperfusion

- Superoxide FR formation
- Pro-radical formation (NPBI) $\rightarrow$ OH• FR
- Inflammation/cytokines / iNOS/Apoptosis
- Downregulation trophic factors

Fetal hypoxia-ischemia

Hypox-Xanthine

FREE-Iron

Ca$^{2+}$

Glutamate

pH

Birth

nNOS $\rightarrow$ NO$^-$ $\rightarrow$ ONOO$^-$

30 min 3h 6h 12h 24h days
Hypoxia-induced Free Radical Formation

Dirnagel U et al, J Cereb Blood flow Metab 1995
Anti-oxidant Therapies

• Vitamin C/E
• Noble gasses
• Allopurinol
• Deferoxamine
• Selective NOS-inhib
• N-Acetyl Cysteine (NAC)
• Erythropoietin (EPO)
• Lazaroids
• Edaravone
Xenon-inhalation

**Advantage**
- highly neuroprotective
- Anesthetic
- Xenon-inhalation mother

**Disadvantage**
- very costly
- Complex Ventilation set-up
Noble Gas Xenon

Inhibition of NMDA-receptor

Xenon
P7 Rats: Hypoxia-Ischemia and Xenon

Hobbs et al, Stroke 2008
Noble Gas Xenon

• Advantage
  - highly neuroprotective

• Disadvantages
  - very costly
  - Complex Ventilation set-up
ALLOPURINOL
Superoxide Formation upon reoxygenation

Severe Fetal Hypoxia

Hypoxanthine

Birth

Hypoxanthine + \( O_2 \)

Xanthin Oxidase (XO)

Superoxide!!

Xanthine + Uric acid

ALLOPURINOL
Maternal Allopurinol Therapy

• Experimental evidence

• Clinical evidence
Fetal sheep HI-model by Umbilical Cord Occlusion

Derks et al, Perinatal Center-Utrecht, Cambridge-UK
Acid-Fuchsin Staining

Loss of Brain Tissue

Damaged Neurons

Kaandorp et al, Pediatr Res 2012
Troponin (mcg/L)

Myocardial Damage

Kane et al, J Physiol 2014
Maternal Allopurinol Therapy

- Experimental evidence
- Clinical evidence
Allopurinol and Superoxide after Ischemia

Superoxide anion radical Prod.

Takeru Ono et al, Brain Research 2009
TimeProfile of ROS/Superoxide Formation

Re-oxygenation

Superoxide-prod

Fetal Hypoxia

- pH ↓
- FREE-Iron ↑
- Ca^{2+} ↑
- glutamate ↑

Free-iron (Fenton Reaction)

Treatment during Reperfusion-reoxygenation (Maternal Therapy)

30 min 3h 6h 12h 24h days
Clinical Randomized Controlled Trial during fetal hypoxia

2010-2011: Multicenter RCT with 500 mg Allopurinol or placebo iv to mother: n= 111/111

Results:
- No Adverse effects
Short-term outcome after maternal ALLO Biomarkers Brain Damage: S100B (pg/ml) /Neuroketal (pg/ml)

Kaandorp et al, accepted ADC Fetal & Neonatal ed
Long-term outcome after maternal ALLO

Currently 5 years outcome investigated:

- CBCL questionnaires
- ASQ questionnaires
Future

Allopurinol on reanimation table?
Optimal intervention strategy, a Dream?

(Maternal) early postnatal allopurinol*
Hypothermia
Anti-Oxidative Therapy/Anti-Inflammatory Therapy

* Gender effect?
Thank you for your attention
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

J Pediatr 2012