The planning and selection of appropriate models and experimental subjects in animal studies

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Animal studies;

✓ History
✓ Planning
✓ The selection of appropriate models and experimental subjects
✓ Reflection of the results from appropriate models to human beings
EU; 2005: 12.1 million vertebrates in experimental studies.

USA, 2005; 1.2 million vertebrates except mice and rats “Data from Ministry of Agriculture”
Animals in Scientific Research and Education

“History”

It is well-known that the improvements in medical research have emerged and accelerated as a result of animal studies and provided support to human studies.
Why do we need laboratory animals for research and education?

- To understand the physiologic and pathologic mechanisms at the level of cell/tissue/organ/tissue,
- To learn the systems and relations between various systems,
- To develop novel treatments against diseases,
- To develop novel diagnostic measures for diseases and/or disorders,
- To contribute to the education of scientists and physicians
Can animal data be implemented for humans?

“There are many similarities between humans and animals”

- Similarities in immune system: Human-Mouse
- Similarities in cardiovascular function: Human-Dog
- Poliomyelitis; Landsteiner and Popper (1908-Monkey), Jonas Salk (1952-test in humans)
- Diabetes; Banting and Best (1921) have shown the impact of insulin in dogs
- Obesity; Mouse and Zucker obese rat models; leptin research
Can animal data be implemented for humans?

- “Hypertension” treatment modalities by research in rats, cats and dogs

- “Infant deaths” hyaline membrane disease was shown to be the leading cause of death in preterm infants by studies in sheep/lambs

- “Cystic fibrosis” the impact of the chloride channels as a causative factor in the death of young adults has been documented in mouse models.

- “Gene therapy approaches” are being tested in mouse models.
Experimental planning in animal studies

“Alternatives” before choosing the appropriate animal model

non-animal models (3R: Replacement-Reduction-Refinement)

Animal model should be considered only if it is inevitable!!!
Experimental planning in animal studies

Experiments; application of the effects of parameters organized at various levels and of obtaining the results.

“The main target” of the researcher is to comprehend the experimental process
Experimental planning in animal studies; “The cost of the experiment”

- Source (Human, equipment, etc.)

- Time

- Material
Organization in animal studies

1. Planning

2. Application

3. Completion
Organization in animal studies

1. Planning

- Problem should be identified and the target(s) should be described

- Information on the research topic should be obtained

- Original hypothesis should be established
Organization in animal studies

2. Application

- Time/cost factors and sources
- Ethical committee approval
- Confirmation of applicability/Planning of experiment
- Determination of appropriate data analysis methods
- Distribution of responsibilities
- Personnel training/Starting research/Data recruitment and processing.
Organization in animal studies

3. Completion

- Interpretation of data,
- Completion and submission of outputs,
- Completion of administrative affairs
- Interpretation of experiences and taking lessons.
- Sharing the results with the scientific world,
Experimental planning in animal studies
“Confirming with animal rule”

- Since animals present individual variations;

  - Studies should be reproducible and verifiable,
  - Eligible tests should be performed,
  - Standardized instruments and procedures should be used,
  - Techniques or methods; should be sensitive enough to enable comparisons with other studies and species,
  - The type, dose and administration route of the applied material and the results should be comparable.
Animals in scientific research and education

Animal model is an experimental setting in which;

- normal biology and behavior can be explored,
- spontaneous or induced pathological conditions can be investigated,
- animals similar to humans or other animal species in terms of a biological function are used.
The measures that should be considered in the selection of experimental model

In the selection of appropriate animal models;

- a good knowledge of;
  - anatomical,
  - physiological,

- behavioral characteristics of the scheduled species,

is essential for an experiment to be conducted and completed in accordance with the purpose of the study.
The measures that should be considered in the selection of experimental model

- The animal model should be selected in a careful and meticulous manner,
- Research should be planned in detail previously,
- The animals having characteristics that fit best to the planned experiment should be chosen,
- Species, strains and spontaneous, transgenic and homozygous characteristics should be considered,
- Ideal data should be obtained with a minimal number of animals,
- Vehicle-treated control group should be used.
The measures that should be considered in the experimental model

- Young healthy animals should be preferred,
- Nulliparous non-pregnant females should be preferred
- Only one sex should be chosen and at least 8 animals should be used in each group,
- Animals should be parasite and pathogen free,
- Animal weights at the start of the study should not exceed ±20% during the study.

