Clinical trials:
Safety drug assessment

Lecture 14
Preclinical toxicology

Before human studies, it is necessary to demonstrate safety \textit{in vitro} and \textit{in vivo}.

We assume that

- \textit{in vitro} assays predict \textit{in vivo} effects
- the effects of chemicals in laboratory animals apply to humans
- the use of high doses in animals is valid for predicting possible toxicity in humans.

These assumptions are broadly true, but despite this, we cannot be certain that a chemical will show \textbf{no} toxic effects in humans.
Why do compounds still fail once they reach clinical trials?

Only about 1 in 9 compounds going into Phase 1 clinical trials will become marketed medicines.

We have improved our ability to predict pharmacokinetics in man. Now we need to improve our toxicology predictions.
What do we already know about toxicity?

Major reasons for toxic effects can include:

- Mechanism based pharmacology
- Formation of reactive metabolites
- Activation of other receptors
- Interactions with other substances
- Idiosyncratic toxicity
Caused when activation of the target causes unwanted effects as well as the desired therapeutic effect.

Balance of good/bad effects.

Usually not predictable from *in vitro* tests, but can sometimes be predicted from animal models.

A big **potential** problem with drugs designed for completely novel targets, rather than new drugs for a known mechanism.
Case study: beta agonists

- β-agonists (e.g. salbutamol) are used to control asthma by causing activation of the $\beta_2$ receptors in the lung. This causes the airways to dilate.

- These compounds are taken by inhalation, so most of the drug stays in the lung.

- If the patient takes too much medicine, the levels in the systemic circulation rise and can now affect the $\beta_2$ receptors in the heart causing palpitations (abnormality of heartbeat).
Formation of reactive metabolites

- We don’t want chemically reactive medicines! What functional groups might we want to avoid? e.g.

\[
\begin{align*}
\text{RCl} & \quad \text{RNH} & \quad \text{R} \equiv \text{O} \\
\end{align*}
\]

- These are all electrophiles, which means that they can covalently bind to nucleophiles in the body, e.g. in proteins and DNA which lead to toxic effects.

- Most common effects are hepatotoxicity (liver) & genotoxicity (DNA).

- But don’t forget that in the body, chemicals are metabolised so we need to consider the fate of our new medicine – will any of the metabolites be chemically reactive?
Some unwanted groups?
Some unwanted groups!

- Electrophilic aromatics
- Chloroamines
- Terminal acetylenes
- Alkylsuphohonate esters
- Nitro
- Alkylhalides
- Epoxides
- Certain phenols
- Disulphides
- Anilines
- Hydrazines
- Masked anilines
- Aziridines
- Electrophilic esters
- Mono-substituted furans and thiophenes
- Michael acceptors
- Azo
- Azo
- Isocyanates
- Acylating agents
- Certain phenols
- Certain phenols
Case study: paracetamol

• mild analgesic.
• used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies.
• in combination with opioid analgesics, paracetamol can also be used in the management of more severe pain such as post-surgical pain and providing palliative care in advanced cancer patients.
• it exhibits only weak anti-inflammatory activity.

http://www.youtube.com/watch?feature=player_detailpage&v=WOavayV9ENk
http://www.youtube.com/watch?feature=player_detailpage&v=N6L5nKe-UuQ
Case study: paracetamol

paracetamol

phase 1 oxidation

\[ \text{paracetamol} \rightarrow \text{N-acetyl-4-benzoquinone imine} \]

normal phase II metabolism

paracetamol

reaction with glutathione

reaction with protein

Toxic effect

phase 1 oxidation

N-acetyl-4-benzoquinone imine

\[ \text{N-acetyl-4-benzoquinone imine} \rightarrow \text{Glutathione} \]

\[ \text{paracetamol} \rightarrow \text{Glucuronide} \]

Urinary excretion

Glutathione

\[ \text{Glutathione} \rightarrow \text{glutathione} \]
How to avoid the problem?

- Most obviously, avoid functional groups known to show reactive metabolites (not an absolute – some are worse than others).
- Test for the presence of reactive groups
  - Look for binding to proteins or glutathione - detect by mass spectroscopy
- ‘Ames’ test to detect mutagenicity
  - Gastrointestinal track bacteria can modify drugs.
  - Expose bacteria to drug candidate and observe changes
  - This test can also be carried out in the presence of liver enzymes to look for mutagenic metabolites.
Activation of other receptors/enzymes

- Sometimes known as ‘off-target toxicity’.

- Screen against other systems – similar targets will be done early on in the project. Before nomination to preclinical studies, the compound will be tested in many other assays to look for activity.

- Potency (and therefore dose) is important as we are looking for a safety margin, i.e. the absolute potency at another receptor is less important than how much less than the potency at the primary receptor it is.
hERG

- hERG = ‘human ether-a-go-go related gene’
- codes for the alpha subunit of the potassium channel
- Activation causes prolongation of electrical impulses regulating the heart beat
- Can lead to fatal arrhythmias

Normal heart beat

Activation of hERG

‘T’ wave is delayed
Why is hERG important?

Lots of marketed drugs bind to it, with apparently diverse structures.

e.g.

- terfenadine (antihistamine)
- grepafloxacin (antibiotic)
- astemizole (antihistamine)
- sertindole (neuroleptic)

Can you find the common binding motif?
Why is hERG important?

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A hERG pharmacophore

Lipophilic base, usually a tertiary amine

X = 2-5 atom chain, may include rings, heteroatoms or polar groups

Now we know about it, we can try and design hERG activity out and can test for activity *in vitro*.
Case study: farnesyltransferase inhibitors

Changing the lipophilic aromatic ring to a polar one reduces hERG activity by >10x.
Drug-drug interactions (DDIs)

It’s complicated enough to look at the pharmacokinetics, toxicology etc of one medicine at a time, but many patients take several medicines, which can interact……

What might cause this?

