

Use of animals in experimental pharmacology

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Introduction

- Selection of an animal model is one of the most important steps in any of the experimental pharmacological study.
- Animal model preferred for the study must be producing similar disease profile as in the human.
- Suitable animal model should be selected which follows three main objectives:
 1. Use of an animal phylogenetically closer to man
 2. Process under investigation as close to man
 3. Anatomy, physiology and biochemistry are considered to be similar to man

Classification

- 1. Rodents :

E.g. Mouse, Rat, Guinea pig, Gerbil, Hamster etc.

- 2. Non rodents :

E.g. Rabbit, Monkey, Dog, Cat, Pig etc.

- 3. Miscellaneous :

E.g. Frog, Pigeon, Zebra fish, Chicken etc.

Mouse

- Smallest laboratory animal
- Uniform bred, cheap and easy to handle, sensitive to very small doses of substance, require small place for housing
- Strains:
 - Commonly used: swiss albino mice
 - Other strains: DBA/2,C-57BL/6,C3H,CBA,A

- Large similarity in mice and human genome provides good model for genetic studies -
 - Knock out mice:
gene is taken out
 - Knock in mice:
gene is introduced
 - Knocked down:
gene is silenced



- Nude mice: Hairless genetic mutant which lacks thymus gland used for tissue immunity and transplantation research.
- Biege mice: lack of NK cells and are susceptible to cancer - used for cancer studies.
- Large similarity in mice and human genome(99% conserved)- good model for research on mammalian biology and human disease.

❖ Used in

- Acute toxicity studies
- Assay of Insulin and analgesics
- Screening of chemotherapeutic and teratogenic agents
- Cancer and genetic research
- Atherosclerosis
- Autoimmune
- Neurological dysfunction
- Endocrine disorders

Rat

- Most commonly used animal
- Most standardized of all lab animals
- Two inbred original strains of albino rats(Pink-Eyed White or PEW) used
 - 1.Wistar rat :
 - Wide head
 - Long ear
 - Tail length is lesser than body length
 - Resistant to infections
 - 2.Sprague Dawley rat :
 - Longer & narrow head
 - Tail is longer than body length
 - Rapidly growing



Peculiarities

- Lack of vomiting centre (due to strong sphincter between the stomach and the esophagus), tonsil and gallbladder
- Diffuse pancreas, so not a good model for type I diabetes
- Coprophagy (eating their own stool)
- Tail of rat helps in thermoregulation of body
- Physiologically, 24 hr old rat = 6 month old infant.

- Some important phenotypic differences between baby rats and mice
- Baby rat has blunt and broad large head relative to body whereas mice have triangular, small head.
- Baby rat has small ears whereas mice have large ears.
- In the baby rat hind paw and body ratio is larger as compared to mice.
- Tail is thick and shorter in baby rat while mice have thin and larger or same length tail as compared to body.

❖ Used in

- Evaluation of Psychopharmacological agents
- Teratogenic and Carcinogenic Activity
- Anti Ulcer agent
- Liver Physiological Studies -after partial hepatectomy the organ regenerates almost completely in a week
- Antihypertensive effects,
- Assay of Hormones & Acute/ Chronic analgesics
- Antihypertensive Effects
- Cardiovascular diseases

Guinea pig (*Cavia porcellus*)



- Most useful and docile laboratory animal
- Newborn GP can eat solid food by 5th day
- Young GP best mated after 3 months - polygamous mating
- Maturation is slow in males than in females
- Not able to synthesize the required vitamin C daily
- Highly sensitive to histamine & penicillin
- Very susceptible to mycobacterial infection and anaphylactic shock
- Uterus - inhibited by adrenaline
- Serum contains asparaginase - anti-leukemic action

❖ Used in

- Evaluation of bronchodilator (anti-asthmatic)
- Sensitization studies - egg albumin and horse serum.
- Study of local anesthetics
- Bioassay of digitalis, histamines, and acetyl choline
- Study of vitamin C metabolism & anti-tb drugs
- Sensitive cochlea - suitable for hearing experiments
- More resistant to hypoxia - suitable for oxygen consumption experiments
- Isolated preparation of ileum - screening of spasmodic and anti-spasmodic compounds
- Other isolations - tracheal chain, vas deferens etc.

