eISSN (Online): 2598-0580

Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Methods of Selection and Maintenance of Experimental Animals for Biomedical Research

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ARTICLE INFO

Keywords:
Experimental animals
In vivo
Biomedical

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.32539/bsm.v5i1.227

ABSTRACT

Research is an activity carried out based on scientific principles and methods systematically to obtain information, data, and information from related subjects, with understanding the theory and proving assumptions and / or hypotheses. The results obtained are conclusions that can be applied or become additional knowledge for the progress of science. However, research activities must still respect the rights and dignity of research subjects. Health research includes biomedical, epidemiological, social, and behavioral research. Some health research can be done in vitro, using mathematical models, or computer simulations. If the research results are to be used for humans, further research is needed using living materials (in vivo) such as cell lines and tissue cultures. However, to observe, study, and conclude all occurrences in living things as a whole, experimental animals are needed because experimental animals have a value for each part of the body and there are interactions between these body parts.

1. Introduction

The decision to use what type of test animal and which line depends on the purpose of the research being carried out. Basically, the selection of test animals must be based on the similarity or closeness of the characteristics and characteristics of the tested animals to humans, especially in the aspects studied and matters related to these aspects. Apart from the closeness of the characteristics and physiological characteristics of the test animals to humans, the selection of the test animals is also based on the ease of obtaining the animals. In terms of proximity of physiological traits and traits to humans, the closest animal is the chimpanzee ape. However, if it is viewed from the practicality of implementation and the costs involved, the use of the animal needs to be carefully considered. The selection of test animals for a pre-clinical study also needs to consider the aspect of caring for the animal. If in a study the oral administration of the test material, the test animals used must have the same characteristics, characteristics and absorption patterns as humans, although there are still quantitative differences. Differences in the absorption between species for certain substances can also be caused or closely related to differences in the type of intestinal flora. In terms of distribution some experts claim that the distribution of

certain compounds in humans is more consistent than in lower mammals, although in humans the binding of these substances by certain proteins is more pronounced.

In general, a pre-clinical research is considered good if it is carried out on rodents such as mice, rats or hamsters and non-rodents such as cats and dogs. However, in pre-clinical trials it can also be done on other animals such as pigeons, guinea pigs, rabbits, pigs or low monkeys such as the galagos. Due to current technological advances, especially in biotechnology, many toxicological studies have been carried out on human tissue cultures and recombinant bacteria.

Maintenance of test animals

Maintenance of tested animals includes aspects of facilities, staff and food. These three aspects should support each other and be closely related. A good attendant will produce something good if there are facilities. Likewise, good facilities without good officers are useless.

a. Amenities

`The facilities needed in the maintenance of test animals include a place for maintenance (buildings and cages) that meet the requirements and supporting facilities such as water, food preparation or preparation facilities and waste disposal facilities.

1. Building or maintenance room

The building or maintenance room must meet the requirements in area, air circulation, lighting, humidity, divided into several rooms as needed. The space required includes a breeding room (captive breeding), a maintenance room, a research room, an autopsy room, a tissue storage room and an isolation room, a workshop and warehouse. If the building for raising test animals is intended to maintain several types of test animals, the space requirement will certainly be more because several types of test animals cannot be placed in the same room.

2. Cages

The size of the cage needs to be taken into account so that the test animals can keep moving freely without any tension caused by the cage being too narrow. The cage should also be easy to clean, free of rust and have no sharp parts that could injure the test animal. The cage must also meet the requirements so that in the test or research everything that needs to be calculated can be done easily, such as counting the amount of food and drink. The size of the cage needs to take into account the type and line of test animals, single test animals or groups.

The cage should be made of strong, non-rusting and durable material. For mice, rats, hamsters, guinea pigs and rabbits, the cage can be made of plastic, aluminum, monel or stainless steel components. Don't use paint for such a cage. For cats and dogs, wooden cages can be made. The cage material should be waterproof and easy to clean.

