Role of Phytosterols and LPS in the Pathogenesis of Parenteral Nutrition Associated Liver Injury in Mice with Intestinal Inflammation

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Parenteral Nutrition Associated Liver Disease (PNALD)

- PNALD develops in PN dependent infants:
  - preterm
  - intestinal failure, SBS

- PNALD is the leading indication for intestinal/ multi-visceral transplant because of cirrhosis and its complications

- PNALD Cholestasis associated with 26 fold relative risk for death in infants with SBS
Pathogenesis of PNALD

- Lack of stimulation of bile flow, immaturity
- Small intestinal bacterial overgrowth
- Absorption of endotoxin – inhibits hepatocellular bile secretion
- Inflammatory cytokines – gut or liver derived
- Oxidative stress & bile acid toxicity
- Absence of essential nutrient(s)
- Toxin(s) in TPN solution or lipid emulsion
- Plant sterols in lipid emulsion
Overarching Hypothesis

Infants have a propensity for cholestasis because of immaturity of bile acid transport systems

Soy lipid based PN

Bile Acid Transporter Expression

Phytosterols

ω6FA

KC

LPS

IL6, TNF, etc.

Bacterial Overgrowth

Portal vein

Hypomotility

Underlying Inflammation

Short bowel syndrome, NEC, Intestinal failure

Permeability

Hepatic Injury & Fibrosis

Activation + recruitment of inflammatory cells

Inflammation

NPO

PN

ω6FA

Hypomotility

Bacterial Overgrowth
PNALD Mouse Model

Induce intestinal inflammation with 2.5% DSS in water x 4 days

Dextran sulphate sodium

i.v. PN+Intralipid
Lipid dose (1.4 g/kg/day)

7 days Sacrifice

AST, ALT, Bile acids, Bilirubin
qRT-PCR (liver)
qRT-PCR isolated and purified KC

El Kasmi et al. 2012, Hepatology
Increased Portal Vein LPS Associated with Increased Intestinal Permeability and PN

Portal vein LPS

Portal vein LPS [EU/L]

- chow
- 4d DSS
  - 1d chow
  - 8d chow
- 4d DSS
- 7d PN (no chow)

*p<0.05
One-Way ANOVA

El Kasmi et al. 2012, Hepatology
PNALD after Combining PN Infusion with Intestinal Injury

**ALT**

![ALT Graph]

**Bile Acids**

![Bile Acids Graph]

* p< 0.05 One-Way ANOVA

El Kasmi et al. 2012, Hepatology
**PNALD in mice is associated with Kupffer cell Activation**

*Il6*

*El Kasmi et al. 2012, Hepatology*

*Similar results for TNFα and TGFβ*


*p< 0.05 One-Way ANOVA*

*El Kasmi et al. 2012, Hepatology*
PNALD and KC activation are attenuated after interruption of TLR4 signaling or suppression of intestinal bacteria.

*pn< 0.05 One-Way ANOVA
In this model both Intestinal Injury and PN are necessary but not sufficient to induce liver injury, cholestasis

- PNALD is associated with KC activation

- KC activation and PNALD depend on LPS-TLR4 signaling and presence of intestinal bacteria
Reversal of PNALD in PN-Dependent Infants with Intestinal Failure/SBS by Omegaven (fish-oil/omega-3-fatty acids)

Lipid Reduction in PN solutions also results in reduced Bilirubin

**Graph:**
- **Control**
- **Lipid reduction**

**Intralipid:**
1 g/kg/d/2x week instead of 3 g/kg/d

# Comparison of Lipid Emulsions

<table>
<thead>
<tr>
<th>Per 10g/100ml</th>
<th>Intralipid&lt;sup&gt;R&lt;/sup&gt;</th>
<th>Omegaven&lt;sup&gt;R&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy bean Oil</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Fish Oil</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Phytosterols mg/L</strong></td>
<td><strong>348</strong></td>
<td>0</td>
</tr>
<tr>
<td>Fatty acids in g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
<td>1</td>
<td>0.25-1</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>0.4</td>
<td>0.05-0.2</td>
</tr>
<tr>
<td>Oleic (18:1)</td>
<td>2.6</td>
<td>0.6-1.3</td>
</tr>
<tr>
<td><strong>Linoleic (18:2)</strong></td>
<td><strong>5</strong></td>
<td>0.1-0.7</td>
</tr>
<tr>
<td>α-Linoleic (18:3)</td>
<td>0.9</td>
<td><strong>0.2</strong></td>
</tr>
<tr>
<td>EPA (20:5)</td>
<td>0</td>
<td>1.28-2.82</td>
</tr>
<tr>
<td>DHA (22:5)</td>
<td>0</td>
<td>1.44-3.09</td>
</tr>
</tbody>
</table>

