Basic Overview of Preclinical Toxicology in Drug Development

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Outline of Presentation

- Background
- In Vitro Toxicology
- In Vivo Toxicology
- Animal Models
Toxicology – the study of the adverse effects of:
- Chemical
- Physical
- Biological agents on
- People
- Animals
- Environment
• Everything we eat, breathe and come in contact with can have a toxicologic effect
• Everything, including water, can be toxic
• “The dose makes the poison”
• Toxicology studies the interaction of these compounds and biological systems
**Why do Toxicology Testing?**

- **Drug Development**: Drugs must be approved by the FDA before they can be marketed in the US.
- **Long, costly, and inherently risky**: only 1 of 10-15,000 reach FDA approval.
- **Toxicology testing** is required to demonstrate that drugs are **safe** before they can be given to humans.
Introduction to Toxicology

• The future of Toxicology may be in computer simulations, today we use animal models to simulate human biologic systems, often over multiple generations

• We have very strict animal guidelines for the care and use of animals

• We use animal models which are closely related to human physiology for the endpoint of interest

• It’s crucial to use the right animal model for the right test. An error, like the one used to study thalidomide can lead to catastrophic failure
• Thalidomide tragedy (1961-1962)
• One of the greatest of all drug disasters
• Introduced as safe and effective during pregnancy to treat nausea
• Potent human teratogen, caused major birth defects in ~10,000 children
• Phocomelia
Importance of Animal Models!

Key Assumptions:

- Other organisms can serve as accurate predictive models of toxicity in humans.
- Selection of an appropriate model is critical to accurate prediction of effects in humans.
- Understanding the strengths and weaknesses of any particular model is essential to understanding the relevance of specific findings to humans.
Good Laboratory Practices

- **GLP** is a Federal Regulation to ensure the integrity of data from nonclinical studies.

- **Definition:** GLP embodies a set of principles that provides a framework within which laboratory studies are planned, performed, monitored, reported and archived.

- In the USA, the GLPs are administered by the **FDA**, and are laid out in **21CFR (Code of Federal Regulations) Part 58**.

- Other regulatory agencies (OECD, EPA) have their own sets of GLP regulations that are similar to but not identical to those of the FDA.

- Definitive preclinical studies (i.e., the ones the FDA uses to make the final decisions regarding approval to start testing in humans) **MUST be GLP-compliant!**
Why GLPs?

- Created in response to the Industrial Bio Test Labs Scandal
  - Early 70’s, FDA became aware of cases of poor laboratory practice all over the US
  - Discovered fraudulent activities
    - Animals would be removed in the data, then later “resurrected”
    - Animal room called “the swamp” due to excessive humidity
    - They deemed their products were safe for human use
In vitro toxicology

- The crossover point between drug discovery and drug development.
- Provides information on mechanism(s) of action of a drug.
- Provides an early indication of the potential for some kinds of toxic effects, allowing a decision to terminate a development program before spending too much money.
• In vitro methods are widely used for:
  – Screening and ranking chemicals
  – Studying cell, tissue, or target specific effects
  – Improve subsequent study design for in vivo studies

In Vitro Toxicology
In vitro methods are usually

- Less expensive to run than in vivo studies
- Faster than in vivo studies (PLUS they don’t bite!)
- Somewhat less predictive of toxicity in intact organisms
• Screening, Some Types of In Vitro Toxicology Tests
  – Cytotoxicity
  – Protein binding
  – CYP inhibition/induction
  – Membrane permeability
  – Metabolic stability
  – Interspecies comparison
• **Cytotoxicity** = toxicity to cells

• Many different types of cells can be used; cells from higher organisms (e.g., liver cells, blood cells); bacteria; fungi; yeast

• Can be used to assess viability, structural effects, and/or function
  – Structural – e.g., effects on membrane integrity
  – Functional – e.g., effects on mitochondrial function
  – Cell proliferation – decreases or increases
In Vitro Toxicology

• Replace in vivo tests such as Dermal Corrosion, Skin Irritation, Draize Eye Irritancy

• Many tests now available in kit form

• **Example: EpiDerm**
  – Normal *human* epidermal keratinocytes
  – Cultured on a permeable polycarbonate membrane
  – Stratified, highly differentiated, model of human epidermis
  – Metabolically and mitotically active cells organized into differentiated layers
• Assess ability of a chemical to induce metabolism of specific substrates, including the chemical itself
• Information about metabolic pathways by which the chemical can be metabolized
• Information on production of toxic/reactive metabolites
• Interspecies comparisons
  – Can provide information on relevance of a particular animal model from a metabolic standpoint
Results from preclinical toxicology studies should, at a minimum:

- Establish a safe starting dose for clinical studies
- Provide information on a drug-treatment regimen that would produce the least toxicity
- Assess target organ toxicity and its reversibility
- Provide insight into biomarkers for clinical monitoring
Types of Testing Required

- Single dose (acute) toxicology testing
  - Combine with preliminary testing
- Repeat dose toxicology testing
  - “Pivotal” testing
- Toxicokinetic and pharmacokinetic studies
  - Distribution within the body and disposition
- Safety Pharmacology studies
  - CV, respiratory and CNS
  - Stand alone studies or combine with toxicology?
- Local tolerance testing
- Genotoxicity testing (some in vivo, some in vitro)
The number and types of studies required depend on the therapeutic indication.

Drugs for life-threatening illnesses require fewer studies to reach the clinic.

In general, animal studies are conducted in two species, one rodent (e.g., rat, mouse) and one non-rodent (e.g., dog, nonhuman primate). Biologics may require only one species.

