Inflammatory Stress and Models of Idiosyncratic DILI

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"Idiosyncratic" Reactions:

- **Idiosyncratic adverse drug response (IADR)** = an adverse reaction that occurs in a minority of patients as a result of drug therapy.
- **Implication**: occurrence is governed by susceptibility factors within individuals.
- **Characteristics of IADRs:**
  - Usually affect a small fraction of people;
  - Occur at doses that do not cause toxicity in most people;
  - Have inconsistent temporal relationship to exposure;
  - Are generally not reproducible in typical animal tests.
Modes of Action of Idiosyncratic Drug-induced Liver Injury (IDILI)?

- Drug metabolism polymorphism hypothesis
- Adaptive immunity hypothesis
- Transporter polymorphism hypothesis
- Multiple determinant hypothesis
- Pharmacological interaction hypothesis
- Mitochondrial toxicity hypothesis
- Failure-to-adapt hypothesis
- Inflammatory stress hypothesis

We really don’t know what causes these reactions: Many hypotheses, no thoroughly convincing proof!
Determinants of Individual Susceptibility to Xenobiotic Toxicity

- Age
- Gender
- Metabolic
- Immunologic reactions
- Reserve capacity
- Absorption/distribution
- Coexisting disease
- Inflammation
- Coexposures
- Nutritional status

- Genetic
- Environmental
Concurrent Inflammation as a Susceptibility Factor

Modest Inflammatory Episode

Increased Susceptibility to Intoxication

Drug Exposure

GI Permeability
Diseases
Obesity?
Alcohol
Surgery
Infection
Diet
Xenobiotics
Hypothesis: Inflammatory Stress Precipitates Idiosyncratic Responses
The inflammatory stress hypothesis can explain the characteristics of many IADRs

- **Characteristics of IADRs:**
  - Usually affect a small fraction of people--
    - Intersection of drug therapy with a sufficient inflammatory episode is likely infrequent.
  - Occur at doses that do not cause toxicity in most people—
    - Inflammatory stress lowers the threshold for toxicity.
  - Have inconsistent temporal relationship to exposure—
    - Inflammatory episodes occur inconsistently within and across individuals.
  - Are generally not reproducible in typical animal tests
    - Preclinical testing does not typically involve animals subjected to inflammatory stresses.
Hepatotoxic interaction between iDILI-associated drugs and inflammatory stress in animal models

<table>
<thead>
<tr>
<th>Drug</th>
<th>Inflammatory mediator</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicloxacillin</td>
<td>IL-4, DK-PGD2</td>
<td>mouse</td>
<td>Higuchi et al., 2011.</td>
</tr>
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<td>Amiodarone</td>
<td>LPS</td>
<td>rat</td>
<td>Lu et al., 2012.</td>
</tr>
<tr>
<td>Halothane</td>
<td>LPS, Poly I:C</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cheng et al., 2009.</td>
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<tr>
<td>Trovafloxacin</td>
<td>LPS, TNF, PGN/LTA</td>
<td>mouse, rat</td>
<td>Shaw et al., 2009a, 2009b.</td>
</tr>
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Abbreviations: DK-PGD2, 13,14-Dihydro-15-keto-PGD2; PGN/LTA, peptidoglycan/lipoteichoic acid.
Trovafl oxacin (TVX)

- A broad spectrum fluoroquinolone antibiotic released in 1998;
- Toxicology: Liver injury not apparent in preclinical safety evaluation or clinical trials.
- In 1999, TVX was linked with hepatotoxicity, and its use was curtailed--14 cases of acute liver failure were linked to TVX, including 6 deaths;
- The incidence of liver injury caused by TVX was about 1 in 18,000 and about 1 in 178,000 for severe hepatotoxicity;
- Levofloxacin (LVX), in the same pharmacologic class, has far less IDILI liability;
Inflammatory Stress Hypothesis: Mouse Model of TVX-Inflammation Interaction

-3 hr  0 hr  2-24 hr

Vehicle or TVX (nontoxic dose, po)  Vehicle or LPS (nontoxic dose, ip)  Serum ALT
Histopathology
Etc.
A modest inflammatory episode renders TVX hepatotoxic.
Liver Histopathology of TVX/LPS-treated Mice
TVX, but not LVX, interacts with LPS to cause liver injury
Tumor Necrosis Factor-alpha (TNF)

- Inflammatory cytokine (17kD) released by macrophages and other cells;
- Its release is stimulated by several inflammatory mediators, including LPS;
- Acts on two receptors TNFR1 (p55) and TNFR2 (p75) – each of which has been reported to be involved in various models of liver injury.
- Actions:
  - Activates the acute phase response
  - Stimulates production of secondary inflammatory mediators
  - Can cause death of hepatocytes (directly).
TVX prolongs the LPS-induced increase in plasma TNF
TNF Receptor Knockout Affords Protection
Etanercept, a soluble TNF receptor, reduces TVX/LPS-induced liver injury
Can TNF replace LPS in the model?

-3 hr  
Vehicle or TVX (150 mg/kg, po)

0 hr  
Vehicle or Murine TNF (50 ug/kg, ip)

21 hr  
Serum ALT 
Histopathology 
Etc.
TVX interacts with TNF to cause hepatotoxicity

H & E (400x)

TUNEL (200x)
Drug-induced enhancement of LPS-mediated TNF appearance is critical for distal events in the pathogenesis.
Effect of TVX on TNF production in vitro: RAW264.7 murine macrophages
TVX enhances LPS-mediated TNF production in RAW cells
TNF production rate after exposure of RAW cells to LPS and/or TVX

(Treatments with different letters differ significantly within a time point, p< 0.05)
TVX enhances LPS-induced TNFα mRNA expression

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*Drugs for which TNF is critically important in the pathogenesis (not examined in most of the other models)*
Conclusions and perspectives:

- Animal studies suggest that some IDILI reactions are likely to result from interaction of drugs with inflammatory episodes (or other stresses);

- The inflammatory stress hypothesis has provided the first animal models of IDILI in which liver injury occurs over a range of drugs;

- TVX and other IDILI-associated drugs interact with LPS to cause liver injury in mice—
  - These drugs increase (prolong) the appearance of TNF;
  - TNF is a proximal mediator of drug-LPS hepatotoxicity;
  - Distal (downstream) mechanisms are complex, involving cytokines, neutrophils, the hemostatic system, and probably other factors;
  - In vitro, TVX
    - (1) increases TNF production by macrophages and
    - (2) increases hepatocellular sensitivity to TNF-mediated killing.
Conclusions and perspectives (cont’d):

- Do other IDILI-associated drugs increase cytokine production in cells stimulated by TLR agonists?

- The Hope: Understanding of mechanisms will lead to high-throughput, cell-based assays that predict IDILI potential.
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