Biochemical Mechanisms of Drug Toxicity: Drug-induced Liver Injury (DILI)

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Presentation Outline

Adverse Drug Reactions

• Adverse Drug Reactions
• Drug-induced Liver Injury
  • Intrinsic vs. Idiosyncratic
• Systemic Drug Reactions
• Chemotherapy Adverse Reactions
• Teratogenic Reactions to Drugs

Adverse Drug Reaction (ADR)

Undesirable Drug Effect

• World Health Organization (WHO)
  • "A response to a drug that is noxious and unintended and occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease, or for modification of physiological function"
Adverse Drug Reaction (ADR)

Undesirable Drug Effect

FDA Warns About Teething Medication: Benzocaine

Benzocaine and Drug-induced Methemoglobinemia

- Benzocaine
  - Local anesthetic
  - Used as topical pain reliever or in cough drops
  - Active ingredient in many over-the-counter anesthetic ointments

Adverse Drug Reaction (ADR)

World Health Organization (WHO)

- “a response to a drug that is noxious and unintended and occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease, or for modification of physiological function”

Contradictory effects of drugs

- Therapeutic benefits and adverse effects (range from very insignificant to serious to fatal)

Target locations of toxicity

- Hepatotoxicity
- Nephrotoxicity
- Gastrointestinal Toxicity
- Neurotoxicity
- Otolotoxicity
- Dermatological Toxicity
- Endocrine System
- Hematological Toxicity
- Pulmonary Toxicity
Drug-induced Liver Injury (DILI)  
Adverse Drug Reactions

- Diverse set of responses after exposure to manufactured or natural chemical compounds
- Leading cause of acute liver failure or liver transplantation
- Common reason for regulatory actions concerning drugs

Etiology of Acute Liver Failure (ALF) in the USA
US ALFSG Adult Registry 1998-2014 (N=2102)

Drug-induced Liver Injury (DILI)  
Adverse Drug Reactions

- Diverse set of responses after exposure to manufactured or natural chemical compounds
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Drug – Non-Toxic Metabolites – Excretion

Further Metabolism and/or Excretion

Principles of Clinical Pharmacology, ed. 3. ch.16, Fig.16.4.
Drug-induced Liver Injury (DILI)

Adverse Drug Reactions

- Diverse set of responses after exposure to manufactured or natural chemical compounds
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![Diagram of Drug-induced Liver Injury (DILI)]

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<thead>
<tr>
<th>Intrinsic DILI</th>
<th>Idiosyncratic DILI</th>
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<td>Immunoallergic (hypersensitivity reaction) and Metabolic (‘Toxic’)</td>
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Intrinsic DILI
Drug-Induced Liver Injury (DILI)

- As exposure increase beyond the therapeutic dose, toxicity occurs
- Severity of liver injury increases as exposure increases (dose-dependent)
- Acetaminophen (APAP)
  - Over-the-counter analgesic and antipyretic
  - Dose-dependent hepatotoxicity
  - Safe at therapeutic concentrations but severe hepatotoxicity above therapeutic range
  - Leading cause of acute liver failure in United States
- Two Phase drug-induced toxicity
  - Metabolic Phase (bioactivation-initiating event)
  - Oxidative Phase
  - Large variations in APAP susceptibility
  - Inflammatory stress (exposure to microbes)
  - Alcohol consumption (increase metabolic activation)

Roth R.A. and Ganey P.E. J Pharmacology and Experimental Therapeutics. 2010; 332(3), 692-697

Etiology of Acute Liver Failure (ALF) in the USA
US ALFSG Adult Registry 1998-2014 (N= 2102)

Acetaminophen (APAP)
Phase 1: Metabolic Phase (Bioactivation)
- Acetaminophen (APAP) \( t_{1/2} = 2.0-2.5 \text{ hour} \)
- With hepatic injury, APAP \( t_{1/2} \) increase to >4 hour
- Daily dose of APAP:
  - Adult (>12 yrs.): 4 g/day
  - Children (<12 yrs.): 50-75 mg/kg/day
- Overdose of APAP:
  - Adult (>12 yrs.): >7.5 g/day
  - Children (<12 yrs.): >150 mg/kg/day


