6. Drug Metabolism and Toxicity (2013)

General Scheme for Metabolism-Induced Toxicity:

A. Many drugs are known to undergo metabolism to reactive metabolites that bind to cellular macromolecules and produce a toxicity. The key is that metabolites are causing the toxicity as opposed to the parent drug and that there are multiple cellular targets.

![Diagram showing the general scheme for metabolism-induced toxicity.](image)

**Figure 1** Relationship between drug metabolism and toxicity. Toxicity may accrue through accumulation of parent drug or, via metabolic activation, through formation of a chemically reactive metabolite, which, if not detoxified, can effect covalent modification of biological macromolecules. The identity of the target macromolecule and the functional consequence of its modification will dictate the resulting toxicological response.

1. While drug metabolite induced toxicities can and do occur in tissues and organs the liver is often the target due to it’s high concentration of metabolizing enzymes and it’s anatomical location. We will look at some examples of drug induced liver toxicity (DILI).

2. Many anticancer drugs are either alkylating agents, are designed to be converted to alkylating agents or promote destructive processes such a redox cycling to reactive oxygen species. They also cause toxicities and often widespread cell death including healthy cells. We won’t talk about those.
3. Examples of Post-marketing Drug Induced Liver Toxicities (DILI)

**Black Box Warnings:** Dacarbazine, Dantrolene, Felbamate, Flutamide, Halothane, Ketoconazole, Leflunomide Tolcapone Valproic Acid Zalcitabine Zidovudine

**Withdrawn from Market:** Benoxaprofen Bromfenac Iproniazid Nefazadone Pemoline Temafloxacin Ticrynafen Troglitazone Trovafloxacin

4. A **Black Box Warning** is mandated by the FDA and warns to the potential for a serious adverse drug reaction (ADR). It must be placed on the label or package insert. It almost always reduces drug utilization and may be a prelude to a drug being removed from the market. See section IV.A in FDA guidance. [http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm075096.pdf](http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm075096.pdf)

5. Quite often the molecular basis for the ADR is not known. Particularly troublesome are rare and unpredictable (idiosyncratic) ADRs.

6. Toxicity may be either acute or cumulative. In some immune-based ADRs the toxicity is observed upon repeat exposure.

7. Certain types of functional groups (moieties) are most suspect. Drugs that contain or can be metabolized to the following structures may cause toxic effects via the formation of reactive metabolites:
   - **Hydrazines** and hydrazides
   - Arylacetic or aryl propionic acids
   - Thiophene, furan, pyrrole, polycyclic aromatics.
   - Anilines or anilides
   - Quinone and quinone imines
   - Medium chain fatty acids
   - **Halogenated hydrocarbons** and some halogenated aromatics
   - **Nitroaromatics**
   - Moieties that can form reactive α,β-unsaturated enal-like structures
   - Thiols, thiono compounds, thiazolidinediones

8. We are discovering that in some cases there is a genetic component to risk of ADR. This area of study is called toxicogenomics

### Table 1 Examples of established genetic ADR risk factors

<table>
<thead>
<tr>
<th>Drug</th>
<th>Adverse drug reaction</th>
<th>Genetic risk factor</th>
<th>Cases required*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diarrhea</strong></td>
<td>ABCG2 Q141K</td>
<td>0.07</td>
<td>29/101 47/&gt; 150</td>
</tr>
<tr>
<td><strong>Hepatotoxicity</strong></td>
<td>CYP2E1*1 &amp; NAT2 slow Ac</td>
<td>0.13*</td>
<td>7</td>
</tr>
<tr>
<td><strong>Neutropenia</strong></td>
<td>UGT1A1*28</td>
<td>0.32</td>
<td>17/36 26/58</td>
</tr>
<tr>
<td><strong>Hypersensitivity</strong></td>
<td>HLA-B*5701</td>
<td>0.04</td>
<td>10/13 15/19</td>
</tr>
<tr>
<td><strong>Hyperbilirubinemia</strong></td>
<td>UGT1A1*28</td>
<td>0.30</td>
<td>28/37 42/54</td>
</tr>
<tr>
<td><strong>Neutropenia, other toxicity</strong></td>
<td>TPM1*2, *3A, *3B,*3C</td>
<td>0.05*</td>
<td>49</td>
</tr>
<tr>
<td><strong>Stevens-Johnson syndrome</strong></td>
<td>HLA-B*5801</td>
<td>0.15</td>
<td>678</td>
</tr>
<tr>
<td><strong>Stevens-Johnson syndrome</strong></td>
<td>HLA-B*1502</td>
<td>0.04</td>
<td>1023</td>
</tr>
</tbody>
</table>

*Cases required = 2 × 10⁻³ 10⁻⁷
9. To common types of liver injury are termed necrotic or apoptotic.

