Central Role for CD68(+) Macrophages in the Etiology of Hepatopulmonary Syndrome (HPS): Reversal by Macrophage Depletion

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Background

- Hepatopulmonary syndrome (HPS) is defined as the triad of hypoxemia, intrapulmonary shunting, and liver disease
- HPS occurs in 5-32% of cirrhotic patients
- The pulmonary circulation in HPS is characterized by the presence of excessive vasodilatation and A-V malformations
- The pathogenesis of HPS is unclear, and currently, there are no effective medical therapies, but liver transplantation is curative
- HPS (high CO and low PVR) can transition to portopulmonary hypertension (low CO and high PVR) following liver transplantation. The basis for this transition is unknown

Rationale

- Common bile duct ligation (CBDL) is an accepted animal model of HPS. HPS occurs in virtually all rats within 2 weeks.
- Prior research in this model has identified increased accumulation of intrapulmonary macrophages and increased levels of both iNOS and VEGF, and has shown benefit from inhibiting NOS and VEGF
- However, the relationship between the macrophage and HPS is unclear, and no prior study has directly targeted the macrophage to determine their role in HPS

Disclosure

I have no financial disclosures

References


Nunes H et al. Am J Respir Crit Care Med 2001;164:979-85
Qian BZ et al. Cell;141:39-51
Hypotheses

• Accumulation of activated pulmonary intravascular macrophage is central to the pathogenesis of HPS because these macrophages are a source of vasodilatory, angiogenic, and proliferative growth factors (e.g. iNOS, VEGF, and PDGF)
• Depletion of pulmonary intravascular macrophages can prevent or reverse HPS

Methods

• Adult, male Sprague Dawley rats
• CBDL vs. Sham surgery on Day 1 (n = 21 per group)
• Animals were studied on Day 28
• Statistics: Values are mean±SEM, ANOVA with Bonferroni post hoc testing, and p<0.05 was considered significant

2 Protocols For Macrophage Depletion Using Agents That Induce Macrophage Apoptosis

• Prevention: Gadolinium Chloride (GdCl₃)
• Regression: Liposomal Clodronate

CBDL Recapitulates Human HPS

P<0.05
Activated CD68(+) Macrophages Accumulate in PA’s

Endotoxemia induces Lung Macrophage Accumulation

Macrophages secrete iNOS, VEGF, and PDGF

HPS Plasma Increases Endothelial Tube Formation and PASMC Proliferation
Macrophage Depletion Prevents and Regresses HPS Histology

**CD68(+) cells**

- Sham
- CBDL
- CBDL + GdCl
- CBDL + Clodronate

**CD31**

- Sham
- CBDL
- CBDL + GdCl
- CBDL + Clodronate

**PCNA(+) cells**

- Sham
- CBDL
- CBDL + GdCl
- CBDL + Clodronate

**Phosphorylation of ERK-1**

- Sham
- CBDL
- CBDL + GdCl
- CBDL + Clodronate

**A-a Gradient**

- Sham
- CBDL
- CBDL + GdCl
- CBDL + Clodronate

**Cardiac Output**

- Sham
- CBDL
- CBDL + GdCl
- CBDL + Clodronate

**PVR**

- Sham
- CBDL
- CBDL + GdCl
- CBDL + Clodronate

**MPAP**

- Sham
- CBDL
- CBDL + GdCl
- CBDL + Clodronate

**Occult Proliferative Vasculopathy in HPS Lungs**

- Sham
- CBDL
- CBDL + GdCl
- CBDL + Clodronate
Depletion of Macrophage Prevents and Regresses Proliferative Vasculopathy

Clinical Characteristics of Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Gender</th>
<th>Diagnosis</th>
<th>(A-a) O2 Gradient</th>
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<tr>
<td>1</td>
<td>46</td>
<td>Female</td>
<td>Autoimmune hepatitis</td>
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<td>2</td>
<td>59</td>
<td>Male</td>
<td>Hepatitis C and Alcoholic cirrhosis</td>
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<td>3</td>
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<td>Male</td>
<td>Alcoholic cirrhosis</td>
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<td>4</td>
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<td>Cryptogenic cirrhosis</td>
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<td>9</td>
<td>64</td>
<td>Female</td>
<td>Non alcoholic steatohepatitis</td>
<td>&gt;15 mm Hg</td>
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<tr>
<td>10</td>
<td>67</td>
<td>Male</td>
<td>Alcoholic cirrhosis</td>
<td>&gt;15 mm Hg</td>
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</table>

Increased Capillary Density in Patients with Cirrhosis

CD68(+) Macrophages Accumulate in Patients with Cirrhosis
Conclusions

- HPS results from intravascular accumulation of activated CD68(+) macrophages
- The macrophages release vasodilatory, angiogenic and proliferative factors
- Macrophage depletion reveals its central role in HPS etiology, and may have therapeutic potential
- Recognition of a proliferative vasculopathy may explain the sporadic transition to portopulmonary hypertension

Acknowledgement

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Macrophage Depletion Reduces Endothelial Tube Formation

![Graph showing the effect of macrophage depletion on endothelial tube formation.](image)
Nuclear Translocation of NFκB

Plasma VEGF and PDGF

Effect of PDGF-AB in PASMC Proliferation and Endothelial Tube Formation

Increased Biological Variability with CBDL Plasma
L-NIL Increases iNOS Expression

Inhibition of iNOS doesn’t normalize HPS Hemodynamics