SOUTHERN RESEARCH
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Basic Overview of Preclinical Toxicology Animal Models

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Outline

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- In Vivo Toxicology

- Animal Models

Background

- What is Toxicology?

  - Toxicology is the study of the adverse effects of chemical, physical, or biological agents on people, animals, and the environment.

  - Toxicologists are scientists trained to investigate, interpret and communicate the nature of those effects.

"Solely the dose determines that a thing is not a poison." *Paracelsus* (German-Swiss physician), 1493-1541.

Water, in excess, is toxic.

Why Do Toxicology Testing

Need to prove new drugs are safe:

- Before first administration to humans

- Before later clinical trials

Good Laboratory Practices

- Regulations to ensure the integrity of data from nonclinical studies.

- In the USA, the GLPs are administered by the FDA, and are laid out in 21CFR 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 must be GLP compliant

In Vitro Toxicology

- In vitro toxicology
  - The crossover point between drug discovery and drug development.

  - Provide 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

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

In Vitro Toxicology

- Screening
  - Cytotoxicity
  - Protein binding
  - CYP inhibition/induction
  - Membrane permeability
  - Metabolic stability
  - Interspecies comparison

In Vitro Toxicology
Cytotoxicity

- 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
Dermal or Ocular Toxicity

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

- Many tests now available in kit form

- Example: EpiDerm
  - Normal <u>human</u> epidermal keratinocytes
  - Cultured on a permeable polycarbonate membrane
  - Stratified, highly differentiated, model of human epidermis
  - Metabolically and mitotically active cells organized into differentiated layers

In Vitro Toxicology Immunotoxicity

- These are, by necessity, functional tests

- Examples
  - Cytokine release. Assess ability of a chemical to induce release of cytokines/chemokines. Can use cells from various sources, including peripheral blood cells. Many variations of this assay. TGN 1412
  - Drug-induced histamine release. Cremophor, polysorbate 80

In Vitro Toxicology
Metabolism and Kinetics

- 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 <u>relevance of a particular animal model</u> from a metabolic standpoint

In Vivo Toxicology - Purpose

- 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

Safety Assessment
Types of Testing Required

- Single dose (acute) toxicology testing
  - Combine with preliminary testing
- Repeat dose toxicology testing
  - The "pivotal" testing
- Toxicokinetic and pharmacokinetic studies
  - Next lecture
- Safety Pharmacology
  - CV, respiratory and CNS
  - Stand alone studies or combine with toxicology?
- Local tolerance testing
  - Parenteral
  - Stand alone studies or combine with toxicology?
- Genotoxicity testing

Types of Preclinical Safety Studies

- 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

Biologic Products

- Biologics (e.g., gene therapy vectors, vaccines, monoclonal antibodies) require <u>some</u> 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)

Issues Pertinent to Specific Classes of Biologic Products

Product Class Examples	Class-Specific Preclinical Issues
Monoclonal Antibodies	Immunogenicity, stability, tissue cross-reactivity, effector mechanisms
Gene Therapy	Biodistribution, persistence, genomic integration of the vector, species specificity of the transduced gene, permissiveness for infections by viral vectors, duration and level of gene expression
Recombinant Proteins	Immunogenicity, autoimmunity
Preventive Vaccines	Enhancement of intended disease, autoimmunity, tolerance, cytokine release, immunogenicity, protection from disease

Animal Models in Toxicology Testing

Key Assumptions

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

– Selection of an appropriate model to use 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

– Drugs showing safety and efficacy in preclinical animal models may show <u>very different pharmacological properties</u> 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 <u>requiring Sponsors to provide data to support selection of the specific species</u> (and even strains) used to support testing of new drugs.

Animal Models

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

- Differences in susceptibility to infection by specific pathogens

Animal Models
Lack of Appropriate Drug Target

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!

Animal Models
Presence of Irrelevant Target

Example:

- Unleaded gasoline-induced nephropathy
    - Unleaded gasoline has been shown to induce a unique type of nephropathy in male rats following inhalation exposure.
    - Accumulation of hyaline droplets containing $\propto$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
    - $\propto$2u-globulin is a <u>male rat-specific protein</u>. Humans have not been found to produce $\propto$2u-globulin.
    - Suggests that <u>humans are probably not at risk for this type of nephropathy</u> 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.

Animal Models
Differences in Susceptibility to Infection by Specific Pathogens

Example:

- Adenovirus vector toxicology

    - Adenoviruses are currently use 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.
    - 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!).

Animal Models
Bottom Line

Pick the right animal model(s)

It is wasteful of resources (time, money), unethical from an animal welfare point of view, and potentially dangerous to humans to perform safety testing in an inappropriate animal model

QUESTIONS?