Drug Induced Liver Injury (DILI): Challenges and Opportunities

Robert H. Squires, MD
Professor of Pediatrics
University of Pittsburgh

Overview

• General aspects of drug-induced liver injury
• Pathogenesis
• Drug-Induced Liver Disease Network
• Examples
  – Minocycline
  – Acetaminophen
  – OxyELITE Pro
• Reporting

Scope of the Problem

• Definition is difficult
  – Lack of systematic reporting
  – Unknown denominator of those taking the drug
  – Inconsistent post-marketing testing
  – Lack of consensus of liver test abnormalities
  – Arbitrating the culprit with multiple medications
  – Co-morbidities (e.g., NAFLD)
• Incidence estimated 13—19 / 100,000

Classification

• Intrinsic
  – Predictable, affects everyone
  – Short latency period
  – Dose related
  – Acetaminophen

• Idiosyncratic
  – Unpredictable, susceptible
  – Longer latency period
  – Not dose dependent; >50 mg/d
  – Amoxacillin/clavulanate, isoniazid

• Biochemical pattern
  – Hepatocellular
  – Cholestatic
  – Mixed

• Histologic features

Histologic Patterns in DILI

Autoimmune Hepatitis
Steatosis / Steatohepatitis
Cholestatic Hepatitis

INH
PTU
Nitrofurantoin
Minocycline
Statins
Hydralazine
Methyldopa

Corticosteroids
Antidepressants
Amiodarone
Methotrexate
Valproate
Linzoloid
Zidovudine

Penicillins
Cephalosporine
Amox/Clavulanate
Rifampin
Methimazole
Many others

INH
Amox/Clavulanate
Phenytoin
Statins
Green Tea
Other Herbals

Carbamazepine
Phenytion
Sulfonamides
Interferon
INH
Mesalamine

Azathioprine
Triquinine
Vitamin A
Methotrexate
Mercaptopurine
Diltiazem

INH
PTU
Nitrofurantoin
Minocycline
Statins
Hydralazine
Methyldopa

Corticosteroids
Antidepressants
Amiodarone
Methotrexate
Valproate
Linzoloid
Zidovudine

Penicillins
Cephalosporine
Rifampin
Methimazole
Many others

INH
Amox/Clavulanate
Phenytoin
Statins
Green Tea
Other Herbals

Carbamazepine
Phenytion
Sulfonamides
Interferon
INH
Mesalamine

Azathioprine
Triquinine
Vitamin A
Methotrexate
Mercaptopurine
Diltiazem

INH
PTU
Nitrofurantoin
Minocycline
Statins
Hydralazine
Methyldopa

Corticosteroids
Antidepressants
Amiodarone
Methotrexate
Valproate
Linzoloid
Zidovudine

Penicillins
Cephalosporine
Rifampin
Methimazole
Many others

INH
Amox/Clavulanate
Phenytoin
Statins
Green Tea
Other Herbals

Carbamazepine
Phenytion
Sulfonamides
Interferon
INH
Mesalamine

Azathioprine
Triquinine
Vitamin A
Methotrexate
Methyldopa
Mercaptopurine
Diltiazem

INH
PTU
Nitrofurantoin
Minocycline
Statins
Hydralazine
Methyldopa

Corticosteroids
Antidepressants
Amiodarone
Methotrexate
Valproate
Linzoloid
Zidovudine

Penicillins
Cephalosporine
Rifampin
Methimazole
Many others

INH
Amox/Clavulanate
Phenytoin
Statins
Green Tea
Other Herbals

Carbamazepine
Phenytion
Sulfonamides
Interferon
INH
Mesalamine

Azathioprine
Triquinine
Vitamin A
Methotrexate
Methyldopa
Mercaptopurine
Diltiazem

INH
PTU
Nitrofurantoin
Minocycline
Statins
Hydralazine
Methyldopa

Corticosteroids
Antidepressants
Amiodarone
Methotrexate
Valproate
Linzoloid
Zidovudine

Penicillins
Cephalosporine
Rifampin
Methimazole
Many others

INH
Amox/Clavulanate
Phenytoin
Statins
Green Tea
Other Herbals

Carbamazepine
Phenytion
Sulfonamides
Interferon
INH
Mesalamine

Azathioprine
Triquinine
Vitamin A
Methotrexate
Methyldopa
Mercaptopurine
Diltiazem

