Drug Toxicity I

Toxicology: Molecular Mechanisms

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Lecture objectives

After this lecture, and further reading as required, students will be able to:

• explain how drugs are important agents for poisoning
• describe the manifestations of toxicity
• outline the major molecular mechanisms of toxicity and how drug metabolites may be toxic
• explain how toxic potential of a drug can be quantified using a variety of methods including carcinogenicity, mutagenicity, teratogenicity, allergy testing
• explain LD_{50} values and therapeutic index
• evaluate the benefits and limitations of animal testing to predict human toxicity
**Pharmacology:**
the study of the effect of drugs on the function of living systems
[origin: Gk *pharmakon* = drug]

**Toxicology:**
the study of the effect of **poisons or toxicants**
on the function of living systems

Chemical agents that cause toxicity include:
• Drugs
• Insecticides/herbicides
• Plant toxins
• Animal toxins
• Chemical weapons
• Radioactive elements
Paracelsus (1493-1541) ‘Grandfather of Toxicology’

"All things are poison and nothing is without poison, only the dose permits something not to be poisonous."

“The dose makes the poison”

therapeutic effect

increasing dose

toxic effect
Adverse Drugs Reactions (ADRs)

ADRs are noxious or unintended responses occurring at **therapeutic** doses (WHO definition) ~ 5% of all acute hospital admissions

| Type A (augmented) ADRs | Effects are:  
| · related to known pharmacology, but undesirable  
| · common, dose-related  
| · predictable | Examples  
| · haemorrhage with anticoagulants  
| · respiratory depression with opioids  
| · sedation with anxiolytic and older antihistamine drugs |

| Type B (bizarre) ADRs | Effects are:  
| · unrelated to known pharmacology  
| · rare  
| · unpredictable  
| · often idiosyncratic | Examples  
| · anaphylaxis with penicillin  
| · allergic liver damage by halothane  
| · bone marrow suppression by chloramphenicol  
| · individual allergy/genetic basis |
Toxicokinetics

the effects of the body on the poison
(relates to Absorption, Distribution, Metabolism, Excretion (ADME)).

With this information it is possible to predict concentration of toxin that reaches the site of injury and the resulting damage.

Absorption

- ingestion
  - mercury and dioxin in fish
  - pesticides in farm produce
  - salmonella (diary), botulinum (meat) toxins
  - inhalation
  - asbestos (Cd), nerve gases

Distribution

as discussed for therapeutic drugs
Toxicokinetics

**Metabolism**
- Phase I by cytochrome P450 (oxidation, reduction, hydrolysis)
- Phase II conjugation to allow excretion in urine and bile

**Detoxification**: compound rendered less toxic
**Toxification**: relatively inert compound converted into toxin

**Excretion**
- Toxins not excreted may be stored in:
  - bone (eg. lead)
  - fat (eg. DDE a metabolite of the pesticide DDT)
  - (Dichlorodiphenyl trichloroethane)
- The toxin may be released slowly into the body
Molecular Mechanisms of Toxicology

1. Allergic responses

Common form of ADR, usually with a different time course to pharmacological effects

4 basic clinical syndromes – types I, II, III & IV (Gell & Combes, 1963)

Type I hypersensitivity reaction – IgE-mediated mast cell degranulation

Type II antibody-mediated cytotoxic hypersensitivity involve haematological reactions i.e. those pertaining to the blood cells and blood-forming organs

Type III immune complex-mediated hypersensitivity

Type IV delayed-type hypersensitivity
Molecular Mechanisms of Toxicology
Type I hypersensitivity reactions can trigger anaphylactic shock

- **hapten**
- **low MW allergen** (e.g. bee venom, peanut oil)
- **immunogenic conjugate** (e.g. penicillin 75% of all deaths)
- **IgE recognition triggers**
  - histamine release
  - bronchoconstriction
  - vasodilation
  - inflammation
  - treated with adrenaline

75% of all deaths
Molecular Mechanisms of Toxicology

Type II hypersensitivity reactions deplete blood cell types

These reactions can deplete:
- Red blood cells (haemolytic anaemia) eg. sulfonamides
- Neutrophiles (agranulocytosis) eg. certain NSAIDs
- Platelets (thrombocytopenia) eg. quinine and heparin
Molecular Mechanisms of Toxicology

