Safety assessment in the discovery and development of a new drug

Anna Ollerstam
Agenda

• What is Toxicology/Safety Pharmacology?
• Toxicology in the Drug Development Process
• Toxicity of a compound
• Therapeutic window/ Safety margin determination
• Early Toxicity Testing
• Regulatory Safety Assessment
What is Toxicology/Safety Pharmacology?

- “Toxicology is the study of the adverse effects of chemical, physical or biological agents on living organisms and the ecosystem, including the prevention and amelioration of such adverse effects.”  
  Society of Toxicology (SOT)

- “Safety Pharmacology studies are defined as those studies that investigate the potential undesirable pharmacodynamic effects of a substance on physiological functions in relation to exposure in the therapeutic range and above.”  
  Safety Pharmacology Society (SPS)
The Science of Poisons

“All substances are poisons, there is none which is not a poison. The right dose (exposure) differentiates a poison and a remedy.”

Paracelsus (1493 -1541)
A typical Development Project

Year
- Phase I: Discovery, Pre-clinic
- Phase II: Clinic, Phase III: Registration

Phase
- Phase I
- Phase II
- Phase III

Chemistry
- Non-regulated
- Regulated

Toxicology
- Non-regulated
- Regulated

Formulation
- Non-regulated
- Regulated

Clinic
- Non-regulated
- Regulated

Compounds
- 50000
- 20
- 10
- 5
- 2
- 1

Proof of Concept
- 1st humane dose
- 1st dose in patient

Patent application
- Effect
- Approval

1st dose in patient

Nature Reviews | Drug Discovery
Vol. 3, August 2004
Drug attrition
AZ example 2014

b Project closures

<table>
<thead>
<tr>
<th>Phase</th>
<th>Preclinical</th>
<th>Phase I</th>
<th>Phase IIa</th>
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<td>(8)</td>
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</table>

a Organ systems involved in safety failures

- Cardiovascular: Preclinical 17%, Clinical 24%
- Liver: Preclinical 14%, Clinical 12%
- Musculoskeletal: Preclinical 12%, Clinical 12%
- Genetic: Preclinical 3%, Clinical 3%
- Respiratory: Preclinical 8%, Clinical 3%
- Renal: Preclinical 8%, Clinical 3%
- CNS: Preclinical 7%, Clinical 34%
- Gastrointestinal: Preclinical 3%, Clinical 9%
- Other (for example, immunity): Preclinical 3%, Clinical 21%
Toxicity of a compound

• Toxicity of a compound can be derived from

  ◦ The intended target (target related)
    — Exaggerated pharmacology
    — E.g. corticosteroids

  ◦ ”Chemical” derived effects
    — Due to interaction of a molecule with a different target than the intended or the reactivity of a molecule

Toxicity of a compound

• Target derived effects
  ◦ Early identification of risk with interaction with a target
    – Expression and function in humans (and relevant animal species)
      • Where is the target expressed and to what level?
    – Reference compounds?
    – Transgenic mouse data?
    – Published literature?
  ◦ Integrate information
  ◦ Design studies to answer target specific questions
Toxicity of a compound

• ”Chemical” derived effects
  ◦ Due to interaction of a molecule with a different target than
    the intended or reactivity of a molecule
    - E.g. phospholipidosis, most genotoxicity, reactive metabolites
  ◦ May be avoided by knowledge of related chemistry and directed screening as well as
    a broader selectivity screen early on
Toxicity can only happen if the human (or animal) body is exposed to the compound!

Need to do an estimation of what the human exposure will be.
Prediction of Safety margin

Predicted human concentration of a drug over 48 hours
Prediction of Safety margin

**Therapeutic window**

The difference or gap between the lowest drug concentration that controls the disease, and the drug concentration that above this level could be harmful.