INH
PTU
Nitrofurantoin
Minocycline
Statins
Hydralazine
Methyldopa

Corticosteroids
Antidepressants
Amiodarone
Methotrexate
Valproate
Linzoloid
Zidovudine

Penicillins
Cephalosporine
Rifampin
Methimazole
Many others

INH
Amox/Clavulanate
Phenytoin
Statins
Green Tea
Other Herbals

Carbamazepine
Phenytion
Sulfonamides
Interferon
INH
Mesalamine

Azathioprine
Triquinine
Vitamin A
Methotrexate
Methyldopa
Mercaptopurine
Diltiazem

INH
PTU
Nitrofurantoin
Minocycline
Statins
Hydralazine
Methyldopa

Corticosteroids
Antidepressants
Amiodarone
Methotrexate
Valproate
Linzoloid
Zidovudine

Penicillins
Cephalosporine
Rifampin
Methimazole
Many others

INH
Amox/Clavulanate
Phenytoin
Statins
Green Tea
Other Herbals

Carbamazepine
Phenytion
Sulfonamides
Interferon
INH
Mesalamine

Azathioprine
Triquinine
Vitamin A
Methotrexate
Methyldopa
Mercaptopurine
Diltiazem

INH
PTU
Nitrofurantoin
Minocycline
Statins
Hydralazine
Methyldopa

Corticosteroids
Antidepressants
Amiodarone
Methotrexate
Valproate
Linzoloid
Zidovudine

Penicillins
Cephalosporine
Rifampin
Methimazole
Many others

INH
Amox/Clavulanate
Phenytoin
Statins
Green Tea
Other Herbals
Pathogenesis of DILI

- **Drug**
  - Chemical structure
  - Molecular weight
  - Lipophilicity
  - Dose

- **Cellular Stress**
  - Apoptosis
  - Necrosis

- **Reactive Metabolite**

- **Innate Immune Response**
  - Danger/Damage Signals
  - Haptan

- **Common MCH II**
  - DILI resistant
  - DILI susceptible

Variability in T-cell Receptor Specificity May Drive Clinical Response

- **Drug**
  - Clinical T-cell receptor repertoire

- **Histocompatibility**
  - Class I and II antigens

- **DAMPs**
  - HMGB1
  - ATP

DILI with Immune Features

<table>
<thead>
<tr>
<th>Feature</th>
<th>Immuno-Allergic</th>
<th>Autoimmune</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency</td>
<td>Short, &lt;30 days</td>
<td>Variable, &gt;3 mo to years</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Fever, rash, pruritus, arthralgia, Stevens-Johnson, toxic epidermal necrolysis, liver failure</td>
<td>Pain, arthralgia, nausea, vomiting, extra-hepatic autoimmune features (gastrointestinal, renal), liver failure</td>
</tr>
<tr>
<td>Laboratory</td>
<td>Eosinophilia</td>
<td>High IgG, (+) autoantibodies</td>
</tr>
<tr>
<td>Histology</td>
<td>Lobular and portal inflammation, eosinophilia, cholestasis/cholestasis-hepatic features</td>
<td>Lobular / portal inflammation, interface hepatitis, lympho-histiocytic and plasma cell infiltrate</td>
</tr>
<tr>
<td>History</td>
<td>Allergies-50%</td>
<td>Other autoimmune disease</td>
</tr>
<tr>
<td>Outcome</td>
<td>Gradual, months</td>
<td>Rapid, more severe</td>
</tr>
<tr>
<td>Drugs</td>
<td>Erythromycin, macrolides, PCN, phenytoin, sulfonamides</td>
<td>Statins, miconazole, hydralazine, procainamide</td>
</tr>
</tbody>
</table>

New Opportunities for Diagnosis

- **Biomarkers**
  - Micro RNAs: miR-192, miR-122 [Hepatology 2011;54:1767]
  - IL-28B genotyping

- **Proteomics** [Aliment Pharmacol Ther. 2012;35:600]
  - Apo E
  - Gelsolin, complement C7, amyloid P, age

- **Genomics**
  - IL-28B for interferon
  - HLA-B*1502 and HLA-A*3101 for carbamazepine

- **Protein adducts** [Hepatology 2011;53:597]