Gerbil

- Small laboratory animal;
- Length in between rat and mice - " Sand rat"
- Originally developed in japan as lab animals
- Ease in handling, mild and quiet in nature
- Used in the field of stroke, epilepsy, auditory studies, parasitic & bacterial infections and lipid metabolism & heart disease studies 24



- This animal is one of the few species which were originally developed in Japan as laboratory animals.

❖ Used

- field of stroke
- epilepsy, auditory studies (hearing curve similar to man)
- parasite and bacterial infections
- lipid metabolism
- heart disease studies (high serum cholesterol levels).

Hamster (Mesocricetus auratus)

- 3rd most commonly used animal
- Strains:
 - Syrian hamster (Golden)
 - Chinese hamster (striped back)
 - European hamster (Gray)
 - Armenian hamster(Gray)



- Advantages

1. Availability and Ease in reproduction
2. Anatomical and physiological features with unique potential for study
3. Rapid development with short life cycles
4. Cheek pouch: lack of lymphatic drainage ; ideal site for tissue transplants such as tumors and grafts(also used for collection or transport of food materials)
5. Pouch isolation - Assay of Prostaglandin E& F

❑ Syrian hamsters:

- Syrian hamster is the most commonly used in biomedical research because of availability and ease of reproduction
- Used in virology, cancer, genetics, toxicology and reproductive and physiology studies

❑ Chinese hamsters:

- Has lowest no. of chromosomes compared to other lab animals; Useful for cytogenetic studies
- Defective β cells in pancreas - used in diabetic studies

- ❑ European hamster (quite larger than other hamster) is a more suitable for highly concentrated and prolonged smoke inhalation studies
- ❑ Armenian hamsters: more specific for mutagenic and carcinogenic studies

❖ Used

- Onco virus, influenza virus, respiratory syncytial virus (RSV) studies
- Vaccine production (Foot and Mouth).
- Diagnostic techniques for numerous infectious agents (i.e. Clostridium spp; Leptospirosis spp).

Non rodent

Rabbit (*Oryctolagus cuniculus*)

- Very docile
- animal of choice for many cardiac studies
- Ideal animal for pharmacokinetic studies
- New zealand white rabbit - most common strain
- Homologous to humans to react similarly to diseases and medications
- Cannot vomit
- Very sensitive to histamine - raise BP, constriction of pulmonary vessels
- Has an ability to taste water - absent in rodents



- Dopamine causes fall in BP
- Presence of atropinesterase - degrades atropine so pupillary light reflex is either not abolished or it recovers in 10-15 min
- lack vasomotor reversal phenomenon - Doesn't have adrenergic vasodilator nerves
- Has huge caecum and long appendix
- Absence of CYP3A4
- Lack of melanin - 'Albinism'
- Uterus - stimulated by adrenaline

❖ Used in

- Pyrogen Testing
- Toxic effects of cosmetics and pharmaceuticals
- Production of antibodies and antiserums
- Anti Diabetic Study & Topical agents study
- Bioassay of Insulin, curare and sex hormones
- Capillary Permeability Study
- Embryo Toxic and Reproductive studies
- Isolated preparation : heart, duodenum, ileum

Monkey(Macaca mulatta)

- Monkeys are one of the most commonly used mammalian
- Rhesus species - commonly used
- Structure and function - closely resemble to man
- Uterus resembles human & exhibiting regular menstrual periods
- Metabolic pattern & Brain structure - same as man
- Require regular check up for rabies, Tuberculosis & timely immunization



❖ Used in

- Infertility studies
- Virology, Parasitology & Immunological studies
- Immunosuppressant & Nutrition related studies
- Ideal model for pharmacokinetic studies
- Best for studying drugs acting on CNS, CVS and GIT

Squirrel monkey

- Most commonly used neotropical primates in US
- Plasmodium species are host specific - important animal model for malaria vaccine development studies
- One of the most susceptible non - human primate species to experimental infection with Creutzfeldt - Jakob disease & spongiform encephalopathies



Dog (Canis familiaris)

- Most preferred large experimental animal
- Can be easily trained for behavioral activity
- Stomach and intestinal tract resembles human
- Distinct structure of pancreas -allowing it as good model for the research on diabetes
- May develop spontaneous Diabetes &Hypertension



- Cervical sympathetic and vagal nerves run together inseparable vagal stimulation - causes reflex variations in blood pressure
- Animal of choice for studies on gastric secretion and digestion
- Preferred for Anti hypertensive, cardiovascular, Diabetes and anti ulcer experiments, ulcerative colitis, open heart surgery, organ transplantation, central nervous system (CNS), safety pharmacology and toxicology.

