Aspartate a New Treatment Modality in the treatment of Acute Liver Failure and Acute Pancreatitis

Ahmad Farooq, M.D
Medical Resident,
University at Buffalo,
Catholic Health System.
Tissue Injury Triggers Innate Immune Responses

Injured Parenchymal Cell \(\rightarrow\) DAMP Sensing Cell \(\rightarrow\) Neighboring Cell

Signal 1

DAMPs

TLRs

Pro-IL-1β, Pro-IL-18

Nlrp3

Caspase-1

ASC

ProCaspase-1

IL-1β, IL-18

independent components
Pancreas

Impact of toll-like receptor 4 on the severity of acute pancreatitis and pancreatitis-associated lung injury in mice

R Sharrir¹, R Dawra¹, K Wasliuk¹, P Phillips¹, V Dudeja¹, E Kurt-Jones², R Finberg², A Saluja¹


TLR9 and the NLRP3 inflammasome link acinar cell death with inflammation in acute pancreatitis.


Role of Kupffer cells and toll-like receptor 4 in acetaminophen-induced acute liver failure.

Lactated Ringer’s solution reduces systemic inflammation compared with saline in patients with acute pancreatitis.

Wu BU¹, Hwang JQ, Gardner TH, Repas K, Delee R, Yu S, Smith B, Banks PA, Conwell DL.
Lactate Reduces Liver and Pancreatic Injury in Toll-Like Receptor– and Inflammasome-Mediated Inflammation via GPR81-Mediated Suppression of Innate Immunity

Rafaz Hoque,1 Ahmad Farooq,1 Ayaz Ghani,1 Fred Gorelick,1,2 and Wajahat Zafar Mehal1,2

1Section of Digestive Diseases, Yale University, New Haven, Connecticut; 2Section of Digestive Diseases, Department of Veterans Affairs Connecticut Healthcare, West Haven, Connecticut
**Protreatment with Lactate**

### Liver expression

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pro-IL1β</th>
<th>Nlrp3</th>
<th>Casp1</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS + GalN + PBS</td>
<td><img src="chart1" alt="Graph" /></td>
<td><img src="chart2" alt="Graph" /></td>
<td><img src="chart3" alt="Graph" /></td>
</tr>
<tr>
<td>LPS + GalN + Lactate</td>
<td><img src="chart4" alt="Graph" /></td>
<td><img src="chart5" alt="Graph" /></td>
<td><img src="chart6" alt="Graph" /></td>
</tr>
</tbody>
</table>

### Serum ALT

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS + GalN + PBS</td>
<td>6000 IU/mL</td>
</tr>
<tr>
<td>LPS + GalN + Lactate</td>
<td>2000 IU/mL</td>
</tr>
</tbody>
</table>

**Post-treatment with Lactate**

### Liver histology score

- **Apoptosis**
  - LPS/GalN + Saline: ![Graph](chart7)
  - LPS/GalN + Lactate: ![Graph](chart8)
- **Hemorrhage**
  - LPS/GalN + Saline: ![Graph](chart9)
  - LPS/GalN + Lactate: ![Graph](chart10)

### Serum AL1β

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS/GalN + Saline</td>
<td>100 pg/mL</td>
</tr>
<tr>
<td>LPS/GalN + Lactate</td>
<td>50 pg/mL</td>
</tr>
</tbody>
</table>

### Liver IL1β

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS/GalN + Saline</td>
<td>500 pg/mg tissue</td>
</tr>
<tr>
<td>LPS/GalN + Lactate</td>
<td>250 pg/mg tissue</td>
</tr>
</tbody>
</table>
Post-treatment with Lactate

C  LPS/Caerulein + Saline  LPS/Caerulein + Lactate

20X H&E Stain

Histology score

Pancreatic histology scoring

NS  *  *

Edema  Inflammation  Necrosis

- LPS/Caerulein + Saline
- LPS/Caerulein + Lactate

D  Serum amylase

Pancreatic trypsin activity

Normalized units

E  Pancreatic caspase 1 activity

Normalized units

F  Pancreatic myeloperoxidase activity

Normalized units
What About Amino Acids and Fatty acids?
Activation of the \textit{N-methyl-d-aspartate} receptor by aspartic acid downregulates inflammasome activity and liver inflammation via a \textit{b-arrestin-2} pathway.
Step 1: Gene Transcription of pro-IL-1β, Nlrp3, CASP1

Step 2: Cleavage of CASP1 and IL-1β release
Aspartate downregulates inflammasome components and IL-1β in mouse macrophages

(A) Relative Expression Pro IL 1β

(B) Relative Expression Nlrp3

(C) Relative Expression Pro caspase 1

(D) Supernatant IL 1β

(E) Western Blot

- Caspase1 P10
- Beta-actin
Mouse Kupffer cells

A

Pro IL 1 β

B

Nlrp3

C

Pro caspase-1

D

Supernatant IL 1β

E

Pro IL 1 β

F

Nlrp3

G

Pro caspase 1

Human Peripheral Blood Mononuclear cells
In vivo aspartate supplementation reduces hepatic inflammasome levels and protects from acute inflammatory liver injury.
LPS/Gal + DPBS

LPS/Gal + Asp

Hemorrhage

Serum ALT

Histology Score

Serum ALT

0 5,000 10,000 15,000

LPS/Gal+ DPBS LPS/Gal+ Asp

* **
In vivo aspartate supplementation reduces pancreas inflammasome levels and protects from caerulein induced pancreatitis
*In vivo aspartate supplementation reduces liver inflammasome levels and protects from acetaminophen induced liver failure.*

**Pro IL 1β**

**Pro caspase-1**

**Nlrp3**

**Serum ALT**
APAP+ DPBS

APAP + Asp

Hemorrhage

Necrosis

Histology Score

10x

0

1

2

3

4

APAP+ DPBS   APAP+ Asp

0

1.5

3

3

1.5

0

APAP+ DPBS   APAP+ Asp

***

***
Aspartate mediated suppression of TLR4 signaling requires the plasma membrane receptor NMDA, and ARRB2

A. Nr2a Expression

B. Nr2a Expression

C. Pro IL 1β

D. Arrb2 Expression

E. Pro IL 1β

F. P Ikkβ

β-actin
**β-arrestin-2 induced immune regulation is providing a significant degree of down-regulation of the inflammatory response, and supplementation with aspartate acid can further increase this protective effect**

(A) Arrb2 Expression

(B) Arrb2 Expression

(C) Pro IL 1β

(D) Serum IL 1β

(E) siRNA Scramble vs siRNA ArrB2

(F) Serum ALT
Conclusion

• Therapeutic potential of aspartate mediated NMDA signaling in limiting TLR4 driven inflammation in acute pancreatitis.
• NMDA as a novel target and highlight selective NMDA agonists and antagonist in clinical use in the treatment of acute inflammation
• Ethanol has been proposed to be a non-competitive antagonist of the NMDA receptor in neuron. If the same effect is present in macrophages it will result in loss of NMDA induced down regulation of inflammasome activity and may provide another mechanism of the hepatic immune dysregulation induced by ethanol in alcoholic pancreatitis.
• In addition use of TPN enriched with glutamate and aspartate has been shown to be associated with reduced inflammation in inflammatory bowel disease.
• Of great interest is the further possibility that the general immunosuppression seen with TPN may be due to activation of this pathway.
Thank you
Dr H Woodman
Dr Khalid Qazi
Dr Mehal
Dr Gorelick
Dr Hoque
macrophage

LPS

BAFF

BAFF receptor

IKK

ADP

ATP

ubiquitination

proteasome

nuclear localization signal

nucleus