DILIN-Adult: Study Population
**DILIN Causality Assessment**

<table>
<thead>
<tr>
<th>Score</th>
<th>Likelihood (%)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Definite</td>
<td>&gt;95 Injury is typical of drug/herbal</td>
</tr>
<tr>
<td>2</td>
<td>Highly likely</td>
<td>75-95 Evidence is clear and convincing; not definite</td>
</tr>
<tr>
<td>3</td>
<td>Probable</td>
<td>50-74 Supported by a preponderance of evidence</td>
</tr>
<tr>
<td>4</td>
<td>Possible</td>
<td>25-49 Cannot definitely exclude the possibility</td>
</tr>
<tr>
<td>5</td>
<td>Unlikely</td>
<td>&lt;25 Highly unlikely base on available information</td>
</tr>
<tr>
<td>6</td>
<td>Insufficient</td>
<td>N/A</td>
</tr>
</tbody>
</table>


**Roussel Uclaf Causality Assessment Method (RUCAM)**

- Type of liver injury
- Time of onset related the first or subsequent exposure
- Duration of exposure to the drug
- Rapidity of ALT decline after stopping drug
- Risk factors
  - Alcohol use
  - Age over 50 years
- Other drug exposures
- Other possible diagnoses
  - Viral hepatitis, biliary obstruction, hypotension, ETOH, biliary obstruction
- Is the drug known to be hepatotoxic
- Was the patient re-exposed to the drug

**DILI Experts vs RUCAM**

Table 7. Cross-Tabulation of Initial DILIN Causality Scores Against Categorized RUCAM Scores

<table>
<thead>
<tr>
<th>DILIN Expert Opinion</th>
<th>RUCAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definite</td>
<td>Highly Likely</td>
</tr>
<tr>
<td>Definite</td>
<td>10</td>
</tr>
<tr>
<td>Very Likely</td>
<td>38</td>
</tr>
<tr>
<td>Probable</td>
<td>10</td>
</tr>
<tr>
<td>Possible</td>
<td>2</td>
</tr>
<tr>
<td>Unlikely</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
</tr>
</tbody>
</table>

This table is restricted to cases in which a single agent was implicated (n = 187 cases). RUCAM scores were missing for 4 reviews, and this resulted in 657 reviews.

Rockey DC, et al. Hepatology 2010;51:2117

**DILIN-Adult: Adverse Outcomes within 6 months (n=62)**

![Graph showing DILIN-Adult: Adverse Outcomes within 6 months (n=62)]


**Implicated Agents for Pediatric DILIN (N=37)**

- Antibiotics
- Anticonvulsants
- ADHD drugs
- Psychoactives
- Others


**Implicated Agents for Pediatric DILI (N=37)**

1. Diclofenac
2. Levothyroxine
3. Amoxicillin
4. Methotrexate
5. Isoniazid
6. Sulfamethoxazole
7. Fosphenytoin
8. Fluoxetine
9. Clozapine
10. Lamotrigine
11. Minocycline
12. Nicotinamide
13. Nicotinic acid
14. Folic acid
15. Daunorubicin
16. Methylphenidate
17. Hydantoins

**EMR-based Method to Detect DILI in Children (Abstract #284)**

- Drug Safety Service at Children’s Mercy Hospital (Kansas City) to detect adverse drug reactions

- Biochemical triggers
  - ALT > 5 x ULN
  - Total bilirubin > 1.5 x ULN

- Adjudication
  - Staff physician / pharmacologist
  - Drug of known risk of hepatotoxicity
  - Liver injury / recovery in relation to drug exposure / withdrawal
  - No other known cause of liver injury
  - RUCAM

---

**DILI: Children’s Mercy Hospital**

- **# Suspected agent, Age (yr), Sex, Type, Peak ALT, Peak ALP, Peak Bili, RUCAM**

