Probiotics in Neonatal NEC

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The speaker has disclosed that he is a consultant for Sigma Tau Pharmaceuticals and is on the Speaker Bureau for Mead Johnston Nutrition. These affiliations could be perceived as having a bearing on the subject matter of his presentation. He has no significant financial interest or relationship with any other companies or the manufacturer(s) of any commercial product and/or service that will be discussed as part of this presentation.

Session Summary

During this session the presenter will review the pathophysiology of NEC and how probiotics may influence the outcomes of this disease.

Session Objectives

Upon completion of this presentation, the participant will:

- understand the pathophysiology of NEC;
- appreciate the biologic mechanisms whereby probiotics might influence gut homeostasis;
- be able to discuss the clinical trial data on probiotics and NEC.

References


Session Outline

See presentation handout on the following pages.
Should probiotics be the standard of care for the prevention of NEC?

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As we consider potential efficacy for probiotics in NEC prevention......

- What is the pathophysiology of NEC?
- How might probiotics influence the pathogenesis of NEC?
- Do probiotics prevent NEC in preterm infants?
- Is there an acceptable safety profile with probiotic administration?

Necrotizing Enterocolitis

- Acute onset
- Inflammatory necrosis of intestine
- > 95% in preterm infants
- > 90% enterally fed
- Diagnosis confirmed by x-ray or surgery
- Breast milk only current standard of care for prevention or treatment

NEC PATHOGENESIS: pre-1990: Multifactorial Theory

- Prematurity
- Formula feeding
- Intestinal ischemia
- Bacterial colonization

NORMAL INTESTINAL ENVIRONMENT

Host defense

Mucosal injury

Prematurity

SIRS
My quest to understand NEC...

**PRESTIGE**

- **YOUR INTENDED CAREER PATH**
- **WHY YOU DRINK**
- **YOUR ACTUAL CAREER PATH**
- **TIME**
- **DEATH**

**PAF cycle**

- Phosphatidylcholine precursors
- LysoPAF
- Phospholipase A2
- Arachidonic acid
- PAF-AH
- PAF
- Acetyltransferase
- PAF receptor

**Plasma PAF and PAF-AH levels in VLBW NEC patients and controls**

Caplan et al, J Peds, 1990

**Serum PAF-AH activity at various ages**

Caplan et al, 1990

**Model for NEC pathogenesis: Role of PAF**

- Premature intestine
- Formula feeding
- Ischemia
- Too much PAF

- Increased PAF production (PLA2-II)
- Decreased degradation (PAF-AH)

- Increased PAFR expression
- Ischemia

- Increased TLR4 expression

- Toll-like receptor signaling

- Production of inflammatory mediators such as iNOS, IL-8 and TNFα

**Toll-like receptor signaling**

- EGF, TGF, EPO, IGF
- MyD88
- IRAK
- NFκB

- Production of inflammatory mediators such as iNOS, IL-8 and TNFα
Based on the available data, the best theoretical approach for the treatment of NEC would be:

A) Probiotic supplementation
B) High dose steroid dosing
C) TLR4 antagonists
D) A Peyton Manning touchdown pass to Wes Welker

Pro-inflammatory signaling outweighs Anti-inflammatory downregulation in preterm infants

Does the Intestinal Microbiome contribute to NEC pathogenesis?

Gut microbiota in term infants at 4 months of life: Azad et al, CMAJ 2013

Stool microflora in ELBW infants

- Paucity of bacterial species (< 3 at 10 days)
- Breast milk increases diversity
- Antibiotic exposure decreases number
- Only 1/29 colonized by Bifidobacteria or Lactobacilli
- ? Risk for overgrowth of pathogenic strains

Gewolb et al, Arch Dis Child 1999;80:F167
Microbiota in ELBW infants using DNA methodology

Jacquot et al, J Peds, 2011

Stool flora differs between NEC patients and GA-matched controls


Which of these statements is true?