Acetaminophen Metabolism via Phase II Conjugation
- Glucuronidation
- Sulfation
- NAC
- Eliminated in Urine
- Reactive Metabolite
- CYP2EI
- 10%
- 5%
- 5%
**Acetaminophen (APA)**

**Phase 1: Metabolic Phase (Bioactivation) and Phase 2: Oxidative Stress**

1. **Phase 1: Metabolic Phase**
   - Acetaminophen (APAP) → N-acetyl-p-benzoquinone imine (NAPQI)
   - NAPQI Reactive Metabolite
   - Covalent Binding to Cell Proteins/Peptides
   - Mitochondrial Permeability (MPT) Increase
   - Hepatocellular Necrosis

2. **Phase 2: Oxidative Stress Phase**
   - Glutathione (GSH) Depletion
   - CYP2EI

*Immune Response to APAP-induced Hepatocellular Necrosis*

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**Drug-induced Liver Injury (DILI)**

- **Adverse Drug Reactions**
- Diverse set of responses after exposure to manufactured or natural chemical compounds
- Leading cause of acute liver failure and transplantation
- Common reason for regulatory actions concerning drugs
- Both types are influenced by pharmacogenetics

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**Idiosyncratic DILI**

- Drug candidates that lead to intrinsic liver injury are usually eliminated during preclinical testing
  - Idiosyncratic DILI account for large percent of postmarketing restrictions by FDA
- Hepatotoxicity not related to pharmacological action of drug
- Limited mechanistic understanding
- Wide range in the severity of toxic effects
- The liver is not the target organ but an acute stress can increase the sensitivity of the liver to injury
  - Example: Stress reduces cytochrome P450 metabolism
  - This reduces drug clearance, which leads to an increase in drug toxicity
- Mechanistic Theories for Idiosyncratic DILI (animal models)
  - Reactive Intermediate Hypothesis
  - Genetic Polymorphism Hypothesis
  - Hapten Hypothesis
  - The Danger Hypothesis
  - Mitochondrial Dysfunction Hypothesis
  - Failure-to-Adapt Hypothesis
  - Multiple Determinant Hypothesis
  - Inflammatory Stress Hypothesis

**Mechanism of Action Theories**

- **Idiosyncratic DILI**
- Reactive Intermediate Hypothesis

**Factors that Affect Sensitivity to Hepatotoxins**

- Age
- Gender
- Metabolism
- Immunologic Reactions
- Pre-existing Condition
- Inflammation
- Environment
- Diet

**Shaw PJ, Ganey PE, and Roth RA. Toxicol Sci. 2010 Nov; 118(1): 7-18**
Mechanism of Action Theories

Idiosyncratic DILI

- Reactive Intermediate Hypothesis
- Genetic Polymorphism Hypothesis

Mechanism of Toxicity Theories

Idiosyncratic DILI

- Reactive Intermediate Hypothesis
- Genetic Polymorphism Hypothesis
- Mitochondrial Dysfunction Hypothesis

Example: Isoniazid
Isoniazid: Mechanism of Toxicity

Idiosyncratic DILI

- First-line agent in the treatment of active or latent tuberculosis
- Mechanism of Action
  - Bactericidal action: Block formation of mycolic acids
  - Mycolic acid: essential component of mycobacterial cell wall synthesis
  - Disruption of cell wall results in cell death
- Animal model used to elucidate biochemical mechanism
  - ~20% of treated patients develop elevated liver enzymes and bilirubin (small percentage develop hepatitis)

NAT2: N-acetyltransferase 2

Isoniazid

\[ \text{CH}_3\text{CO-NH}_2 \text{NH} \text{H}_2 \text{H} \]

Hydrazine

Isonicotinic Acid

\[ \text{CH}_3\text{CO-NH}_2 \text{NH} \text{H}_2 \text{H} \]

Aceetylhydrazine

\[ \text{CH}_3\text{CO-NH}_2 \text{NH} \text{H}_2 \text{H} \]

Diaceetylhydrazine

↑ Mitochondrial Permeability (MPT)