<table>
<thead>
<tr>
<th>#</th>
<th>Suspected agent</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Type</th>
<th>Peak ALT</th>
<th>Peak ALP</th>
<th>Peak Bili</th>
<th>RUCAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Minocycline</td>
<td>15.5</td>
<td>F</td>
<td>Hepatocellular</td>
<td>839</td>
<td>288</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Carbamazepine</td>
<td>17.9</td>
<td>F</td>
<td>Hepatocellular</td>
<td>831</td>
<td>68</td>
<td>0.2</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>Trimethoprim</td>
<td>1.4</td>
<td>M</td>
<td>Hepatocellular</td>
<td>944</td>
<td>269</td>
<td>0.2</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>Trimethoprim</td>
<td>14.9</td>
<td>F</td>
<td>Hepatocellular</td>
<td>427</td>
<td>297</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>Minocycline</td>
<td>14.8</td>
<td>F</td>
<td>Mixed</td>
<td>95</td>
<td>64</td>
<td>0.6</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>Doxycycline</td>
<td>17.8</td>
<td>F</td>
<td>Hepatocellular</td>
<td>337</td>
<td>61</td>
<td>0.6</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>Oxacillin</td>
<td>6.3</td>
<td>M</td>
<td>Hepatocellular</td>
<td>488</td>
<td>222</td>
<td>0.4</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>Cefepime</td>
<td>10.8</td>
<td>M</td>
<td>Hepatocellular</td>
<td>428</td>
<td>234</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>Metronidazole</td>
<td>1.3</td>
<td>F</td>
<td>Mixed</td>
<td>163</td>
<td>210</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>Antipyrine</td>
<td>16.9</td>
<td>M</td>
<td>Cholestatic</td>
<td>105</td>
<td>423</td>
<td>0.6</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>Sulfasalazine</td>
<td>14.9</td>
<td>F</td>
<td>Hepatocellular</td>
<td>425</td>
<td>193</td>
<td>0.6</td>
<td>7</td>
</tr>
<tr>
<td>12</td>
<td>Lamotrigine</td>
<td>17</td>
<td>F</td>
<td>Cholestatic</td>
<td>346</td>
<td>261</td>
<td>5.9</td>
<td>4</td>
</tr>
<tr>
<td>13</td>
<td>Minocycline</td>
<td>15.5</td>
<td>F</td>
<td>Hepatocellular</td>
<td>1763</td>
<td>184</td>
<td>3.9</td>
<td>8</td>
</tr>
</tbody>
</table>

---

**Etiology of PALF (N = 945)**

- **Number of patients in each age category**

<table>
<thead>
<tr>
<th>Age Category</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4 wks</td>
<td>117</td>
</tr>
<tr>
<td>4-8 wk</td>
<td>28</td>
</tr>
<tr>
<td>9-52 wk</td>
<td>113</td>
</tr>
<tr>
<td>1-5 yr</td>
<td>257</td>
</tr>
<tr>
<td>6-10 yr</td>
<td>115</td>
</tr>
<tr>
<td>10-17 yr</td>
<td>315</td>
</tr>
<tr>
<td>Adult</td>
<td>308</td>
</tr>
</tbody>
</table>

---

**Non-APAP Drug Induced**

- **27/945 (2.9%)**

---

**Minocycline**

- Age at onset of disease: 16.5 yr (range 13-18)
- Female: 70%
- Duration on minocycline before Sx: 13 mo (range 3-48)
- Duration of Sx before diagnosis: 4.3 mo (range 1-12)
- Cumulative dose: 72 grams (range 18-288)
- Constitutional sx
  - Polyarthralgia, polyarthritis, Raynaud’s, a.m. stiffness
- Outcomes (n=27)
  - Transient = 14 (rapid resolution)
  - Intermediate = 6 (resolve within 12 mo)
  - Chronic (active at last f/u) = 7 (31.6 mo; range 13-48)
Acetaminophen = 115 (12.2%)

• First introduced in 1893
  • Exposure
    – ~200,000 million people/yr take acetaminophen (APAP)
    – In 2005: US consumers purchased 28 billion doses of APAP products
      Maximum daily dose (Adult: 325 or 500 mg/tab; Child: 80 or 160 mg/tab or 160 mg/5ml)
        Adult = 4 gm/d
        Child = 10-15 mg/kg/dose; 75 mg/kg/day
  • Mechanism of action is not well defined
    – Weak inhibitor of cyclooxygenase
  • Toxicity
    – Estimated 500 deaths / year from acute ingestion (50% unintentional)
    – Median acute dose is 24 gm; as low as 2.5 gm / day

Chronic APAP Exposure in PALF

• Widely available
  • Safe dose: 10-15mg/kg (single), ≤ 5 times per day, 75 mg/kg/day
  • ALF in adults with unintentional overdose following exposure to >4-6 gm/day (8-12 extra-strength APAP)
  • 895 children grouped by APAP exposure history
    – 83: Chronic Exposure: multiple doses ≥ 2 days
    – 85: Single dose exposure
    – 498: No exposure: No history, measured and undetectable APAP level, final dx that is not APAP toxicity
    – 229: Criteria not met: History of exposure w/o documentation
  • Single dose and total daily dose per day recorded