2. Receptor, ion channel and enzyme-mediated toxicity

Molecular drug/toxin targets

**Receptors** (4 major superfamilies)
- Ligand-gated ion channels
  - ionotropic receptors
  - voltage-gated ion channels
- GPCRs - G protein coupled receptors (metabotropic receptors)
- Enzyme-linked receptors (tyrosine kinase activity)
- Nuclear receptors (regulate gene transcription)

**Enzymes** metabolic and catabolic pathways

**Carriers** uptake/transport systems

**Others** proteins involved in vesicle release
## Molecular Mechanisms of Toxicology
### Sources of toxins

<table>
<thead>
<tr>
<th>Source</th>
<th>Active agent</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Amanita phalloides</em></td>
<td>α-amanitin</td>
<td>inhibits RNA polymerase</td>
</tr>
<tr>
<td><em>Digitalis lanata</em></td>
<td>digoxin/digitoxin</td>
<td>Na⁺/K⁺ ATPase inhibitor</td>
</tr>
<tr>
<td>Calabar (ordeal) bean</td>
<td>physostigmine</td>
<td>anticholinesterase</td>
</tr>
<tr>
<td><em>Atropine belladonna</em></td>
<td>atropine</td>
<td>blocks muscarinic AChR</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clostridium botulinum</em></td>
<td>botulinum toxin</td>
<td>inhibits synaptic protein</td>
</tr>
<tr>
<td><em>Cholera vibrio</em></td>
<td>cholera toxin</td>
<td>activates Gα₉ proteins</td>
</tr>
<tr>
<td><em>Bordetella pertussis</em></td>
<td>pertussis toxin</td>
<td>inhibits Gα₁ₒ proteins</td>
</tr>
</tbody>
</table>
### Molecular Mechanisms of Toxicology

#### Animal sources of venoms and toxins

<table>
<thead>
<tr>
<th>Source</th>
<th>Active agent</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kraits (elapid snakes)</td>
<td>α-bungarotoxin</td>
<td>blocks nicotinic AChR</td>
</tr>
<tr>
<td>Green mamba snakes</td>
<td>dendrotoxins</td>
<td>block K⁺ channels</td>
</tr>
<tr>
<td>Funnel web spider</td>
<td>ω-agatoxin</td>
<td>blocks Caᵥ2.1 Ca²⁺ channels</td>
</tr>
<tr>
<td>Coneshell</td>
<td>ω-conotoxin</td>
<td>blocks Caᵥ2.2 Ca²⁺ channels</td>
</tr>
<tr>
<td>Tarantula spider</td>
<td>SNX-482</td>
<td>blocks Caᵥ2.3 Ca²⁺ channels</td>
</tr>
<tr>
<td>Puffer fish</td>
<td>tetrodotoxin</td>
<td>blocks Na⁺ channels</td>
</tr>
<tr>
<td>Frog (<em>Dendrobates</em>) skin</td>
<td>cardiac glycosides</td>
<td>Na⁺/K⁺ ATPase inhibitor</td>
</tr>
</tbody>
</table>
Animal toxins block ion-conduction

$\alpha$-bungarotoxin on nicotinic acetylcholine receptor (nAChR)

Banded krait
($Bungarus$ $multicinctus$)
Voltage-gated $K^+$ channels are blocked by dendrotoxins

Black mamba
(Dendroaspis polylepis)

Green mamba
(Dendroaspis angusticeps)
Voltage-gated Ca$^{2+}$ channels are important toxin targets

Funnel web spider

ω-agatoxin (Ca$_{V}$2.1)

Coneshell

ω-conotoxin (Ca$_{V}$2.2)

Tarantula spider

SNX-482 (Ca$_{V}$2.3)

Ca$^{2+}$ current recording from a sensory neuron in pain pathway (Wilson et al. 2001)
Tetrodotoxin acts on Na\(^+\) channels to block action potentials

