Hepatic Encephalopathy in acute liver failure:
a primary neurogliopathy

Roger F. Butterworth, Ph.D., D.Sc.

Classification of hepatic encephalopathy (HE)

- Type A - Associated with acute liver failure
- Type B - Associated with portosystemic bypass
  with no intrinsic hepatocellular disease (rare)
- Type C - Associated with cirrhosis
  and portal hypertension

Acute liver failure (ALF)

- Causes: viral hepatitis, drug toxicity, Wilson’s disease
- Symptoms: disorientation, stupor, coma
- Rapidly progressing course (days to weeks)
- High mortality (30%-50%)
- The presence of hepatic encephalopathy defines severe stage
  of ALF, and heralds listing for liver transplantation
- Brain edema leading to intracranial hypertension/brain herniation
  remains a major cause of mortality in ALF worldwide
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Neuropathology of ALF

- Astrocytic swelling leading to cytotoxic brain edema, intracranial hypertension and brain herniation
- Microglial activation, neuroinflammation
- Blood-brain barrier (BBB) breakdown when infection/sepsis present

Neuropathology of ALF (2)

- Astrocytic swelling leading to cytotoxic brain edema, intracranial hypertension and brain herniation

Microglial activation and neuroinflammation in ALF (Hepatic devascularized rat model)

SHAM

ALF-COMA

Cortex
Hippocampus
Thalamus

Kato et al., Hepatology, 1992 15(6)
Jiang et al., J Neurochem, 2009 109(2): 489

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Blood-brain barrier (BBB) breakdown occurs in ALF when infection/sepsis (LPS) present

Pathogenesis of HE in ALF: key role of ammonia

- Ammonia produced in the gut, removed by the liver as urea or glutamine
- Arterial ammonia predicts brain herniation in ALF
- Arterial ammonia independent risk factor for intracranial hypertension in ALF
- Ammonia removal by brain: a neuroglial responsibility

Ammonia produced in the gut, removed by the liver as urea or glutamine
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**Arterial ammonia predicts brain herniation in ALF**

Kaplan-Meier plot of ICH in 165 patients with ALF according to the arterial ammonia concentration on admission; For the log-rank test, $P < 0.01$

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**Arterial ammonia: an independent risk factor for intracranial hypertension in ALF**

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**Ammonia removal by brain: a neuroglial responsibility**

- Brain ammonia as high as 5mM reported in ALF
- No urea cycle in brain; removal via glutamine synthetase (GS)
- GS: predominantly, if not exclusively, astrocytic

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Ammonia removal by brain

- No urea cycle
- Glutamine synthetase (GS), the major system involved

\[
\begin{align*}
\text{Glutamate} & \xrightarrow{\text{GS}} \text{Glutamine} \\
& \xrightarrow{\text{ATP}} \text{NH}_3
\end{align*}
\]
- GS uniquely astrocytic

Selective alterations in expression of genes coding for neuroglial proteins in ALF

- Decreased: - GFAP (astrocytes)
  - Glutamate transporter (astrocytes) EAAT-2
- Increased: - Pro-inflammatory cytokines (TNF-\(\alpha\), IL-1\(\beta\), IL-6)

Glial fibrillary acidic protein (GFAP)

- 50 kDa protein
- Astrocyte marker protein
- Principal constituent of neurofilaments (astrocytes)
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Loss of GFAP mRNA in brain in ALF


Negative correlation between GFAP expression and brain edema in ALF

r² = 0.9954

Glutamate transporter EAAT-2

- Formerly GLT-1
- Major high-affinity high-capacity glutamate transporter in forebrain
- Primarily astrocytic localization

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Glutamate transporters in forebrain

Decreased EAAT-2 mRNA and protein in brain in ALF

Consequence of loss of EAAT-2 expression
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Extracellular brain glutamate in relation to neurological status in ALF

Systemic inflammation in ALF
- Sources: infection, hepatocyte cell death
- Systemic Inflammatory Response Syndrome (SIRS) worsens HE and brain edema in ALF
- Increased plasma pro-inflammatory cytokines (TNFα, IL-1β, IL-6) reported in ALF

Neuroinflammation in ALF
- Two models:
  1) Hepatic devascularized rat (ischemic liver failure)
  2) Azoxymethane mouse (toxic liver injury)
- Microglial activation by OX-6 mRNA and OX-42 immunohistochemistry
- Brain cytokines by ELISA, cytokine mRNA's by RT-PCR
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Microglial activation and neuroinflammation in ALF
(Hepatic devascularized rat model)

Brain cytokines in ALF
(Hepatic devascularized rat model)

Brain cytokine gene expression in ALF
(Hepatic devascularized rat model)

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Brain cytokine gene expression in ALF (AOM mouse model)

Therapeutic implications

Do treatments currently used to manage ALF act on neuroinflammatory mechanisms?

- Mild hypothermia
- N-Acetylcysteine
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Mild hypothermia limits microglial activation in experimental ALF

Mild hypothermia reduces brain cytokines in experimental ALF (Hepatic devascularized rat model)

Mild hypothermia attenuates brain cytokine gene expression in ALF (Hepatic devascularized rat model)

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Minocycline

- Antibiotic
- Crosses blood-brain barrier
- Potent inhibitor of microglial activation

Brain edema in ALF: protective effect of minocycline

Jiang et al., J Neurochem, 2009 109(2): 488
Take home messages

1. HE in acute liver failure is characterized neuropathologically by alterations in neuroglial function that include:
   - Astrocyte swelling
   - Microglial activation
   - BBB disruption (if infection/sepsis are present)
2. Ammonia toxicity remains a major cause of HE in ALF
   - Arterial ammonia predicts brain edema/intracranial hypertension
   - Removal of excess ammonia by brain relies on the astrocyte
3. Neuroinflammation occurs in ALF characterized by:
   - Microglial activation
   - Increased expression of genes coding for proinflammatory cytokines
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Take home messages (2)

4. ALF causes alterations in expression of genes coding for other neuroglial proteins including:
   • Loss of GFAP that correlates with brain edema
   • Down-regulation of the glutamate transporter EAAT-2 leading to increased extracellular brain glutamate

5. Novel therapeutic opportunities for HE in ALF currently under evaluation include:
   • Mild hypothermia
   • N-Acetyl cysteine (antioxidant)
   • Minocycline (antibiotic with anti-microbial activation properties)
   • Etanercept (anti-TNFα)

6. The above treatments act by prevention of neuroglial responses

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