• First introduced in 1893
  • Exposure
    – ~200,000 million people/yr take acetaminophen (APAP)
    – In 2005: US consumers purchased 28 billion doses of APAP products
      Maximum daily dose (Adult: 325 or 500 mg/tab; Child: 80 or 160 mg/tab or 160 mg/5ml)
        Adult = 4 gm/d
        Child = 10-15 mg/kg/dose; 75 mg/kg/day
  • Mechanism of action is not well defined
    – Weak inhibitor of cyclooxygenase
  • Toxicity
    – Estimated 500 deaths / year from acute ingestion (50% unintentional)
    – Median acute dose is 24 gm; as low as 2.5 gm / day

Chronic APAP Exposure in PALF

• Widely available
  • Safe dose: 10-15mg/kg (single), ≤ 5 times per day, 75 mg/kg/day
  • ALF in adults with unintentional overdose following exposure to >4-6 gm/day (8-12 extra-strength APAP)
  • 895 children grouped by APAP exposure history
    – 83: Chronic Exposure: multiple doses ≥ 2 days
    – 85: Single dose exposure
    – 498: No exposure: No history, measured and undetectable APAP level, final dx that is not APAP toxicity
    – 229: Criteria not met: History of exposure w/o documentation
  • Single dose and total daily dose per day recorded

### APAP Characteristics and Diagnoses

<table>
<thead>
<tr>
<th></th>
<th>Chronic Exposure N=83</th>
<th>Acute Exposure N=85</th>
<th>No Exposure N=498</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>3.5</td>
<td>15.2</td>
<td>3.2</td>
</tr>
<tr>
<td>(25th , 75th )</td>
<td>(1.2, 10.1)</td>
<td>(14.3, 16.3)</td>
<td>(0.1, 10.1)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>45 (54.2%)</td>
<td>15 (17.6%)</td>
<td>278 (55.8%)</td>
</tr>
<tr>
<td>Female</td>
<td>38 (45.8%)</td>
<td>20 (22.4%)</td>
<td>220 (44.2%)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not Hispanic or Latino</td>
<td>62 (74.7%)</td>
<td>79 (92.9%)</td>
<td>398 (79.9%)</td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>21 (25.3%)</td>
<td>6 (7.1%)</td>
<td>100 (20.1%)</td>
</tr>
<tr>
<td><strong>Encephalopathy at study entry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>5 (-)</td>
<td>2 (-)</td>
<td>36 (-)</td>
</tr>
<tr>
<td>Grade I</td>
<td>30 (38.5%)</td>
<td>51 (61.4%)</td>
<td>247 (53.3%)</td>
</tr>
<tr>
<td>Grade II</td>
<td>27 (34.6%)</td>
<td>16 (19.3%)</td>
<td>119 (23.8%)</td>
</tr>
<tr>
<td>Grade III</td>
<td>9 (11.5%)</td>
<td>5 (6.0%)</td>
<td>46 (9.2%)</td>
</tr>
<tr>
<td>Grade IV</td>
<td>7 (9.0%)</td>
<td>6 (7.1%)</td>
<td>33 (6.6%)</td>
</tr>
<tr>
<td></td>
<td>5 (6.4%)</td>
<td>5 (6.0%)</td>
<td>17 (3.4%)</td>
</tr>
</tbody>
</table>

### Biochemical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Chronic Exposure N=83</th>
<th>Acute Exposure N=85</th>
<th>No Exposure N=498</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (25th, 75th)</td>
<td>2.6 (2.4)</td>
<td>2.2 (1.7, 3.3)</td>
<td>2.7 (2.1, 3.8)</td>
</tr>
<tr>
<td><strong>Total bilirubin (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (25th, 75th)</td>
<td>76 (1.8, 12.8)</td>
<td>75 (2.0, 1.1, 3.5)</td>
<td>420 (13.1, 5.7, 19.7)</td>
</tr>
<tr>
<td><strong>ALT (IU/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (25th, 75th)</td>
<td>2384.0 (1018, 4344)</td>
<td>5140.0 (2600, 7050)</td>
<td>363 (555.0, 149, 2067)</td>
</tr>
</tbody>
</table>