“In general rats or mice are preferred in experimental studies”
The measures that should be considered in the selection of animal model and species

» It should represent the normal living conditions of target species,

» It should be well-described regardless of the selected model,

» Genus, strain and genetic characteristics (if possible) should be expressed for the model to guide other researchers,

» Microbiological status and flora of animals should be taken into account,

“Normal mouse" and "pathogen-free mouse" respond differently against various agents.
Factors that influence selection of animal models

- The number of animals to be used according to appropriate plan and statistical method,
- The compliance of the model or organ systems in the proposed study,
- Genetic status of the model,
- The nature of experimentally induced models,
- The response of animals to applications,
- The effect of the environment on specific animal model,
- Prior information about the animal used.
The measures that should be considered in the experimental model

- Species selection should be based on facilities, experience and cost,

- If researcher is not familiar with the model, he should contact with experienced person,

- Experienced researcher may provide "tips" or suggestions that are not specified in his publications.
Non-experimental factors affecting the experimental data

- Animal factor
- Physical and environmental factors
- Care facility
- Animal care and treatment
  - If animal require special care (Genetic models or pigs, monkeys, etc.);
    - Care conditions
    - Experienced caregiver
Non-experimental factors affecting the experimental data “Animal factors”

- Body volume
- Life expectancy/age/sex /pregnancy status
- Genetics; inbred-outbred-mutant-genetic modification
- Biological Rhythms
- Microbiological ambience/Latent (subclinical or silent) infections
- The presence of stress/Disease
- Price and care
Non-experimental factors affecting the experimental data
Non-experimental factors affecting the experimental data
“Quality of life in laboratory animals”

- Care facility;
- Normal life of animal (diet, housing conditions),
- "Pathogen-free animals" and "normal laboratory animals" live in areas with different requirements; it should be taken into consideration that this situation could affect the outcome of the study.
Non-experimental factors affecting the experimental data

“Quality of life in laboratory animals”

- The variables that influence metabolism should be checked;
  - Dietary habits, sex and age,
  - Administration route, time and dose
  - Sampling location and time,
  - Season of the year,
  - Daily body temperature and diurnal variations

- More rapid metabolism in rodents than humans (e.g., rat heart rate 325-350/min),

- Control and function of metabolism slows down as body size increases and dosage of drugs should be adjusted accordingly.
Non-experimental factors affecting the experimental data
“Sex and Development”

- At the same environmental conditions, young "male" rats gain weight more quickly than "female" rats do.
The measures that should be considered in the selection of animal model and species

- One of the best examples of selection of appropriate animal is encountered in “vascular restenosis” research.

- In pigs and monkeys the results on "restenosis" after angioplasty is closer to humans when compared to data obtained from small animals.

- Literature should be reviewed before describing new models and deciding which model is feasible and realistic.
Key data elements in an animal model

- Animal model selection criteria;
  - Species
  - Pathogenesis
  - Required procedures
  - Cost

- Test System
  - Vaccine / Therapeutic Dose
  - Characteristics of the agent
  - Administration of the agent
  - Effective dose
The measures that should be considered in the experimental model

- Characteristics of the agent;
  - The administration route should be as it is in humans; inhalation, oral, etc.,
  - The effect should be quantifiable
    - Results should be reproduced when the effective dose is repeated,
    - Scalable relationship between dose and results should be demonstrated,
  - The disease-causing agent should be the same as that in humans,

- Pathogenic determinants
  - How does an agent cause a pathology?
    - Toxin production of bacteria
The measures that should be considered in the experimental model

Pathogenesis

- The similarities to course of the disease in humans
  - SEB (Staphylococcal enterotoxin B):
    - Vomiting and fever in humans and monkey
    - Death in high doses,
  - Anthrax (Bacillus anthracis): enlarged mediastinum in humans and monkey,
  - Q-Fever (Coxiella burnetii): pneumonia in humans and monkeys,
  - Botulinum (Clostridium botulinum): is a cause of paralysis in almost every species.
The selection of appropriate animal in respiratory system research

- Required Procedures
  - Larger animal model
  - The amount of blood to be taken
- Limits in body weight
- Chest X-ray
- Constant physiological monitoring, etc., clinical observations
- Telemetry, etc.,
- Administration route
  - Inhalation (Head, Nose or whole body)
  - Parenteral
  - Oral
  - Dermal
Selection of animal consistent with experimental procedure; “An example of experimental planning”

- **Goal:** To develop a drug to eliminate the symptoms of a disease A

- **Specific objectives:** To investigate the effects of a drug on lung function variables in rats exposed to a disease A

- **Experimental Design:** 32 animals in each group (4 subgroups, n = 8) and 3 sub-groups will be exposed to different levels of a carcinogen, one group will serve as control.

- Then the animals in 3 subgroups will be exposed to 3 different doses of the drug,

- Lung function variables will be measured in each rat.
The measures that should be considered in the selection of animal model and species

- Lack of confidence in animal test results may lead to misinterpretations and could cost the life or health of thousands of people.

- A substance that is toxic or ineffective in a laboratory animal may be non-toxic or effective in humans.

- Phylogenetic relationships or anatomical suitability are not reliable features physiological behaviors,

- Rats are inefficient as a "predictive model" in studies of asthma and bronchitis.
The measures that should be considered in the selection of animal model and species

- The animal model does not always fully represent the original condition.