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(NO Observed Adverse Effect Level) NOAEL

Minimum Effective Concentration) MEC

Concentration

Time (h)

0 12 24 36 48
Prediction of Safety margin

The fold difference between the drug concentration that causes toxicity and the $C_{\text{max}}$ in humans.
Early Toxicity Testing

- **Genotoxicity**
  - eg. *In vitro* Ames, *in vitro* Micronucleus, GreenScreen
  - *eg. In Vivo* Micronucleus screen

- **Cytotoxicity**
  - Liver toxicity
  - Skin toxicity (for topical drugs)

- **Safety Pharmacology**
  - hERG QT prolongation
  - In vivo cardiac toxicity
  - other test ...

- **Selectivity**

- **In vivo rat study to determine NOAEL**
What is Genetic Toxicology?

... the study of chemical, physical or biological agents that can change the sequence or structure of DNA

• DNA damage can be:
  ◦ at nucleotide level in DNA, or at the chromosomal level
  ◦ induced by direct mechanisms (chemical or metabolite interacts with DNA)
  ◦ induced by indirect mechanisms (chemical or metabolite affects other cellular macromolecules, e.g. mitotic spindle fibers)
Why Are We Worried About Genotoxicity?

DNA damage is associated with many human diseases

Germ Cells
spermatocytes, oocytes

Somatic Cells

Heritable Damage (genetic damage to offspring)
Infertility
Cancer
Other Diseases
Some Terminology

• **Mutagen**
  ◦ agent that induces mutations (change in the DNA sequence)

• **Clastogen**
  ◦ agent that induces breaks in chromosome (structural damage)

• **Aneugen**
  ◦ agent that induces changes in the number of chromosomes

• **Genotoxicity**
  ◦ mutations, chromosome damage, non-specific damage/repair

• **Mutagenicity**
  ◦ often used interchangeably with genotoxicity
Ames test

**Objective:** the detection of induction of gene mutations in bacteria in the absence and in the presence of a rat liver metabolic activation system (S9)
Ames test strains

**Genotypes of the *S. typhimurium* strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Amino acid Mutation</th>
<th>Type of mutation</th>
<th>DNA target</th>
<th>Cell wall mutation</th>
<th>DNA-repair</th>
<th>Plasmid</th>
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<td>GC</td>
<td>rfa</td>
<td>uvrB</td>
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<td>His G46</td>
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<tr>
<td>TA1537</td>
<td>His C3076</td>
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<tr>
<td>TA1538</td>
<td>His D3052</td>
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<td>GC</td>
<td>rfa</td>
<td>uvrB</td>
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</table>

Base pair substitution
Early Toxicity Testing

- Genotoxicity
- Cytotoxicity
  - Liver toxicity
  - Skin toxicity (for topical drugs)
- Safety Pharmacology
  - hERG QT prolongation
  - In vivo cardiac toxicity
- Selectivity
- In vivo rat study to determine NOAEL

Cytotox in liver cells

- Cell Loss
- DNA Fragmentation
- Nuclear Size
- Apoptosis
- Steatosis
- Phospholipidosis
- Mitochondrial Function
- DNA Damage Response

High Content Screening

Automated platform for performing fluorescence microscopy and quantitative image analysis
Skin toxicity assessment

- **Local tolerance *in vitro***
  - Keratinocyte cytotoxicity
  - Reconstructed human epidermis model

- **Local tolerance *in vivo***
  - Mini-pig 14 day local tolerance
    - Histology
    - Clinical scoring

- **Phototoxicity**
  - 3T3 NRU *in vitro* test
    - Cell toxicity test in the presence of UV light

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3T3 NRU Phototoxicity Assay
From iivs.org webpage
**In vitro** skin irritation testing in Reconstructed Human Epidermis (RHE)

RHE closely mimics the upper parts of the human skin, i.e. the epidermis

Irritant chemicals can be identified by their ability to decrease cell viability which can be measured by MTT reduction, cytokine release and histology scoring.