### 21-day Outcome: Chronic APAP Exposure

<table>
<thead>
<tr>
<th></th>
<th>Chronic Exposure N=83</th>
<th>Acute Exposure N=85</th>
<th>No Exposure N=498</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death w/o transplantation</td>
<td>10 (12.0%)</td>
<td>2 (2.4%)</td>
<td>78 (15.7%)</td>
</tr>
<tr>
<td>Transplantation</td>
<td>17 (20.5%)</td>
<td>5 (5.9%)</td>
<td>174 (34.9%)</td>
</tr>
<tr>
<td>Alive w/o transplantation</td>
<td>56 (67.5%)</td>
<td>78 (91.8%)</td>
<td>246 (49.4%)</td>
</tr>
</tbody>
</table>

### APAP Chronic Exposure in PALF

- Children with CE
  - Dose history revealed doses within the usual daily dose
  - Had lower bilirubin and higher ALT than NE; similar to SE
  - APAP levels were elevated in 67%; 3 were >100 mg/L
  - Clinical outcomes were worse than SE, but better than NE
- Obtaining the dose and frequency of APAP exposure is important
- Characterizing the pharmacokinetics of APAP in the setting of CE in ill children is necessary

### 1,3 dimethylamylamine (DMAA) toxicity

- DMAA banned by FDA
- Sept 2013 Hawaii DOH
  - 7 cases acute liver injury
- National advisory 10/13
- Feb 2013 FDA reported
  - 97 cases
  - 47 hospitalizations
  - 3 liver transplants
  - 1 death
OxyELITE Pro© associated liver injury

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Duration</th>
<th>Total bilirubin (mg/dl)</th>
<th>ALT (IU/ml)</th>
<th>INR</th>
<th>ENC</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>F</td>
<td>2 yr</td>
<td>26.4</td>
<td>1,980</td>
<td>3.8</td>
<td>Yes</td>
<td>LTx</td>
</tr>
<tr>
<td>28</td>
<td>M</td>
<td>8 wk</td>
<td>32</td>
<td>2,379</td>
<td>3.4</td>
<td>Yes</td>
<td>LTx</td>
</tr>
<tr>
<td>19</td>
<td>M</td>
<td>3 yr</td>
<td>1.2</td>
<td>189</td>
<td>1.3</td>
<td>No</td>
<td>Resolved</td>
</tr>
<tr>
<td>28</td>
<td>F</td>
<td>4 wk</td>
<td>6.7</td>
<td>1,162</td>
<td>1.2</td>
<td>No</td>
<td>Resolved</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>2 yr</td>
<td>17.5</td>
<td>194</td>
<td>--</td>
<td>No</td>
<td>Resolved</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>1 wk</td>
<td>6.3</td>
<td>176</td>
<td>0.9</td>
<td>No</td>
<td>Resolved</td>
</tr>
<tr>
<td>24</td>
<td>F</td>
<td>1 yr</td>
<td>8</td>
<td>3,348</td>
<td>--</td>
<td>No</td>
<td>Resolved</td>
</tr>
</tbody>
</table>

Diggs Dk Sci. 2014

Minocycline

- Overview
- Hepatotoxicity
- Mechanism of injury
- Outcome and Management
- Illustrative case reports
- Product information
- Chemical formula and structure
- Links
  - Recent references on PubMed
  - ClinicalTrials.gov (165 studies)
  - Toxline citations:

www.fda.gov/safety/medwatch

MedWatch: The FDA Safety Information and Adverse Event Reporting Program

Report a problem:

Evaluation and Assessment

- Careful drug and supplement use history
- Timing of exposure
- Characterize injury pattern
- Evaluate for alternative liver disease
- Remove the drug
- Monitor response
- Report to MedWatch
<table>
<thead>
<tr>
<th>Characteristics for those with APAP adducts tested and those without</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants with APAP adduct tested</td>
</tr>
<tr>
<td>N(%)</td>
</tr>
<tr>
<td>Age at randomization</td>
</tr>
<tr>
<td>Less than 2 years</td>
</tr>
<tr>
<td>At least 2 years</td>
</tr>
<tr>
<td>Coma grade at randomization</td>
</tr>
<tr>
<td>0-1</td>
</tr>
<tr>
<td>2-4</td>
</tr>
<tr>
<td>Race</td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>African-American</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Final diagnosis</td>
</tr>
<tr>
<td>Indeterminate</td>
</tr>
<tr>
<td>Autoimmune</td>
</tr>
<tr>
<td>Metabolic</td>
</tr>
<tr>
<td>Infection</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>