Hepatocellular Apoptosis

GSH Binding/Depletion

Toxic Metabolite

↑ Oxidative Stress

CYP2E1

Principles of Clinical Pharmacology, ed 3. ch.16, Fig. 16.6.
Isoniazid: Mechanism of Toxicity

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![Diagram of Isoniazid metabolic pathway](image)

**Theories of Toxicity**

- **Reactive Intermediate Hypothesis**
- **Genetic Polymorphism Hypothesis**
- **Mitochondrial Dysfunction Hypothesis**

![Diagram of mitochondrial dysfunction](image)

**NAT2: N-Acetyltransferase 2**

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**Hapten Hypothesis**

**Idiosyncratic DILI**

- Drug-induced hepatotoxic reaction mediated by an immune response
- Chemically reactive drug or reactive metabolite (hapten) binds endogenous protein that initiates an immune response
  - Macromolecule-drug adduct: neoantigen
- Example Drug: Halothane
  - Volatile general anesthetic
  - 2 types of hepatotoxicity of Halothane
    1. Subclinical increase in transaminase enzymes (in blood)
    2. Fatal hepatitis-like reaction (severe hepatocellular necrosis)

![Diagram of Halothane metabolic pathway](image)

**Hapten Hypothesis**

**Idiosyncratic DILI**

- Drug-induced hepatotoxic reaction mediated by an immune response
- Chemically reactive drug or reactive metabolite (hapten) binds endogenous protein that initiates an immune response
  - Macromolecule-drug adduct: neoantigen
- Example Drug: Tienilic Acid (Ticrynafen)
  - Uricosuric diuretic (marketed in 1979 and withdrawn within a few months)
  - Hepatitis-like adverse reaction (Immunologic response)

![Diagram of Tienilic Acid metabolic pathway](image)
**Inflammatory Stress Hypothesis**

*Idiosyncratic DILI*

- Drug therapy coupled to inflammatory stress precipitates drug-induced liver injury
- Inflammatory stress induced by several factors
  - Infection, intestinal microbial disturbance, cell death, etc.
- Example Drug: Trovafloxacin (Trovan, Pfizer)
  - Broad-spectrum fluoroquinolone antibiotic
  - ~140 severe hepatic reactions reported; 14 resulted in liver failure
  - Restrictions in 1999 and withdrawn in 2001—due to hepatotoxicity
  - Mode of action for hepatotoxicity identified via animal model
  - Mechanism of action:
    - Bactericidal activity exerted by parent compound (no reactive metabolite)
    - Inhibit bacterial DNA gyrase (topoisomerase II) and Topoisomerase IV

**Trovafloxacin (TVX)**

**TVX and Inflammatory Stress via Animal Model**

- Mode of action for hepatotoxicity: inflammation-drug interaction animal models

**Effect of TVX/LPS co-treatment on liver in mice model**

- Detectable liver injury
  - Global Gene Profiling:
    - Genes involved in interferon signaling (IFN-γ) play a role in TVX/LPS co-exposure
    - Early Marker of Hepatotoxicity
Trovafloxacin (TVX)

TVX and Inflammatory Stress via Animal Model

- Mode of action for hepatotoxicity: inflammation-drug interaction animal models

Mechanism of Toxicity Theories

**Idiosyncratic DILI**

- Inflammatory Stress Hypothesis: common link between the six theories

Systemic Drug Reactions

**Drug-induced Adverse Effects (Drug Allergy)**

- Drug hypersensitivity
  - Immunologically mediated response to drug in a sensitized person (allergy or autoimmunity)
  - Reactions are classified into four groups