**Necrosis:**
- Anoxia
- Membrane-damaging xenobiotics
- Mitochondrial toxins
- Blebs
- ATP depletion
- Loss of ion homeostasis
- Cell lysis

**Apoptosis:**
- Signals:
  - Oxidative stress
  - Death receptors
  - Mitochondria
- Caspase- and non-caspase-mediated proteolysis (lamin, fodrin, PARP, actin)
- Internucleosomal DNA degradation
- Phagocytes
- Apoptotic bodies
10. Liver Injury is usually accompanied by a rise of enzymes and other markers in the blood as the cells undergo cell destruction (lyse). Examples are

- **AST** (Aspartate Amino Transferase) Ref. Levels = (0-35 Units/L)
- **ALT** (Aspartate Amino Transferase) Ref. Levels = (0-65 Units/L)
- **BIL** (Total Bilirubin) Ref. Levels = (0.1-1 mg/dL)

AST and ALT levels also rise after a heart attack but BIL does not.

In some cases a liver biopsy is obtained. One type of liver injury occurs with acetaminophen. This is called centrolobular necrosis which is defined as necrosis restricted to the hepatocytes immediately surrounding the central venule. (A below after acetaminophen; B is normal). The clear area in the middle is the central vein.

**B. Flutamide and liver failure.**

Flutamide is an anti-androgen that is used on the treatment of advanced prostate cancer, acne and hirsutism. Flutamide administration has been associated with acute hepatitis and it’s more serious stage acute liver failure (fulminant). As in many cases of DLIL the incidence is fairly low but can be fatal. **Flutamide is a nitroaromatic compound.**

![Proposed Mechanism](image-url)
1. The reduction steps has been shown to be catalyzed by cytochrome P450 reductase (CPR). In general nitroaromatic structures are avoided in new drugs as they are readily reduced and lead to hepatic toxicity.

2. The oxidation is a P450 catalyzed oxidative dehydrogenation. The same type of reaction that produces NAPQI from acetaminophen.

3. Multiple isomers of the GSH adducts are formed. The protein adducts have never been characterized.

4. The blood panels of some affected patients in Chile is shown below. BIL, AST and ALT are massively higher than normal. PT% less than 100 means prolonged bleeding times due to underproduction of prothrombin by the damaged liver. The liver is also unable to convert bilirubin to bilirubin diglucuronide for excretion in bile so bilirubin levels in the blood rise (jaundice). Note that all but one patient recovered after removal of the drug.

5. Cytology indicates that hepatocytes undergo apoptosis rather than necrosis.

<table>
<thead>
<tr>
<th>Indications for treatment</th>
<th>Age</th>
<th>Days of ingestion</th>
<th>Hepatic alterations (maximum)</th>
<th>Hepatitis</th>
<th>Outcome after withdrawal of flutamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Prostate cancer</td>
<td>77</td>
<td>40</td>
<td>2.4  200  159  100%</td>
<td>Acute</td>
<td>Good (1)</td>
</tr>
<tr>
<td>2. Prostate cancer</td>
<td>80</td>
<td>65</td>
<td>0.6  225  273  90%</td>
<td>Acute</td>
<td>Good (2)</td>
</tr>
<tr>
<td>3. Prostate cancer</td>
<td>67</td>
<td>50</td>
<td>40  2,040  2,410  73%</td>
<td>Acute</td>
<td>Good (3)</td>
</tr>
</tbody>
</table>

PT: Prothrombin time (%). BIL: Bilirubin (mg/dL). AST: Aspartate aminotransferase (IU/ml). ALT: Alanine aminotransferase (IU/ml). (1) 60 days; (2) 30 days; (3) 90 days.

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<tr>
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<th>Hepatic alterations (maximum)</th>
<th>Hepatitis</th>
<th>Outcome after withdrawal of flutamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hirsutism</td>
<td>44</td>
<td>90</td>
<td>4.2  390  605  90%</td>
<td>Acute</td>
<td>Good (1)</td>
</tr>
<tr>
<td>2. Hirsutism</td>
<td>27</td>
<td>60</td>
<td>27  1,064  1,803  10%</td>
<td>Fulminant</td>
<td>Good (2)</td>
</tr>
<tr>
<td>3. Hirsutism</td>
<td>32</td>
<td>60</td>
<td>25  1,189  925  38%</td>
<td>Acute</td>
<td>Good (3)</td>
</tr>
<tr>
<td>4. Hirsutism</td>
<td>20</td>
<td>180</td>
<td>20  850  590  15%</td>
<td>Fulminant</td>
<td>Good (4)</td>
</tr>
<tr>
<td>5. Acne</td>
<td>22</td>
<td>180</td>
<td>25  1,576  1,116  14%</td>
<td>Fulminant</td>
<td>Good (5)</td>
</tr>
<tr>
<td>6. Acne</td>
<td>21</td>
<td>65</td>
<td>29  2,778  3,360  10%</td>
<td>Fulminant</td>
<td>Good (6)</td>
</tr>
<tr>
<td>7. Alopecia</td>
<td>38</td>
<td>90</td>
<td>44  2,130  1,430  24%</td>
<td>Fulminant</td>
<td>Died (7)</td>
</tr>
</tbody>
</table>

PT: Prothrombin time (%). BIL: Bilirubin (mg/dL). AST: Aspartate aminotransferase (IU/ml). ALT: Alanine aminotransferase (IU/ml). (1) 1 month. (2) 4 months post transplant. (3) 3 months. (4) 7 months post transplant. (5) 53 months post transplant. (6) 53 months post transplant. (7) Post operative period.