---

**Drug Induced Liver Injury Network (Adult and Pediatric)**

- **Inclusion**
  - Five Sites: UConnecticut, UCSF, Indiana, UMchigan, UNoCarolina
  - Over 2 years of age
  - Enrolled within 6 mo of liver injury
  - AST/ALT >5 x ULN or >5 x pre-drug average
  - Total bilirubin >2.5 mg/dl + elevated AST, ALT or SAP
  - INR >1.5 with 4 elevated AST, ALT or SAP
- **Exclusion**
  - Acetaminophen toxicity
  - Pre-existing liver disease (e.g., PBC, PSC, AIH, or biliary disease)
  - Liver / bone marrow transplant
  - Identifiable competing cause of liver injury other than HIV, HBV, HCV, unexplained abnormal liver tests

1-year overall survival, spontaneous survival and transplantation rate for death as a competing risk between those with APAP adducts tested and those without

<table>
<thead>
<tr>
<th></th>
<th>Participants with APAP adduct tested</th>
<th>Participants without APAP adduct tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>Cum. %*</td>
<td>78%</td>
<td>78%</td>
</tr>
<tr>
<td>p-value &amp;</td>
<td>0.998</td>
<td>0</td>
</tr>
<tr>
<td>1y overall survival</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>1y spont survival</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>1y transplant rate</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>(death as a competing risk event)</td>
<td>36%</td>
<td>43%</td>
</tr>
</tbody>
</table>

Note: * Cum. % = Cumulative percent for survival of 1 year after randomization
+ Cum. Inc. = Cumulative percent of incidence of transplantation within 1 year after randomization for death as a competing risk
* From Log-rank test
* From Chi-square test

Children surviving 1 year tested for APAP adducts

<table>
<thead>
<tr>
<th>APAP Adduct* (nmol/ml)</th>
<th>NAC (N=68)</th>
<th>Placebo (N=76)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>33</td>
<td>45</td>
</tr>
<tr>
<td>No LTx</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>LTx</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>&lt;1</td>
<td>32</td>
<td>24</td>
</tr>
<tr>
<td>≥1</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

* = APAP adduct levels ≥1 nmol/ml are considered (+)

There were no children who died by 1 year who tested positive for APAP adducts.

DILIN Severity Index

<table>
<thead>
<tr>
<th>Score</th>
<th>Grade</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mild</td>
<td>Elevated Alt/SAP: TB &lt;2.5; INR &lt;1.5</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>Elevated Alt/SAP: either TB or INR elevated</td>
</tr>
<tr>
<td>3</td>
<td>Mod-Severe</td>
<td>Elevated Alt/SAP: either TB or INR elevated; hospitalized</td>
</tr>
<tr>
<td>4</td>
<td>Severe</td>
<td>Elevated Alt/SAP: TB &lt;2.5; Liver failure or other organ failure due to DILI event</td>
</tr>
<tr>
<td>5</td>
<td>Fatal</td>
<td>Death or Liver Transplant</td>
</tr>
</tbody>
</table>

With / without Symptoms: nausea, vomiting, rash, itching, fatigue, weight loss

Children who tested positive for APAP adducts in NAC trial

9 / 84 tested positive
All 9 survived to 1 year

6 Placebo
- All with native liver

Diagnosis
- 3 indeterminate
- 1 viral hepatitis
- 1 sepsis

3 NAC
- 2 received LT
- 1 native liver

Diagnosis
- All indeterminate

Characteristics for those with APAP adducts tested and those without enrolled in NAC trial

