MOUSE MODEL OF HALOTHANE-INDUCED LIVER INJURY

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Topics

• The Rationale for developing a mouse model of halothane-induced liver injury (HILI)

• Development and characterization of the mouse model of HILI --- involvement of neutrophils

• PolyI:C co-treatment exacerbates HILI --- concurrent infection as a risk factor
# Similarities and Differences between AILI and IDILI

<table>
<thead>
<tr>
<th></th>
<th>AILI</th>
<th>IDILI</th>
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<tbody>
<tr>
<td><strong>Dose dependence</strong></td>
<td>Overdose</td>
<td>Therapeutic dose</td>
</tr>
<tr>
<td><strong>Patient dependence</strong></td>
<td>Not really</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Timing</strong></td>
<td>Predictable</td>
<td>Variable</td>
</tr>
<tr>
<td><strong>CRM involvement</strong></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Innate immunity</strong></td>
<td>Controversial</td>
<td>Not known, probably involved</td>
</tr>
<tr>
<td><strong>Adaptive immunity</strong></td>
<td>Not involved</td>
<td>Not known, probably involved</td>
</tr>
</tbody>
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Rationales for Developing a Mouse Model of Halothane-Induced Liver Injury

- Halothane is known to cause both mild and severe forms of hepatotoxicity
- It has been well established that halothane toxicity is associated with its metabolism to a reactive metabolite (TFA)

![Chemical Structures]

Halothane  Trifluoroacetyl (TFA)  TFA-protein
Chloride
Rationales for Developing a Mouse Model of Halothane-Induced Liver Injury

- It has been shown that guinea pigs develop liver toxicity after treated with halothane. However, the detailed mechanisms have not been studies because of the high cost of guinea pigs and the limited availability of various immunological tools.
- Mouse immunology is by far the most advanced for research purposes.
- The availability of various strains of wild-type and trasgenic mice enables investigation of the genetic elements potentially involved in the mechanism(s).
Development and Characterization of the Mouse Model of HILI

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Comparison of Halothane-Induced Liver Injury in Balb/c, DBA/1 and C57BL/6J Mice
Histological presentation of liver injury in female Balb/c mice treated with halothane
Immunochemical detection of TFA-protein adducts in mouse liver homogenates
Increased expression of hepatic mRNA levels of pro-inflammatory mediators in female Balb/c mice treated with halothane.
Comparison of hepatic mRNA levels of various pro-inflammatory mediators among C57BL/6J, DBA/1 and Balb/c strains of mice
Halothane treatment significantly induced hepatic recruitment of neutrophils in female Balb/c mice
Comparison of halothane-induced hepatic neutrophil infiltration among C57BL/6J, DBA/1 and Balb/c strains of mice
Neutrophil depletion prevents hepatic recruitment of these cells
Neutrophil depletion reduces halothane-induced liver injury
Summary I

• Three common strains of mice vary in the degree of liver injury caused by halothane treatment. The strain-dependent susceptibility is not due to variations in halothane metabolism, or the pattern of TFA-protein adducts

• Hepatic message levels of pro-inflammatory cytokines and the number of infiltrated neutrophils were significantly higher in the most susceptible Balb/c strain compared with DBA/1 and C57BL/6J mice

• Depletion of neutrophils significantly decreased halothane hepatotoxicity in Balb/c mice, indicating a crucial role of these cells in causing and/or aggravating liver injury initiated by halothane
Effect of PolyI:C Cotreatment on Halothane-Induced Liver Injury in Mice

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PolyI:C post-treatment exacerbated HILI in mice
PolyI:C/Halothane co-treatment induced hepatocyte apoptosis
Caspase inhibition suppressed PolyI:C/Halothane-induced liver injury
Depletion of NK partially attenuated PolyI:C/Halothane-induced liver injury
Depletion of KC attenuated PolyI:C/Halothane-induced liver injury
PolyI:C/halothane co-treatment induced TNF-α expression
KC, but not NK, depletion abrogated TNF-α expression
TNF-α neutralization partially inhibited polyI:C/haothane-induced liver injury
Summary II

- Compared with mice treated with halothane alone, those treated with polyI:C/post-halothane exhibited a profound increase in tissue damage.
- The exacerbation of liver injury correlated with enhanced hepatocyte apoptosis.
- Innate immune cells are activated by polyI:C and produce pro-apoptotic factors that mediate polyI:C-induced hepatocyte apoptosis.
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