6. The text of the black box warning from Facts and Comparisons. Note first signs and symptoms. Note: Hepatic encephalopathy refers to the loss of mental function the occurs when the liver cannot remove normal toxic substances such as ammonia from the blood.

Hepatic injury:
There have been postmarketing reports of hospitalization and rarely death due to liver failure in patients taking flutamide. Evidence of hepatic injury included elevated serum transaminase levels, jaundice, hepatic encephalopathy and death related to acute hepatic
failure. The hepatic injury was reversible after discontinuation of therapy in some patients. Approximately half of the reported cases occurred within the initial 3 months of treatment with flutamide.

**Serum transaminase levels should be measured prior to starting treatment with flutamide.** Flutamide is not recommended in patients whose ALT values exceed twice the upper limit of normal. Serum transaminase levels should then be measured monthly for the first 4 months of therapy, and periodically thereafter. Liver function tests also should be obtained at the first signs and symptoms suggestive of liver dysfunction (eg, nausea, vomiting, abdominal pain, fatigue, anorexia, “flu-like” symptoms, hyperbilirubinuria, jaundice, right upper quadrant tenderness). If at any time, a patient has jaundice, or their ALT rises above 2 times the upper limit of normal, flutamide should be immediately discontinued with close follow-up of liver function tests until resolution.

C. Halothane is an inhalation anesthetic used in surgery and is hepatotoxic.

1. The use of halothane began in the late 1950s. It was hailed as a new generation of safe, non-flammable inhalation anesthetics. It is discontinued in the USA where it has been supplanted by less toxic alternatives such as sevoflurane and desflurane which are halogenated ethers and do not cause hepatotoxicity. However, it is on the WHO list of essential medicines as it is inexpensive and widely used in the third world.

2. In 1 in 10,000 cases severe liver toxicity is observed. This is often referred to as halothane hepatitis.

3. During halothane anesthesia as much as 30% of the dose is metabolized by P450 enzymes (CYP2E1 mainly) to the acid chloride which undergoes rapid reaction with water to generate trifluoroacetic acid which is found in the urine. Lysine residues of proteins become trifluoro-acylated.

4. Halothane hepatitis is usually seen after repeated use and is believed an immune mediated toxicity where the immune system responds to trifluoroacetylated proteins that are haptons. There may be a genetic component and glutathione depletion is observed in animals.

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“Children are thought to be less susceptible to halothane hepatotoxicity, with an estimated incidence of 1 in 80,000 to 200,000”

-- “The occurrence of rare halothane hepatitis among closely related family members and an increased frequency of the HLA-DR2 haplotype in patients with severe halothane hepatitis suggest a genetic predisposition”
5. There is an example of an idiosyncratic hepatotoxic drug that has been removed from the market. Here the antigen may well be the drug metabolite covalently adducted to the P450 that made it. (Ticrynafen (tienilic acid) and CYP2C9).

C. Isoniazid (anti-tuberculosis; widely used; liver toxicity)


2. Major clearance route via a conjugation reaction with N-acetyl transferase, a liver enzyme. Slow acetylator phenotype (50% of the population) have higher concentrations of the drug however dose does not undergo adjustment for PM’s.
3. We (I) forgot to mention this enzyme when we talked about Phase II reactions. A number of drugs are acetylated by NAT. NAT uses acetyl-CoA as the cosubstrate.

4. There is fairly good evidence that the fast acetylators are at lower risk because the formation of diacetylhydrazine is more rapid and the toxification route is disfavored. However there is some controversy about the mechanism of the human toxicity since the toxicology has been carried out in animals.

5. A portion of the black box warning is shown below. Note the increased risk with alcohol. Alcohol induces CYP2E1 which is believed to be involved in the bioactivation of the acetylhydrazine metabolite (see figure).

**Hepatitis:**
Severe and sometimes fatal hepatitis associated with isoniazid therapy has been reported and may occur or may develop even after many months of treatment. The risk of developing hepatitis is age related. Approximate case rates by age are as follows: less than 1/1,000 for persons younger than 20 years of age, 3/1,000 for persons in the 20- to 34-years of age group, 12/1,000 for persons in the 35- to 49-years of age group, 23/1,000 for persons in the 50- to 64-years of age group, and 8/1,000 for persons older than 65 years of age. The risk of hepatitis is increased with daily consumption of alcohol. Precise data to provide a fatality rate for isoniazid-related hepatitis is not available; however, in a US public health service surveillance study of 13,838 persons taking isoniazid, there were 8 deaths among 174 cases of hepatitis.