<table>
<thead>
<tr>
<th>Type</th>
<th>Complex Type</th>
<th>Mechanism</th>
<th>Clinical Manifestations</th>
<th>Time of Onset</th>
<th>Example Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I (IgE-mediated)</td>
<td>Specific IgE bind to mast cells and cause release of histamine, inflammatory mediators</td>
<td>Anaphylaxis, Urticaria</td>
<td>Minutes to hours after exposure (immediate)</td>
<td>Penicillin</td>
<td></td>
</tr>
<tr>
<td>Type II (IgG or IgM)</td>
<td>Specific IgG or IgM antibodies directed at drug epitopes coated cell</td>
<td>Hemolytic anemia, Necrotic pneumonia, Thrombocytopenia</td>
<td>Hours to days (variable)</td>
<td>Cefotetan</td>
<td></td>
</tr>
<tr>
<td>Type III (Immune complex)</td>
<td>Tissue deposition of drug-antibody complexes with complement activation and inflammation</td>
<td>Arthralgia, Fever, Gastroenteritis, Lymphadenopathy, Joint stiffness, Rash, Urticaria, Vasculitis</td>
<td>1 to 3 weeks after exposure</td>
<td>Infliximab</td>
<td></td>
</tr>
<tr>
<td>Type IV (delayed, cell-mediated)</td>
<td>Delayed presentation of drug, involvement of T cells with cytokine and inflammatory mediator release</td>
<td>Contact dermatis, Necrolytic migratory drug rash</td>
<td>2 to 7 days after exposure</td>
<td>Penicillin</td>
<td></td>
</tr>
</tbody>
</table>
Hypersensitivity Reactions
Classification of Reactions - Gell and Coombs

Type I
Type II
Type III
Type IV

Hapten: chemical moiety too small to elicit an immune response

Panels: (Diagrams illustrating the mechanisms of Type I, II, III, and IV reactions)

Penicillin
Hypersensitivity Reactions (Type I and IV)

Penicillinergic Reactions
Panels: (Diagrams showing the chemical structure of penicillin and its derivatives)
Penicillin

Hypersensitivity Reactions (Type I and IV)

Type I Reaction (IgE-mediated)
- Urticaria, Angioedema, Wheezing, Anaphylaxis
- Penicillin skin test
- Positive
- Graded-dose challenge
- Desensitization

Type IV Reaction (Cell-mediated)
- Typical lesions of chronic urticaria

Penicillenic Acid
- Penicilloyl-Protein Haptenation Product
- Penicillamine Haptenation Product
- Side Chain
- Beta-lactam ring
- Thiazolidine ring

Clinical Decision for Penicillin Allergy

Penicillin

Hypersensitivity Reactions (Type I and IV)

Infliximab (Remicade®)

Hypersensitivity Reactions (Type III)

- Infliximab
  - Chimeric monoclonal antibody (mAb) to human tumor necrosis factor (TNF-α)
  - mouse (variable region)-human (IgG1 constant region)
  - Approved by FDA for treatment of Crohn’s disease (1998) and Rheumatoid Arthritis (RA-1999)
  - Also used in Psoriasis, Ankylosing spondylitis (AS), and Behçet’s disease

Mouse (TNF-α binding site)

Infliximab (Remicade®)

Human (IgG1)

Type III
Infliximab (Remicade®)
Mechanism of Tumor Necrosis Factor (TNF-α)


Infliximab (Remicade®)
Mechanism of Drug Activity


Infliximab (Remicade®)
Mechanism of Drug Activity

Infliximab (Remicad®)

Drug Toxicity

  - Association with a 2- to 8-fold increase in risk for tuberculosis, listeriosis and histoplasmosis
  - Increased incidence in European countries as well
- Anti-TNF-α activity leads to increased risk of granulomatous infections
  - TNF: essential for host defenses against Mycobacterium tuberculosis
  - TNF is involved in the activation of specific macrophages enzymes (iNOS) which are vital for bacterial elimination

Distribution of Tuberculosis cases in relation to Anti-TNF therapy


Effect of Anti-TNF therapy on M. tuberculosis Infection in Mice

Control Mouse Lung
Transgenic Mouse Lung
Adverse Effect of Chemotherapy

**Drug Toxicity**

- Basic principle: inhibit the mitotic and metabolic process of cancer cells
  - Cancer drugs either inhibit cellular proliferation or target specific proteins vital to the expansion of abnormal cells
- Normal cells and tissues are also affected, leading to various mild and severe adverse effects
- Chemotherapy has improved significantly but the applicability of the drugs are limited due to the risk of chemotherapy-induced cardiotoxicity
- Two types of chemotherapy-induced cardiotoxicity
  - **Type I**
    - Irreversible myocyte destruction
    - Example: Anthracyclines (Doxorubicin)
  - **Type II**
    - Reversible myocyte damage (broad range of incidence and severity)
    - Example: Trastuzumab