Puffer fish

Tetrodotoxin (TTX)
Molecular Mechanisms of Toxicology

Enzyme-mediated toxicology

“You are walking through a crowded shopping mall, when you hear a soft ‘pop’ and see smoke coming from the other end of the mall. You immediately notice dim vision, and your nose begins to run severely. Less than 1 minute later, you notice shoppers collapsing to the floor, breathing heavily, some of them losing consciousness and developing seizure activity. You notice that their pupils are constricted. You immediately grab 2 small children near you, cover your nose and mouth with your jacket, and run out of the mall”


US Army Medical Research Institute of Chemical Defense
Irreversible anticholinesterase eg. parathion and sarin

Enzyme active site

Catalytic site

Histidine

Serine

Glutamate

Histidine

No hydrolysis - de novo synthesis needed
Oximes are strong nucleophiles that reactivate AChesterase

\[ \text{pralidoxime} \]

\[ \text{histidine} \]

\[ \text{serine} \]

\[ \text{glutamate} \]

\[ \text{catalytic site} \]

\[ \text{anionic site} \]
First line of defence against biological nerve gases:

- Atropine - mAChR blocker - central respiratory depression
- Pralidoxime - reactivation of acetylcholinesterase

Reactivation of plasma cholinesterase (ChE) in a volunteer subject by intravenous injection of pralidoxime. (Sim V M 1965 J Am Med Assoc 192: 404.)
Molecular Mechanisms of Toxicology

3. Biochemical pathways

(i) Cyanide inhibits mitochondrial cytochrome c oxidase to prevent cellular respiration

(ii) Carbon monoxide: displaces oxygen from haemoglobin causing hypoxia
Molecular Mechanisms of Toxicology
4. Organ-Directed Toxicity

Organs particularly susceptible to toxin damage are the liver and kidney

Hepatotoxicity
(i) hepatic necrosis
   paracetamol poisoning

(ii) hepatic inflammation (hepatitis)
   halothane can covalently bind to liver proteins to trigger an autoimmune reaction

(iii) chronic liver damage (cirrhosis)
   long-term ethanol abuse causes cellular toxicity and inflammation and malnutrition as ethanol becomes a food source
Paracetamol is a prominent cause of hepatic poisoning (48% of all poison admissions and >200 deaths/year)

Overdose:
(i) enzymes saturation
(ii) glutathione depletion

Treatment:
Acetylcysteine
Methionine
(glutathione precursors)
Molecular Mechanisms of Toxicology
Organ-Directed Toxicity

Nephrotoxicity

(i) changes in glomerular filtration rate (GFR)
   Largely due to drugs that alter blood flow:
   NSAIDs (eg. aspirin) reduce prostaglandins which in turn reduces blood flow/GFR
   ACE inhibitors (eg. ramipril) increase blood flow/GFR

(ii) allergic nephritis
   allergic reaction to NSAIDs (eg. fenoprofen) and antibiotics (eg. metacillin)

(iii) chronic nephritis
   long-term NSAID and paracetamol use
Molecular Mechanisms of Toxicology
5. Mutagenesis and carcinogenesis

Mutagens cause changes to cell DNA that are passed on when cell divides, if this produces a neoplastic cell the agent is termed a carcinogen.

2 major classes of gene are involved in carcinogenesis:
• Proto-oncogenes: promote cell cycle progression eg. constitutive activity of growth factor tyrosine-kinase receptors can cause neoplastic transformation

• Tumour-suppressor genes: inhibit cell cycle progression eg. mutations in tumour suppression gene product p53 (prevalent in smokers)
Molecular Mechanisms of Toxicology

6. Teratogenicity

**Teratogenesis**: the creation of birth defects during fetal development

**Teratogens**: substances that induce birth defects

Thalidomide

(R)-enantiomer
sedative

(S)-enantiomer
teratogen
The thalidomide disaster heralded modern teratogenicity testing

• 1950’s- thalidomide was synthesized by the Grünenthal

• Non-toxic at high doses in all animals species tested

• 1957 - marketed throughout Europe in as Contergan a non-lethal hypnotic and sedative, recommended as an anti-emetic to treat morning sickness in pregnant women

• 1961 - thalidomide was the best-selling sleeping pill in West Germany and the UK

• However, thalidomide produced teratogenic effects in 100% of foetuses exposed between 3-6 weeks gestation
The thalidomide disaster heralded modern teratogenicity testing