*Data as provided by manufacturers*
Based on these clinical observations we asked what component(s) of the PN solution promotes liver injury

Translation of these clinical observations back into the mouse model
**PNALD Mouse Model**

- **Central venous catheter in jugular vein**

- **Induce intestinal inflammation with 2.5% DSS in water x 4 days**

- sacrificed

- **PNALD Mouse Model**

  - a) PN+Intralipid
  - b) PN+Omegaven
  - c) PN without lipid (iso-caloric to Intralipid and Omegaven)

  - Equal Lipid dose (1.4 g/kg/day);

- **7 days** → **Sacrifice** → **AST, ALT, Bile acids, Bilirubin**

- qRT-PCR (liver and isolated KCs)
Omegaven Prevents PNALD

**ALT [U/L]**

* * * * - - - 
- - - - NS 
PN Intra-lipid 
PN Omegaven

**Total Serum Bile Acids [µmol/L]**

* * * * - - - 
- - - - NS 
PN Intra-lipid 
PN Omegaven

*p<0.05 compared to controls and compared to PN-Omegaven. One-Way ANOVA*
Removal of all lipids also prevents PNALD

**ALT [U/L]**

- PN Intra-lipid
- PN Ome-gaven
- PN w/o lipid

**Total Serum Bile Acids [µmol/L]**

- PN Intra-lipid
- PN Ome-gaven
- PN w/o lipid
Omegaven and Lipid removal
Attenuated KC activation

Relative Kupffer cell mRNA levels

Chow          DSS           i.v.          PN intra-lipid          PN Omegaven          PN w/o lipid

**Il6**

* p < 0.05

NS: Not significant
Similar effects of Omegaven and Lipid removal on attenuation/prevention of PNALD suggested that in Omegaven a “toxic” component that was present in Intralipid was removed rather than a “protective” component added.

As the most likely factor plant sterols (Phytosterols) were considered.

Intralipid 20% contains Stigmasterol (28mg/L), Campasterol (26mg/L), Sitosterol 108mg/L

We chose to study stigmasterol because of the data reported by Dr. Karpen that show stigmasterol as the most potent sterol in suppressing BSEP expression in primary hepatocytes (Carter et al, Ped. Res. 2007)
**PNALD Mouse Model**

- Induce intestinal inflammation with 2.5% DSS in water x 4 days

- Dextran sulphate sodium

- **Central venous catheter in jugular vein**

- 7 days

- **Sacrifice**

- AST, ALT, Bile acids, Bilirubin

- qRT-PCR (liver and isolated KCs)

- a) PN+Omegaven + 2mg/100ml Stigmasterol
  (conc. reflecting Stigmasterol conc. in PN-Intralipid infused mice)

- b) PN+Omegaven + 6mg/100ml Stigmasterol
  (conc. reflecting Stigmasterol conc. in PN-Intralipid infused infants)
Stigmasterol spiked PN solution Promotes Liver Injury

**AST [U/L]**

- Chow: + + + +
- DSS: - + + +
- i.v.: NS

**ALT [U/L]**

- Chow: + + + +
- DSS: - + + +
- i.v.: NS

* ns

**Note:**

- PN Intra-lipid
- PN Omegaven
- PN Omegaven 2mg Stig
- PN Omegaven 6mg Stig

Promotes Liver Injury
Stigmasterol containing PN solution Promotes Cholestasis

Total Serum Bilirubin [mg/dl]

Total Serum Bile Acids [µmol/L]
Phytosterol - PN solutions
Suppress Hepatic Abcb11 (BSEP)

Similar results for Abcc2 (MRP2) mRNA expression
Phytosterol containing PN solutions Suppress FXR

Liver Fxr mRNA expression

Similar results for LXR mRNA expression
Stigmasterol Added to PN+ Omegaven Promotes Kupffer Cell Activation

Relative Kupffer cell mRNA levels

Chow + + + -
DSS - + + +
i.v. - - NS -

PN Intra-lipid PN Omegaven PN w/o lipid PN Omegaven 6mg Stig

* Il6

* NS

*
Provisional Working Model for the Pathogenesis of PNALI

1) DSS → LPS/MAMPS → TLR4 dep. KC activation → \( \uparrow \) cytokines → Hepatocyte Injury Apoptosis Inflammation

2) Phytosterols Stigmasterol → \( \downarrow \) BSEP/MRP2 → \( \downarrow \) G5/G8 → Cholestasis Accumulation of Phytosterols
Credits

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