This *in vitro* model is used to select compounds/formulations to progress to further local tolerance testing in *in vivo* mini-pig → REPLACE/REDUCE
Local tolerance, *in vivo* mini-pig

**Justification**
- Complete inflammatory response present
- Full thickness skin
- Permeability closer to human skin
- Repeated dosing regimen possible

**Set-up optimized**
- Is combined with dermal PK -> REDUCE
- Including systemic PK with a wash-out period -> REDUCE
- Multiple test sites -> all tested compounds/ formulation are tested in the same individual -> variation is reduced -> REFINE
Early Toxicity Testing

- Genotoxicity
- Cytotoxicity
  - Liver toxicity
  - Skin toxicity (for topical drugs)
- Safety Pharmacology
  - hERG QT prolongation
  - In vivo cardiac toxicity
  - Other studies CNS, respiration
- Selectivity
- In vivo rat study to determine NOAEL

Cardiomyocyte action potential

Membrane Potential

Time
hERG channel block causes QT prolongation

Acquired or congenital long QT syndromes may cause cardiac arrhythmias (torsades de pointes) and sudden death in otherwise young and healthy persons.
Torsades de Pointes

Several drugs have been withdrawn from the market

- Multiple classes/pharmacophores implicated

  Seldane® (*terfenadine* - *antihistamine*)
  Hismanal® (*astemizole* - *antihistamine*)
  Propulsid® (*cisapride* - *prokinetic*)
  Serlect® (*sertindole* - *antipsychotic*)
  Raxar® (*grepafloxin* - *antibiotic*)
  Zagam® (*sparfloxacin* - *antibiotic*)
Early Toxicity Testing

- **Genotoxicity**
- **Cytotoxicity**
  - Liver toxicity
  - Skin toxicity (for topical drugs)
- **Safety Pharmacology**
  - hERG QT prolongation
  - In vivo cardiac toxicity
- **Selectivity**
- **In vivo rat study to determine NOAEL**
  - No Observable Adverse Effect Level and safety margin

Panel of tens to several hundreds of targets depending of phase in development and chemical class

<table>
<thead>
<tr>
<th>Targets</th>
<th>Targets</th>
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</thead>
<tbody>
<tr>
<td>Adenosine A2A</td>
<td>Adenosine A1</td>
</tr>
<tr>
<td>Adrenergic α2A</td>
<td>Adrenergic α1A</td>
</tr>
<tr>
<td>Adrenergic α1B</td>
<td>Adrenergic β2</td>
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<td>Adrenergic β1</td>
<td>Calcium Channel L-Type, Dihydropyridine</td>
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<tr>
<td>Cannabinoid CB1</td>
<td>Dopamine D2S</td>
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<tr>
<td>Dopamine D1</td>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;, Flunitrazepam, Central</td>
</tr>
<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;, Muscimol, Central</td>
<td>Glutamate, NMDA, Phencyclidine</td>
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<tr>
<td>Histamine H1</td>
<td>Imidazoline I2, Central</td>
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<td>Muscarinic M2</td>
<td>Muscarinic M3</td>
</tr>
<tr>
<td>Muscarinic M3</td>
<td>Nicotinic Acetylcholine</td>
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<td>Nicotinic Acetylcholine α1, Bungarotoxin</td>
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<td>Phorbol Ester</td>
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<tr>
<td>Phorbol Ester</td>
<td>Potassium Channel [KATP]</td>
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<tr>
<td>Potassium Channel hERG</td>
<td>Prostanoid EP4</td>
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<td>Prostanoid EP4</td>
<td>Rolipram</td>
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<td>Rolipram</td>
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<td>Sigma σ1</td>
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<td>Sigma σ1</td>
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<tr>
<td>Sodium Channel, Site 2</td>
<td>Transporter, Norepinephrine (NET)</td>
</tr>
</tbody>
</table>
Early Toxicity Testing

- Genotoxicity
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- Safety Pharmacology
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  - In vivo cardiac toxicity
- Selectivity

- *In vivo* study to determine NOAEL No

Observed Adverse Effect Level and safety margin
Cross Species Metabolism Assessment

Metabolite profile analysis of a drug from human, rat mouse, dog and mini-pig hepatocyte cultures.
In vivo rodent toxicity study

1-2-week treatment

Ref drug

Comp. 1

Comp. 2

Comp. 3

- Clinical chem.
- Hematology
- Biomarkers

- MNT genotox assay
  - Exposure assessment incl metabolites

- Biomarkers for eg. liver-, kidney- or cardiac tox

- Histology
- Organ weights

Exposure assessment incl metabolites
Next step…..