• An estimated 8-12,000 infants were born with deformities caused by thalidomide, and only about 5,000 of these survived beyond childhood

• 1968 - Contergan case was brought to trial

• 1970 - court dismissed the case due to only minor responsibility of Grünenthal and "minor importance to the public of the Federal Republic of Germany"

• In fact, thalidomide is a useful drug, used today to treat leprosy and multiple myeloma (probably due to inhibitory activity on tumour necrosis factor (TNF)-α production)
### Drug effects on fetal development

<table>
<thead>
<tr>
<th>Stage</th>
<th>Gestation period</th>
<th>Cellular process</th>
<th>Affected by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastocyte formation</td>
<td>0-16 days</td>
<td>Cell division</td>
<td>Cytotoxic drugs, Alcohol</td>
</tr>
<tr>
<td>Organogenesis</td>
<td>~17-60 days</td>
<td>Division, migration, differentiation, death</td>
<td>Teratogens (thalidomide, retinoids, antiepiletics, warfarin)</td>
</tr>
<tr>
<td>Maturation</td>
<td>&gt;60 days</td>
<td>As above</td>
<td>Alcohol, Nicotine, ACE inhibitors, Steroids</td>
</tr>
</tbody>
</table>
Drug Toxicity II

Toxicology:
Treatment and prevention
Stages of drug development

~50 projects

12

Drug discovery

Preclinical development

5

pharmacological selection

toxicity testing

3

Phase I

test in healthy (~20-80) volunteers

2-5

Phase II

~2

small scale test in (~100-300) patients

2-5

Phase III

~2

large scale (~1000-5000) controlled trial

5-7

Phase IV

1

post-marketing surveillance
## Stages of drug development

<table>
<thead>
<tr>
<th>Phase</th>
<th>Number of Patients</th>
<th>Questions</th>
</tr>
</thead>
</table>
| Phase I | 100 – 200 Healthy Subjects | • Does it *seem* safe in humans?  
• What does the body do to the drug (pharmacokinetics)?  
• What does the drug do to the body (pharmacodynamics)?  
• *Might* it work in patients? |
| Phase II| 200 – 300 Patients   | • Does it *seem* safe in patients?  
• Does it *seem* to work in patients? |
| Phase III| 1,000 – 3,000 Patients | • Does it *seem* safe in patients?  
• Does it *really* work? |
| Phase IIIb | Hundreds - Thousands Patients | • Does it *seem* safe in a different group of patients?  
• Does it *really* work in a different group of patients? |
| Phase IV | Tens to many thousands Patients | • Is it *truly* safe?  
• How does it compare with similar drugs? |
Preclinical drug development testing

To assess genotoxic potential a battery of tests are used:

*in vitro* tests for mutagenicity *eg* Ames test
- strains of *Salmonella typhimurium* bacteria cannot synthesis histidine
- mutant grown on histidine-containing media
- drug and a liver microsomal enzyme preparation (to test for reactive metabolites) added
- histidine becomes depleted and only back-mutants can grow
- mutation rate measured
Preclinical drug development testing

*in vitro* cytogenetic evaluation of chromosome damage *in* response to drug

- **carcinogenicity testing:**
  chronic drug dosing; look for tumours

- **reproductive (teratogenicity) testing:**
  pregnant females from one rodent species and one non-rodent (usually rabbit) species dosed with drug at different organogenesis stages outlined previously; look for birth defects
Preliminary toxicity testing

Maximum non-toxic dose (given for 28 days to 2 species). Animals examined post-mortem and tissue damaged assessed

Lethal dose $\text{LD}_{50}$ - the dose of drug which kills 50% of treated animals within a specified short amount of time

![Graph showing the relationship between log [drug] (M) and toxic response. The graph shows a curve that reaches a maximum at about $\text{LD}_{50}$.](image-url)
Preliminary toxicity testing

**NOAEL** (no observed adverse effects level)
Highest concentration that does not Produce a toxic response

**LOAEL** - lowest observed adverse effects level
Lowest concentration that produces a toxic response
Preliminary toxicity testing

NOAEL (no observed adverse effects level)
Highest concentration that does not a toxic response