- Models certainly don't have the last word, but they give an idea.
Induced animal models

- Disease models induced by surgery or chemical agents

- e.g. inducing diabetes with STZ, or liver failure with CCl$_4$

- Although scientists wish to implement information from one species to another, "phylogenetic similarity" does not warrant the consistency of the conclusion.
Induced animal models

- Transgenic models "animals with a foreign DNA which is artificially placed in their genome" also fall into the category of induced models.

- Recently "knock-out" model has begun to take the agenda,

- In practice, mouse is the preferred species as a transgenic model, but other species are also increasingly used for this purpose,

- Transgenic animals need special care owing to the facts that they may develop unknown diseases and it is difficult to show signs of distress.
**Spontaneous animal models**

- They are models that genetic characteristics develop spontaneously in animals which show pathologic features and symptoms similar to the disease in humans.

- Natural genetic variants are used to create models of various diseases.
Negative animal models

- They are used to overcome difficulties encountered in elucidating a physiological event.

- Some animal species/strains never develop certain diseases

- Rabbits account for a negative model in that they do not develop gonococcal infections

- The animal does not react to a certain impulse.
Orphan animal models

- Some diseases are first described in animals and then continued to be studied as a model owing to the possibility that may also be described in humans in future.

- If later a similar disease is seen in humans, then the model is adapted accordingly.

- For example, papillomatosis, Marek's disease and mad cow disease.
Genetic classification of laboratory animals

- **INBRED ANIMALS;**
  - Animals that are obtained after at least 20 generations of sibling matings.
  - All animals are genetically similar, because genetic mutations are eliminated after sibling matings.

- **OUTBRED ANIMALS;**
  - They are obtained by non-sibling matings.
  - They are phenotypically similar and give birth to many pups, but they differ genetically.
F1 hybrids and mutant strains

- **F1 HYBRIDS**
  - They result from cross mating of distinctly different inbred strains.
  - They are genetically similar, but have no ability to reproduce.

- **MUTANT STRAINS**
  - More than 500 mutations have been reported in mice.
  - Rats, hamsters, rabbits, guinea pigs, cats, dogs and pigs have mutants.
Frequently used laboratory animals

MICE, RATS, GUINEA PIGS, RABBITS ....

- They are preferred in experimental studies since they are anatomically small, their lifetime is short and are easily manipulated and reproduced.

- In particular, these mammals may be identical models for many specific human diseases.
Frequently used laboratory animals

“Mouse”

They are the most preferred species in biomedical studies, since they have small bodies and they are the vertebrates with best known genetic structure,

More than 500 different inbred and transgenic strains exist,

Anatomical and physiological differences exist between the inbred strains,

The presence of a large number of mutations is a preference in genetic studies.
Frequently used laboratory animals
“Mouse”

- In studies of behavior, embryology, teratology and virology,
- In investigations of immunosuppressive agents and drugs,
- In determination of the safety and efficacy of pharmaceutical products,
- They are considered a suitable animal model in chronic toxicity studies as they are short-lived.
- They constitute 70-80% of all animals used in experiments.
Frequently used laboratory animals

“Rat”

- Its anatomical structure is similar to mouse and it is the most commonly used vertebrate after mouse,

- It also shows great physiological similarity to humans,

- Many of the inbred strains are obtained from the first rat strain, "Wistar albino”

- Their great ability of adaptation and endurance render this animal the most appropriate model for various experimental studies,

- They are used in elucidation of physio/pathological mechanisms and nutrition studies owing to their high tolerance against the deprivation of food.
Frequently used laboratory animals
“Guinea pig”

- Preferred in studies since they are cheap, easily cared for and docile,

- They are used mainly in production of serum, vaccines and other biological substances,

- in studies of immune response and anaphylactic shock,

- in toxicological studies,

- in studies of diseases such as "tuberculosis, diphtheria and brucellosis" they serve as hosts for microorganisms.
Frequently used laboratory animals
“Rabbit”

- "German breed" with less than 2 kg of body weight and "New Zealand White rabbits" with 2-5 kg of body weight are preferred.

- the first animal model of atherosclerosis,

- preferred in hypertension and immunological studies,

- Chinchilla rabbits have features of “von Willebrand's disease; a type of hemophilia due to deficiency of von Willebrand factor",

- in addition they are preferred in antiserum production, hereditary glaucoma studies, and routine toxicological and
Human reflections of results from experimental models

The results obtained from experimental animal models;

- are qualitatively or quantitatively adaptable to humans,
- provide pharmaco-kinetic and/or -dynamic contributions to determination of an effective dose in humans,
- cause a decrease in morbidity and mortality rates in humans,
- provide an obvious benefit to human health.
A direct implementation of the information obtained from animals to humans can not be warranted and remains to be accomplished as a next step.

Today's animal test results will continue to be the trues and falses of the future.
Human reflections of results from experimental models

In experimental studies, the basic rule is to respect the animals rights and “Animal Models" currently maintain their validity in providing useful information for humanity.
Thanks for your patience

Hitit/Hattusa (Çorum)
International Holiday
B.C., 2000