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Type I Cardiotoxicity

**Chemotherapy-induced Drug Toxicity**

- Anthracyclines (Doxorubicin)
  - Well-studied class of cardiotoxic anti-cancer agents
  - Dose-dependent toxicity
  - Mechanism of action: target Topoisomerase II (Top 2) and form a Top 2-DNA complex that ultimately triggers cell death

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**Three proposed mechanisms for cardiotoxicity**

1. Formation of ROS to induce oxidative stress
2. Impaired Diastolic Relaxation
3. Inhibit prosurvival signaling pathways (NKG2-D1 and EnkB inhibition)
Type II Cardiotoxicity
Chemotherapy-induced Drug Toxicity

- Trastuzumab (Herceptin)
- New generation of targeted chemotherapy
- Humanized monoclonal antibody (mAb)
- Mechanism of action: Binds to extracellular domain of human epidermal growth factor 2 (HER2 or ErbB2) - inhibits proliferation of cancer cells
- HER2-positive breast carcinoma treatment
- Cardiotoxicity occurs in ~20-33% of patients treated with drug (mild and reversible)

Teratogenic Reactions to Drugs
Drug Toxicity

- A teratogen is an agent that can disturb the development of the embryo or fetus
- Use of drugs in pregnancy is complicated due to potential for harmful effects on growing fetus
- Pharmacokinetic changes in pregnancy
- Placental transfer of drugs from mother to fetus
Teratogenic Reactions to Drugs

Drug Toxicity

• A teratogen is an agent that can disturb the development of the embryo or fetus
• Use of drugs in pregnancy is complicated due to potential for harmful effects on growing fetus
• FDA established a system to classify drugs to indicate the potential of a drug to cause birth defects during pregnancy (based on human and animal data)

- **A** - "Safe", adequate data to demonstrate no risk (1st trimester) - Examples: Levothyroxine, Folic Acid, Magnesium Sulphate
- **B** - Fetal risk confirmed in human studies but not animal study - Examples: Metformin, Amoxicillin, Pantoprazole
- **C** - Fetal risk confirmed in animal studies but not human study - Examples: Tramadol, Gabapentin, Amitriptyline, Prednisone
- **D** - Fetal risk shown in humans, use if benefit outweighs risk - Examples: Lisinopril, Alprazolam, Losartan, Lorazepam
- **X** - "Proven teratogen", benefit does not outweigh risk - Examples: ACE inhibitors, Metformin, Flutamide

Teratogenic Reactions to Drugs

Drug Toxicity

• A teratogen is an agent that can disturb the development of the embryo or fetus
• Use of drugs in pregnancy is complicated due to potential for harmful effects on growing fetus
• Angiotensin-converting enzyme (ACE)-inhibitors
  • Treatment of hypertension and congestive heart failure
  • Cause relaxation of blood vessels
  • Mechanism of action: reduce activity of renin-angiotensin-aldosterone (RAAS)

Presentation Summary

Adverse Drug Reactions

I. Adverse Drug Reactions
   a. Methemoglobin-Benzoic Acid
II. Drug-induced Liver Injury (DILI)
   a. Intrinsic
      1. Acetaminophen (APAP)
   b. Idiosyncratic
      1. Mechanistic Theories (8)
         2. Immune
      3. Hepatitis C Virus (HCV)
      4. Pseudoreversion (PVR)
III. Systemic Drug Reactions
    a. Gell and Coombs Classification of Drug Reactions
    b. Penicillin
    c. Infliximab
IV. Chemotherapy Adverse Reactions
    a. Cardiotoxicity
    b. 2 Types
       1. Type I: Doxorubicin
    c. Type II: Trastuzumab
V. Teratogenic Reactions to Drugs
   a. FDA Classification of Drugs
   b. ACE Inhibitors