Cat (*Felis catus*)

- Similar physiological features with humans so used in cardiovascular (CVS), behavioral, nerve impulse transmission.
- Ease of collecting blood samples - useful in studying the transmission of vitamins & minerals to the fetus & new born
- Thick nictitating membrane – used in screening of ganglionic blocking drugs
- Able to produce methemoglobinemia - suitable for toxicity studies



❖ Use

- Cats are used for the studies where stable BP is required
- Stimulates the pregnant uterus & inhibits the null uterus
- Essential in the study of the nerve centres in the brain
- It is not a good model in the experiment on the loss of righting reflex, because it regain its righting reflex even fall from a high altitude.
- Used in the neuropharmacology (particularly the testing of psychotropic drugs), toxicology, oncology and chromosomal abnormalities studies.

Pig (*Sus scrofa domestica*)

- Similar in Human physiology
- Less hairy skin - preferred for skin related studies
- Small lungs - susceptible to bronchitis & pneumonia
- Alimentary tract resembles human used in - digestive system studies
- Important model - Cardiovascular research such as MI, atherosclerosis
- investigation of skin permeation and for digestive systems, pig as an experimental animal.
- Dissimilarities are in the vascularization (rich in man, poor in the pig) and in the sebaceous glands.
- Humans have mostly eccrine sweat glands over the body surface, whereas the pig has only apocrine glands.



Zebra fish (Danio rerio)

- Dr. George streisinger - Father of zebra fish research
- Optically translucent embryos
- Clear eggs - developed outside the mother's body; allows watching a egg grow into a newly formed fish in 2-4 days under a microscope
- Length is 5-6 cm; Life span is approx. 5 years



❖ Advantages :

- Cheaper to maintain than other animals
- Suitable model for vertebrate development and genetic studies

❖ Drawback :

- Not closely related to humans
- Genetic modification has not been possible as it is in the mouse (Knock out/Knock in).
- Glofish: Genetically modified fluorescent fish
- Tool for examining the erythrocyte development & circulation defects in embryo

Frog (*Rana tigrina*)

- Only tailless experimental amphibian
- Frog and toad - differentiated by their appearance only;
- Heart contain 3 chambers
- Barbouruta (flat headed frog) – 1st species of frog with out lung
- Oxygen can pass through their permeable skin – breath from skin
- Camouflage – common defensive mechanism
- • Vagal response - varies by species & seasonal



❖ Used in

- Retinal toxicity of drugs
- Action of drugs on the CNS, CVS, Neuromuscular junction and pregnancy assays
- Cloning and embryological researches
- Substances obtained from frog – epibatidine , a pain killer 200 times more potent than morphine, convulsants, nerve poisons

• Chicken (Gallus domesticus)

- Widely used due to easy availability and maintenance
- Only model - studying avian diseases
- Studies related to breeding, genetics, performance testing, embryology, fertility, toxicology
- Important models - chick comb method, scleroderma models, autoimmune thyroiditis



• Pigeon (Columbia livia)

- Mainly preferred for screening of anti emetic activity
- CVS – like arteriosclerosis and stantarization of cardiac glycosides
CNS- anxiety
- Screening of intravenous anesthetics
- Pigeon crop method - prolactin bioassay



- Summary

- Animal selection - based on how it is closer to human
- Mice & Rats - commonest experimental model for various studies
- Guinea pig - very docile; requires vitamin C daily ,Asthmatic studies
- Gerbil - auditory studies
- Rabbit - able to taste water; pyrogen testing .

- Hamster - presence of cheek pouch - site for tissue transplant & graft studies
- Monkey - very closer to human; metabolic studies
- Dog - GI drug studies; nerve conduction studies
- Pig - skin related studies
- Frog - pregnancy assays; chemicals isolation
- Pigeon - screening model for anti emetics

References

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THANK YOU