One substance can affect the metabolism of another. This is why many medicines have a warning on them to say that the patient shouldn’t drink alcohol whilst taking the medication, because alcohol metabolism can affect drug metabolism.

http://www.youtube.com/watch?v=FQJ4NO6SDqg&feature=player_detailpage
Cyclosporin A

- selective immunosuppressive agent
- prevent rejection of a transplanted organs
- treats severe cases of psoriasis and rheumatoid arthritis

http://www.youtube.com/watch?feature=player_detailpage&v=5fjynugHBXs#t=40

Problems: High blood pressure; Bleeding, tender, or enlarged gums; Convulsions; fever or chills; frequent urge to urinate; vomiting
Cyclosporin A drug interaction

- Ketoconazole (e.g., Nizoral) or
- Nefazodone (e.g., Serzone) or
- Nicardipine (e.g., Cardene) or
- Verapamil (e.g., Calan, Covera-HS, Isoptin, Verelan)
- Azathioprine (e.g., Imuran) or
- Chlorambucil (e.g., Leukeran) or
- Corticosteroids (cortisone-like medicine) or
- Cyclophosphamide (e.g., Cytoxan) or
- Mercaptopurine (e.g., Purinethol) or
- Muromonab-CD3 (monoclonal antibody) Coal tar (e.g., Balnetar, Zetar) or
- Methoxsalen (e.g., Oxsoralen) or
- Radiation therapy or
- Trioxsalen (e.g., Trisoralen)—There may be increased risk of some skin cancers
- Lovastatin (e.g., Mevacor)
- Simvastatin (e.g., Zocor)—
- Amiloride (e.g., Midamor) or
- Spironolactone (e.g., Aldactone) or
- Triamterene (e.g., Dyrenium)—Since both cyclosporine and these medicines increase the amount of potassium in the body, potassium levels could become too high
- Allopurinol (e.g., Zyloprim) or
- Androgens (male hormones) or
- Bromocriptine (e.g., Parlodel) or
- Cimetidine (e.g., Tagamet) or
- Clarithromycin (e.g., Biaxin) or
- Danazol (e.g., Danocrine) or
- Diltiazem (e.g., Cardizem) or
- Erythromycins (medicine for infection) or
- Estrogens (female hormones) or
- Fluconazole (e.g., Diflucan) or
- Human immunodeficiency virus (HIV) protease inhibitors (e.g., Crixivan, Fortovase, Invirase, Norvir, Viracept) or
- Itraconazole (e.g., Sporanox) or
Cytochrome P_{450} (CYP)

Top 200 drugs in the USA in 2002

Primary route of clearance

Primary metabolic enzymes

In addition, compounds which inhibit and induce CYPs have the potential to interact with many other drugs.
Case study 1: terfenadine & ketoconazole

- Terfenadine – antihistamine drug on market for many years as an ‘over the counter’ remedy for hayfever (allergic rhinitis).
- Found to cause life threatening cardiac arrhythmias when co-administered with medicines such as erythromycin (antibiotic) or ketoconazole (antifungal).
- Caused by inhibition of hepatic P₄₅₀ enzymes.
Case study 1: terfenadine & ketoconazole

- Found that the major metabolite of terfenadine, caused by oxidation of the tert-butyl group, is the active species.
- This compound, fexofenadine, has little hERG activity as it is a zwitterion, and is now a medicine in its own right.

terfenadine (hERG pIC\textsubscript{50} ~ 7.6)  
fexofenadine hERG pIC\textsubscript{50} ~ 4.8
Case study 2: MAOIs and the ‘cheese effect’

- Monoamine oxidase inhibitors (MAOIs) have antidepressant activity.
- Depressed individuals often have decreased levels of amines such as noradrenaline, serotonin and dopamine in the brain.
- MAOIs increases these levels by reducing oxidation of the amines.
- However, they are not the drug of choice as they are sometimes associated with cardiovascular side effects.
Case study 2: MAOIs and the ‘cheese effect’

- Side effects caused when patient has eaten food which contains high levels of tyramine, e.g. cheese, wine, beer.
- Ingested tyramine causes the release of noradrenaline (NA), which would normally be metabolised by MAOIs.
- But because these enzymes have been inhibited, the NA levels rise. As NA is a vasoconstrictor, the blood pressure rises uncontrollably, which can trigger a cardiovascular event.

\[
\begin{align*}
\text{tyramine (R = H)} & \quad \text{noradrenaline} \\
\text{serotonin (R = OH)} &
\end{align*}
\]
Idiosyncratic toxicity

- ‘Idiosyncratic toxicity’ is something of a catch-all term to include other toxic effects that we don’t currently understand.

- Note that increased potency reduces the possibility of this.

- It is desirable to have two or more compounds in development which are structurally different – this reduces the possibility of both being hit by idiosyncratic toxicity problems.

- It’s a continuous challenge to understand the causes of idiosyncratic toxicity therefore to be able to avoid them at an early stage.
How safety is assessed during clinical trials?
Safety Assessment during Clinical Trial I

- Effectiveness: expected result, prespecified endpoint
- Safety: open-ended, uncertain endpoint
Clinical Trial II

- Anticipated risks (e.g., aminoglycosides and ototoxicity)
- Risks of concern (e.g., hepatic, renal, hematologic, cardiac/QTc)
- Serious adverse events of particular concern even if infrequent (1 per 1000)
- some risks may be missed
Clinical Trials (III)

Events that may be missed

- rare events
- events occurring after long-term use
- events occurring in special populations
- events occurring in association with specific diseases
- events occurring in association with concomitant therapy
Clinical trials:
Safety drug assessment

Questions?