<table>
<thead>
<tr>
<th></th>
<th>Participants with APAP adduct tested (N=84)</th>
<th>Participants without APAP adduct tested (N=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at randomization</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>&lt; 2 years</td>
<td>24 (28.6)</td>
<td>41 (41.0)</td>
</tr>
<tr>
<td>≥ 2 years</td>
<td>60 (71.4)</td>
<td>59 (59.0)</td>
</tr>
<tr>
<td>Comet grade at randomization</td>
<td>0-1</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>43 (51.2)</td>
<td>58 (58.0)</td>
</tr>
<tr>
<td></td>
<td>41 (48.8)</td>
<td>42 (42.2)</td>
</tr>
<tr>
<td></td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td>White</td>
<td>African American</td>
</tr>
<tr>
<td></td>
<td>61 (72.6)</td>
<td>13 (13.5)</td>
</tr>
<tr>
<td></td>
<td>13 (15.5)</td>
<td>17 (17.0)</td>
</tr>
<tr>
<td></td>
<td>10 (11.9)</td>
<td>11 (11.0)</td>
</tr>
<tr>
<td></td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Final diagnosis</td>
<td>Autoimmune</td>
<td>Other</td>
</tr>
<tr>
<td></td>
<td>9 (10.7)</td>
<td>10 (10.0)</td>
</tr>
<tr>
<td></td>
<td>12 (14.3)</td>
<td>12 (12.0)</td>
</tr>
<tr>
<td></td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (3.6)</td>
<td>6 (6.0)</td>
</tr>
<tr>
<td></td>
<td>11 (13.0)</td>
<td>11 (11.0)</td>
</tr>
</tbody>
</table>


1-year overall survival, spontaneous survival and transplantation rate for death as a competing risk between those with APAP adducts tested and those without

<table>
<thead>
<tr>
<th></th>
<th>Participants with APAP adduct tested</th>
<th>Participants without APAP adduct tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>Cum. %*</td>
<td>78%</td>
<td>78%</td>
</tr>
<tr>
<td>p-value &amp;</td>
<td>0.998</td>
<td>0</td>
</tr>
<tr>
<td>1y overall survival</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>1y spont survival</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>1y transplant rate</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>(death as a competing risk event)</td>
<td>36%</td>
<td>43%</td>
</tr>
</tbody>
</table>

Note: * Cum. % = Cumulative percent for survival of 1 year after randomization
+ Cum. Inc. = Cumulative percent of incidence of transplantation within 1 year after randomization for death as a competing risk
* From Log-rank test
* From Chi-square test

Children surviving 1 year tested for APAP adducts

<table>
<thead>
<tr>
<th>APAP Adduct* (nmol/ml)</th>
<th>NAC (N=68)</th>
<th>Placebo (N=76)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>33</td>
<td>45</td>
</tr>
<tr>
<td>No LTx</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>LTx</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>&lt;1</td>
<td>32</td>
<td>24</td>
</tr>
<tr>
<td>≥1</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

* = APAP adduct levels ≥1 nmol/ml are considered (+)

There were no children who died by 1 year who tested positive for APAP adducts.
APAP adducts in PALF participants with indeterminate etiology

<table>
<thead>
<tr>
<th>Adduct positive %</th>
<th>Adduct negative %</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic Encephalopathy at Enrollment*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1 or more</td>
<td>11/15 (73.3%)</td>
<td>3/19 (15.8%)</td>
</tr>
<tr>
<td>Grade 2 or more</td>
<td>2/8 (25.0%)</td>
<td>1/11 (9.1%)</td>
</tr>
</tbody>
</table>

Prolonged NAC treatment delays recovery from APAP toxicity in mice

- **Treatment group A**
  - Saline or APAP (350 mg/kg) by IP injection
  - After 2 hrs, randomized to saline or NAC (100 mg/kg/dose) every 12 hrs for 72 hrs
- **Treatment group B**
  - Randomized to treatment for 24 hrs
- **Control group**
  - Saline injected but not randomized the treatment

**Outcome**
- All animals sacrificed at 72 hrs
- Measured AST/ALT, histology, cyclin D1

Western blot of liver tissue to assess protein levels of Cyclin D1 after 72 hrs of NAC

<table>
<thead>
<tr>
<th>Protein</th>
<th>Control</th>
<th>Saline</th>
<th>NAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclin D1</td>
<td>[Image]</td>
<td>[Image]</td>
<td>[Image]</td>
</tr>
<tr>
<td>β-actin</td>
<td>[Image]</td>
<td>[Image]</td>
<td>[Image]</td>
</tr>
</tbody>
</table>

AST/ALT levels after 24 (top) or 72 hrs (bottom) of NAC

**Challenges**

- Definition of biochemical profile for DILI
- Multiple medication exposures
- Pathogenesis is multifactorial; “personalized”
- Confounding features (e.g., NAFLD, preexisting condition)
- Lack of systematic reporting
Findings of APAP challenged mice receiving prolonged NAC treatment

- Increased serum ALT/AST
- Increased hepatocyte vacuolation
- Delayed hepatocyte regeneration