• A) Carefully delineating the whole genome sequence of the gut microbial flora in preterm infants will identify the cause of NEC
• B) Bifidobacterium strains of bacteria reside in the phylum Actinobacteria while Lactobacilli are Firmicutes
• C) Bovine lactoferrin reduces the risk of NEC in carefully controlled clinical trials
• D) Chicago weather is better than Denver weather

NEC Pathophysiology (in the preterm infant)

Probiotics

• ‘Microbial strains of human origin, non-pathogenic, adherent to gut epithelium and colonizes intestinal tract, produces antimicrobial substances and modulates immune responses’
• Possibly beneficial in infectious and antibiotic-associated diarrhea, pouchitis, atopic dermatitis, colic, and irritable bowel syndrome
• Used throughout the world; rapidly growing market in the US
Effectiveness of Probiotics: are there rational biologic pathomechanisms?

- **LPS**
- **TLR4**
- **MyD88**
- **DNA-CpG**
- **TLR9**
- **IRAK**
- **IkBα**
- **NFκB**

Production of inflammatory mediators such as iNOS, IL-8 and TNFα.

Bacteriocins: secreted modulatory proteins

Crowding out of 'pathogens'

**Bifidobacterium infantis** in neonatal rat model of NEC

<table>
<thead>
<tr>
<th></th>
<th>NEC</th>
<th>LPS (EU/ml)</th>
<th>Mucosal permeability</th>
<th>PLA2-II (mol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19/27</td>
<td>191 ± 31</td>
<td>0.55 ± 0.23</td>
<td>802 ± 320</td>
</tr>
<tr>
<td>Probiotic</td>
<td>7/24*</td>
<td>21 ± 3*</td>
<td>0.46 ± 0.13</td>
<td>42 ± 29*</td>
</tr>
</tbody>
</table>

* p < 0.01

Caplan et al, Gastroenterol 1999

Probiotic effects in experimental NEC animal models

- Gnotobiotic quails (lactase deficient) fed lactose and challenged with *Clostridia* species and/or *Bifidobacteria* species
- Develop cecal inflammatory necrosis with *Clostridia: Bifidobacteria* decreased colonization by pathogens, prevented cecal injury, and decreased butyric acid fermentation from lactose


Do these prevent NEC?


<table>
<thead>
<tr>
<th>Study or Reference</th>
<th>Probiotics</th>
<th>Control</th>
<th>Relative Risk</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birthweight g, mean (SD)</td>
<td>1063 (259)</td>
<td>1048 (260)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Definite late onset sepsis n(%)</td>
<td>72 (13.1)</td>
<td>89 (16.2)</td>
<td>.46 (1.59)</td>
<td>16</td>
</tr>
<tr>
<td>NEC (stage II or III) n(%)</td>
<td>11 (2.0)</td>
<td>24 (4.4)</td>
<td>.46 (2.39)</td>
<td>23</td>
</tr>
<tr>
<td>Death n(%)</td>
<td>27 (4.9)</td>
<td>28 (5.1)</td>
<td>.97 (.58-1.67)</td>
<td>91</td>
</tr>
</tbody>
</table>

Jacobs et al, Pediatrics Dec, 2013

Answered Question:
Effect size significant; probiotics reduce risk of NEC

Unanswered Questions:
- Safety in large study with long-term flu?
- Best strain(s), species combination, dose?
- Are our populations the same as the meta-analyses?
- Effect on infection and mortality?
- Do meta-analyses predict large RCT results?
- Appropriate quality control of available product?

Do neonatologists have equipoise?

Safety of Probiotics in Neonates
- No cases of probiotic sepsis in previous prevention trials, but cases reported in immunocompromised patients, and recently premature infants: anaerobic culture techniques not done in most published studies
- Accuracy and quality control of the formulation requires consideration: 69% of U.S. preparations have contaminants and/or do not contain quantity of organisms (Drago et al, J Chemother, 2011)
- Unexpected outcomes in adults and children
Probiotics given during infancy associated with increased respiratory disease

At 2 years, Kopp et al, 2008
At 7 years, Kalliomaki et al, 2007

Probiotic prophylaxis in adult ICU patients with pancreatitis: 298 patients total, no difference in infectious complications, but...


Conclusions

* Pathophysiology of NEC complex; evidence suggests unbalanced pro-inflammatory response and altered intestinal microbiome
* Probiotics reduce NEC in clinical trials of preterm infants and influence disease by multiple mechanisms
* Additional studies and regulatory approval suggested before probiotics become standard-of-care