• We have chosen our candidate!
  ◦ Is next step to test our candidate drug in humans? Is it proven to be safe enough?

• No, many studies to be preformed to be able to test in humans…. 

**Regulatory framework determines what studies are needed**

• ICH guidelines (International Conference of Harmonisation)
• National/regional guidelines (EMA, FDA…)
• OECD guidelines
• GLP
GLP, Good Laboratory Practice

- Most studies needs to be performed according to Good Laboratory Practice (GLP)
  - Framework for studies that need to be planned, performed, monitored, recorded, reported and archived
- Helps assure regulatory authorities that the data submitted are trustworthy and can be relied upon
Minimum tests before phase I study

- **Safety assessment:**
  - Genotoxicity
    - Eg. two *in vitro*, one *in vivo*
  - Safety pharmacology
    - Cardiovascular, Respiratory, CNS studies
  - Repeated dose toxicity (min 14 days)
    - Rodent and non/rodent
      - Using Intended Route of administration
  - Toxicokinetics
    - Tmax, Cmax, AUC
  - Metabolism
Expectations from animal studies

• To investigate and characterize potential adverse effects in several animal species
  ◦ Target organs, reversibility?, local vs. systemic effects, dose-response
• Eliminate overtly toxic compounds
• Alert clinicians
• Establish margin of safety over proposed clinical dose

Toxicity studies, however exhaustive, can never demonstrate absolute safety of a drug (Hayes 1990)
How do we compensate for the limited number of animals in toxicity studies?

- Increased dose
- Increased duration of treatment
- Two or more animal species
  (typical rodent + non-rodent)

- **What species should be used?**
  - Similar expression pattern in the body
  - Similar biological effect
  - Pharmacological activity (potency) on target
  - Similar metabolism pattern
  - Practicalities in dosing (topical, inhalation)
Preclinical Safety Assessment before first dose in man

- The choice of the first dose to be administered

Always a challenge - no strict rules

Some criteria:
It is a non-toxic dose in toxicology studies
It is a no-effect dose in safety pharmacology 1/10th or 1/50th of the NOAEL (Cmax or AUC)

<table>
<thead>
<tr>
<th>Step</th>
<th>Mini-Pig</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NOAEL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 mg/kg/day after 7 days</td>
<td>30 mg/kg/day</td>
</tr>
<tr>
<td></td>
<td>0.5 mg/kg/day after 28 days</td>
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</tr>
<tr>
<td>2</td>
<td>HED</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5 x 0.95 = 0.475 mg/kg</td>
<td>30 x 0.16 = 4.8 mg/kg</td>
</tr>
<tr>
<td>3</td>
<td>Most appropriate or sensitive</td>
<td>0.475 mg/kg</td>
</tr>
<tr>
<td>4</td>
<td>Safety factor</td>
<td>( \frac{0.475}{10} = 0.0475 \text{ mg/kg} \approx 3.1 \text{ mg per subject (65 kg) per day} )</td>
</tr>
<tr>
<td>5</td>
<td>Pharmacology dose consideration</td>
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</table>

Human equivalent dose (HED)
Later Studies to support clinical studies in Patients

• Extended duration of repeat dose
  ◦ 3 months to “chronic” duration (depending on how long patients are to be treated)

• Reproduction toxicity studies
  ◦ Inclusion of women of childbearing potential

• Carcinogenicity studies
  ◦ Depending on duration of drug treatment
Reproduction Toxicity Studies