Other species (e.g., rabbits, ferrets, hamsters, mini-pigs) may be used for special studies (e.g., vaccine studies).
Types of Preclinical Safety Studies

Usually start with:

**Single Dose (Acute/Range-Finding)**

- Used to determine the most appropriate dose range in the species to be tested.
- Used to get an idea of target organs
- Includes minimal number of animals and evaluations (e.g., body weights, clinical signs of toxicity)
- Usually not required to be GLP-compliant
Types of Preclinical Safety Studies

• Repeat Dose Toxicity
  • Animal models
    • Small molecules – two species (one rodent, one non-rodent)
    • Biologics – may require only one species if only one relevant species can be identified
  • Should mimic as closely as possible the planned clinical design
    • Route
    • Duration
    • Schedule
  • Requirements vary between the different regulatory agencies.
Types of Preclinical Safety Studies

• **Repeat Dose Toxicity**
  - Extensive evaluations of toxic effects
    - Body weights
    - Clinical signs of toxicity
    - Food consumption
    - Clinical pathology
    - Histopathology
    - Other

  • Large animals usually undergo more extensive evaluation (e.g., ECGs)

  • At least one dose should produce dose-limiting toxicity.

  • At least one dose should be non-toxic.
Types of Preclinical Safety Studies

- **Safety Pharmacology**
  - Used to determine the effects of the drug on specialized organ systems (e.g., cardiovascular, respiratory, neurologic)

- **Chronic Toxicity/Carcinogenicity**
  - Used to determine the effects of long-term exposure to the drug, including the ability to produce cancer.
  - May not be required for drugs that are intended for only short-term use (e.g., antibiotics) and that are expected to have no permanent effects on DNA.

- **Reproductive Toxicity/Teratogenicity**
  - Evaluates effects on reproductive function and ability to produce birth defects
• Biologics (e.g., gene therapy vectors, vaccines, monoclonal antibodies) require some of the same tests as small molecules

• Typically each biologic has its own set of unique additional requirements

• Frequently require different animal models than small molecules (e.g., hamsters for adenovirus gene therapy vectors)
Key Assumptions

– Other organisms can serve as accurate predictive models of toxicity in humans.

– Selection of an appropriate model is key to accurate prediction in humans.

– Understanding the strengths and weaknesses of any particular model is essential to understanding the relevance of specific findings to humans.

Caveat

– Assumptions notwithstanding, remember that drugs showing safety and efficacy in preclinical animal models may show very different pharmacological properties when administered to humans.
Animal Models

• Development of proper preclinical models which can efficiently predict drug behavior in humans is essential prior to testing a drug in a human subject.

• The FDA and other regulatory agencies are more and more requiring Sponsors to provide data to support selection of the specific species (and even strains) used to support testing of new drugs.
Some (of the many) reasons that a given animal model may be inappropriate are:

- Lack of appropriate drug target in the preclinical animal model
- Presence of irrelevant target
- Differences in metabolic fate
  - The complement of drug-metabolizing enzymes can vary significantly from one animal species to another, and even between strains of the same species. Significant variability between sexes for some enzymes.
- Differences in susceptibility to infection by specific pathogens
  - Cotton rats and hamsters for adenovirus vectors
Examples:

• Testing of therapeutic antibodies
  • Relevant species is one in which the antibody is pharmacologically active, the target antigen should be present or expressed and the tissue cross-reactivity profile should be similar to that in humans.

• Sex-specific drugs
  • Don’t test TOXICITY of a drug intended for treatment of prostate cancer in FEMALE rats.
  • No, really! This happens.
Example:

- **Unleaded gasoline-induced nephropathy**
  - Unleaded gasoline induces a unique type of kidney damage in male rats following inhalation exposure.
  - Accumulation of hyaline droplets containing $\alpha_{2u}$-globulin in the proximal tubules, leading to cell death and denudation of the lining of specific segments of the proximal tubules.
  - Similar syndrome not seen in female rats, or in mice and nonhuman primates of either sex.
  - $\alpha_{2u}$-globulin is a *male rat-specific protein*. Humans have not been found to produce $\alpha_{2u}$-globulin.
  - Suggests that humans are probably not at risk for this type of nephropathy after exposure to unleaded gasoline.
Animal Models
Differences in Metabolic Fate

Example:

- **Tamoxifen carcinogenicity**
  - Genotoxic in the livers of rats and mice, but produces liver cancer only in rats
  - Has not been shown to produce DNA adducts or liver tumors in human patients
  - Enzymatic pathway responsible for production of tamoxifen metabolites that form adducts with DNA is several-fold higher in rats than humans while the activity of "detoxification" pathways is lower in rats than in humans
  - Thus, the carcinogenic effects of tamoxifen observed in rats have limited relevance to assessment of the safety of the drug in humans.
Example:

- **Adenovirus vector toxicology**
  - Adenoviruses are currently used in gene therapy and in particular for development of oncolytic virus vectors for treatment of cancer.
  - Mice and rats are the most commonly used rodent models for safety testing, BUT
  - The problem with rats and mice is that tissues of the rat and mouse are not permissive for human adenovirus replication. Therefore, it is not possible to assess the possible adverse effects associated with replication of the vector in non-tumor tissue.
  - The only two known small animal models that are permissive (or semi-permissive) for adenovirus replication are Syrian hamsters and cotton rats (MEAN, MEAN, little creatures!).
Performing safety testing in an inappropriate animal model is:

- Wasteful of resources (time, money)
- Unethical from an animal welfare point of view
- Potentially dangerous to humans

Pick the right animal model(s)!
QUESTIONS?