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

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Tissue Injury Triggers Innate Immune Responses

Signal 1

DAMPS

TLRs

Nlrp3

Caspase-1

ASC

ProCaspase-1

Pro-IL-1β Pro-IL-18

IL-1β IL-18

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 Sharrt¹, 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.
BASIC & TRANSLATIONAL—LIVER & PANCREAS

Lactate Reduces Liver and Pancreatic Injury in Toll-Like Receptor-- and Inflammasome-Mediated Inflammation via GPR81-Mediated Suppression of Innate Immunity

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Murine peritoneal macrophages

A
Lactate

Lactate concentration:
- Untreated
- LPS

B
LPS induced Pro-IL1β

Relative expression:
- pH 6.80
- pH 7.40

C
Pro-IL1β, Nlrp3, Casp1, Il10, Pro-IL18

Relative expression:
- LPS
- LPS + Lactate 15 mM

Significance:
- * denotes statistical significance
- NS denotes no significant difference
Pretreatment with Lactate

A. Liver expression

- **Pro-IL1β**
  - LPS + GaIN + PBS
  - LPS + GaIN + Lactate

- **Nlrp3**
  - LPS + GaIN + PBS
  - LPS + GaIN + Lactate

- **Casp1**
  - LPS + GaIN + PBS
  - LPS + GaIN + Lactate

B. Serum ALT

- LPS + GaIN + PBS
- LPS + GaIN + Lactate

C. Liver histology score

- 10X H&E Stain
  - LPS/GaIN + PBS
  - LPS/GaIN + Lactate

Post-treatment with Lactate

D. Liver histology score

- 10X H&E Stain
  - LPS/GaIN + Saline
  - LPS/GaIN + Lactate

- 20X H&E Stain
  - LPS/GaIN + Saline
  - LPS/GaIN + Lactate

E. Serum ALT

- LPS/GaIN + Saline
- LPS/GaIN + Lactate

F. Serum IL1β

- LPS/GaIN + Saline
- LPS/GaIN + Lactate

G. Liver IL1β

- LPS/GaIN + Saline
- LPS/GaIN + Lactate
Post-treatment with Lactate

C

20X H&E Stain

Pancreatic histology scoring

NS * *

Edema Inflammation Necrosis

Histology score

LPS/Caerulein + Saline LPS/Caerulein + Lactate

D

Serum amylase

Pancreatic trypsin activity

E

Pancreatic caspase 1 activity

F

Pancreatic myeloperoxidase activity

Normalized units

Normalized units

Untreated LPS/Caerulein LPS/Caerulein + Saline LPS/Caerulein + Lactate

Untreated LPS/Caerulein LPS/Caerulein + Saline LPS/Caerulein + Lactate
What About Amino Acids and Fatty acids?
Activation of the $N$-methyl-$d$-aspartate receptor by aspartic acid downregulates inflammasome activity and liver inflammation via a 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-1b in mouse macrophages

A) Pro IL 1β

B) Nlrp3

C) Pro caspase 1

D) Supernatant IL 1β

E) Caspase1 Western Blot
Mouse Kupffer cells

A. **Pro IL 1β**

B. Nlrp3

C. **Pro caspase-1**

D. Supernatant IL 1β

Human Peripheral Blood Mononuclear cells

E. **Pro IL 1β**

F. Nlrp3

G. **Pro caspase 1**
In vivo aspartate supplementation reduces hepatic inflammasome levels and protects from acute inflammatory liver injury.
Hemorrhage

Serum ALT

LPS/Gal + DPBS

LPS/Gal + Asp

Hemorrhage Score

Serum ALT

LPS/Gal+ DPBS  LPS/Gal +Asp

LPS/Gal+ Asp

0  1.8  3.6

Histology Score

IU/L

0  5,000  10,000  15,000

Statistical Analysis:

** p < 0.01

* p < 0.05
In vivo aspartate supplementation reduces pancreas inflammasome levels and protects from caerulein induced pancreatitis

**Serum Amylase**

![Bar chart showing Serum Amylase levels for different treatments.](chart)

**Pro IL 1β**

![Bar chart showing Pro IL 1β expression for different treatments.](chart)

**Nlrp3**

![Bar chart showing Nlrp3 expression for different treatments.](chart)

**Pro caspase 1**

![Bar chart showing Pro caspase 1 expression for different treatments.](chart)
**In vivo aspartate supplementation reduces liver inflammasome levels and protects from acetaminophen induced liver failure.**

**Pro IL 1β**

![Graph showing relative expression of Pro IL 1β](image)

**Nlrp3**

![Graph showing relative expression of Nlrp3](image)

**Pro caspase-1**

![Graph showing relative expression of Pro caspase-1](image)

**Serum ALT**

![Graph showing serum ALT](image)
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

Protein Levels

G
\(\beta\)-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.
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
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