- Fertility  Males
  Females

- Embryo-fetal developmental
  Maternal toxicity
  Embryo-fetal toxicity (teratogenicity)

- Peri-post natal developmental
  $F_0$ - Maternal toxicity
  $F_1$ - Toxicity

http://www.slideshare.net/elegacki/reproductive-toxicology
Thalidomide is a potent teratogen in rabbits and humans

It was marketed as a sedative and anti-emetic drug 1957-62

10,000 malformed children were born

50% died
Cancerogenicity studies

- The objectives of carcinogenicity studies are to identify a tumorigenic potential in animals and to assess the relevant risk in humans

- Lifespan studies in rats and mice (6 mon-2 years)

- Data overview and protocols reviewed by regulators prior to testing
Summary

• Pre regulatory toxicology
  • Non GLP, Flexible
  • Aim: Select the best candidate

• Regulatory toxicological studies
  • Done according to GLP
  • Aim: Characterize one compound, investigate if it is safe to give to humans and set a safe starting dose in clinical studies
Thank you – Questions?

“You’re going to be the first to try this. They won’t let us use animals anymore.”
### Conversion of animal doses to human equivalent doses (HED) based on body surface area

<table>
<thead>
<tr>
<th>Species</th>
<th>To convert animal dose in mg/kg to dose in mg/m², multiple by km below</th>
<th>To convert animal dose in mg/kg to HED⁸ in mg/kg, either</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Divide Animal dose by</td>
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<tr>
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<tr>
<td>Dog</td>
<td>20</td>
<td>1.8</td>
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<tr>
<td>Primates</td>
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<tr>
<td>Monkeys⁹</td>
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<td>Baboon</td>
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<td>1.8</td>
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<td>Micro-pig</td>
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<tr>
<td>Mini-pig</td>
<td>35</td>
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</tr>
</tbody>
</table>
Duration of studies

- Exposure duration:
  - **Acute**: exposure for a duration of less than 24 hr; often a single exposure...
  - **Subacute**: generally refers to repeated exposure for a month or less.
  - **Subchronic**: exposure duration from between 1-3 months.
  - **Chronic**: exposure often greater than 3 months. Usually continual daily dietary exposure. For animal studies, often for a lifetime of the animal.
Preclinical Safety Assessment before first dose in man

Step 1: NOAEL (mg/kg/day) in toxicity studies
= the highest dose level that does not produce a significant increase in adverse effects such as:
- overt toxicity (signs, macro-, micro-lesions)
- surrogate markers (e.g. ALAT, ECG)
- exaggerated pharmacodynamic
Step 2: Calculation of human equivalent dose (body surface area)

<table>
<thead>
<tr>
<th>Species</th>
<th>To convert animal dose in mg/kg to dose in mg/m², multiple by km below</th>
<th>To convert animal dose in mg/kg to HED in mg/kg, either</th>
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<td>Divide Animal dose by</td>
<td>Multiply Animal dose by</td>
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<td>Human Child (20 kg)</td>
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<td></td>
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<tr>
<td>Rabbit</td>
<td>12</td>
<td>3.1</td>
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</table>
Preclinical Safety Assessment before first dose in man

Step 3: NOAEL from the most appropriate species
- No data to support choice = most sensitive species
- ADME
- Class experience
- Limited biological cross-species reactions (biological therapeutics)
Preclinical Safety Assessment before first dose in man

Step 4: Application of safety factor

10 or higher:
steep dose response curve, severe & non-monitorable toxicity, variable bioavailability, irreversible toxicity, unexplained mortality etc.

lower:
severe indication, well-characterized class, short duration of administration, similar toxicity across species etc.
Preclinical Safety Assessment before first dose in man

Step 5: Consideration of the pharmacologically active dose (PAD)
- In vivo PAD (mg/kg) → Human equivalent dose (HED)
- HED should be compared with the maximum recommended starting dose

MRSD is found