1. Determine NOAEL

2. Convert NOAEL to a ‘Human Equivalent Dose’ (HED)
   - Adjust for anticipated exposure in humans
   - Adjust for inter-species difference in affinity and potency

3. Apply >10 fold safety factor
Preliminary toxicity testing

Calculating HED (Human Equivalent Dose)

**NOAEL:** dog 50 mg/kg
**LD$_{50}$ values for different toxins**

<table>
<thead>
<tr>
<th>Toxicity rating</th>
<th>Example</th>
<th>LD$_{50}$ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slightly toxic (5-15 g/kg)</td>
<td>Ethanol</td>
<td>8000</td>
</tr>
<tr>
<td>Moderately toxic (0.5-5 g/kg)</td>
<td>Sodium chloride</td>
<td>4000</td>
</tr>
<tr>
<td></td>
<td>Parathion</td>
<td>1300</td>
</tr>
<tr>
<td>Very toxic (50-500 mg/kg)</td>
<td>Aspirin</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>Paracetamol</td>
<td>300</td>
</tr>
<tr>
<td>Extremely toxic (5-50 mg/kg)</td>
<td>Theophylline</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Diphenhydramine</td>
<td>25</td>
</tr>
<tr>
<td>Super Toxic (&lt;5 mg/kg)</td>
<td>Potassium cyanide</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Digoxin</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Tetrodotoxin</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Botulinum toxin</td>
<td>0.00001 (10 ng/kg)</td>
</tr>
</tbody>
</table>
Therapeutic index

The ratio of the dose of the drug that produces an unwanted (toxic) effect to that producing a wanted (therapeutic) effect

\[ \text{TI} = \frac{\text{LD}_{50}}{\text{ED}_{50}} \]

Small TI: e.g. warfarin

Large TI: e.g. penicillin, aspirin
Preliminary toxicity testing

The oral LD$_{50}$ of a new drug was determined in rats.

Q. What can this value tell us:

A. Short term, lethal effects
B. Long-term, lethal effects
C. Long-term, non-lethal effects
D. Potential Type B adverse drug reactions
E. Lethal dosage when injected
F. Toxicity in young and old humans
Why do we need toxicity testing……..

The **Elixir Sulfanilamide** disaster of 1937 was one of the most consequential mass poisonings of the 20th century.

Sulfanilamide was diluted in diethylene glycol to give a red Elixir Sulfanilamide.

One hundred and five patients died from its therapeutic use.

Under the existing drug regulations, premarketing toxicity testing was not required.

In reaction, the U.S. Congress passed the **1938 Federal Food, Drug and Cosmetic Act**, which required proof of safety before the release of a new drug.
The TGN1412 disaster has highlighted need for accurate toxicity testing

- TGN1412 is a **monoclonal antibody** (MAB) designed to bind CD28 protein to activate leucocytes

- TGN1412 could fight leukaemia by triggering cytokine release

- Animal studies of TGN1412 indicated **no toxicity**

- 6 volunteers were given **1:500** dilutions of doses used in animal studies at 30 minute intervals according to agreed protocols. A further 2 volunteers received a **placebo**

- Within minutes of the 6\textsuperscript{th} volunteer receiving the dose, **serious side-effects** occurred severe headache, backache, fever and pain leading to brief coma, kidney failure, head swelling
Potential flaws in the TGN1412 study

- Lack of biological knowledge (of how CD28 works)
- Use of healthy volunteers with intact immune response could trigger a ‘cytokine storm’
- TGN1412 works differently between species (mainly human protein)
- Dose regime too short (i.e. given too frequently)
- Testing should have been staggered over several days
- Problem with contaminants in formulation (later discounted)
- Suggested improvement: **Blister test** - expose small amount of skin to drug to check adverse reaction prior to whole body exposure
Summary: Treatment and prevention of toxicity

1. Preclinical toxicity testing is a vital part of drug development

2. New compounds must be assessed in particular for mutagenic, carcinogenic and teratogenic potential

3. Preliminary toxicity testing typically uses LD$_{50}$ and NOAEL, LOAEL values

4. LD$_{50}$ experiments are not perfect

5. Prevention of toxicity is based on knowledge of molecular mechanisms of toxin action