3. Sleeping pad

For the purposes of sleeping mats, materials that are also intended to suck urine are often used so that the cage is always dry. The requirements for the material to be used as bedding for test animals are that it can absorb water, does not injure the test animal, is not attractive to eat, does not smell and does not contain substances that can interfere with the health of the tested animal. These materials include rice husks and sawn wood. Other materials that can also be used, although not as good as the husks, are corn cobs, sugar cane and peanut shells.

In confining test animals, several types of test animals can be caged in groups in a cage, but several other types must be caged individually in each cage.

4. Grouping

Mice after weaning (generally 3 weeks old and weighing about 15 grams) can be collected in groups of 10-20 animals. For mice that are generally weaned at about 3 weeks of age with a weight of about 50 grams, each individual can be grouped. If the weight is 125-

150 grams, each group should contain at most 6 birds. If it weighs more than 250 grams, grouped into 4 heads. For guinea pigs can be grouped every 10 heads

For rabbits, cats and dogs should be kept separately individually.

b. Food

Test animal food is made based on the need for components for each type of test animal. Sometimes the composition of the test animal feed is made freely from certain components according to need, for example a salt free diet, fat free, a certain number of calories and preferably.

Food preparation must be done hygienically so that the food is not contaminated with eggs or parasite spores which can infect and affect the health of the test animals so that it can affect the results of the study.

Things to be attention to pre-clinic test

Trials in pre-clinical trials are very complex multidisciplinary studies. Extrapolating data from animals to humans requires information from many fields of science. From the pre-clinical trial research, more detailed information and data on the efficacy and safety were obtained, especially at doses equivalent to the human dose, and the presence or absence of a cumulative effect and whether the effect could return to normal *(reversible)* after the administration of the test material was stopped. This test can be used to predict the negative impact on humans if he is exposed to the material for a long time.

In extrapolating data from animals to humans, many factors must be considered, including the extent or threshold where there is no toxic effect, what is the shape of the dose logarithmic curve and how the manifestation of the toxic effect occurs. The no toxic effect threshold is usually calculated statistically at the 95% confidence level with a 5% probability of error. From this calculation it can be imagined that if a toxic effect occurs only in 0.1% of the tested animals it means that the effect cannot be observed if only 100 animals are used. Likewise, if anomalies can arise spontaneously in control test animals then the anomalies that can arise in animals can be greater and if this data is directly extrapolated to humans it can be surprising.

The prediction of toxicity in humans based on the toxicity test carried out on tested animals depends on the relationship between the test and humans, the environment and other living things. It is also greatly influenced by heredity, nutrition, general health and the environment.

Hereditary disposition factors in humans can also play a role in determining the susceptibility of humans to toxic substances such as the tendency to develop tumors and so on.

A person with stress or on treatment with *immunisuppressive* drugs may have a greater risk for poisoning or carcinogenesis. Abnormal people like this can be members of the population who are normal. This risk cannot be estimated from animal tests or studies conducted on healthy test animals. Genetic variations in the test animals that determine the variation in response, of course, can also be considered a limitation in pre-clinical trials.

Bodies or parties and experts with an interest in pre-clinical trials are advised not to be rigid and always follow methodological developments in pre-clinical research, especially fundamental developments in understanding the mechanisms of efficacy or toxicity. The introduction and use of new methods in pre-clinical research may be more meaningful and more informative than the old methods that are commonly used. However, this new method should not be used as a substitute for the old method or used immediately for its reliability, validity and accuracy.

Whether old or new methods are used, it is necessary to state that the special conditions applied to research must also be carried out by other researchers so that the results of research carried out by other laboratory researchers can be compared. It is therefore advisable in the pre-clinical trial report to describe the method of the study in more detail.

Data extrapolation

Pre-clinical research in the laboratory with test animals can be used to predict the efficacy or safety that can arise if the test material enters the human body. However, it must be realized that research using test animals as models has many limitations in its accuracy and reliability as a means of estimating the effects on humans quantitatively. The accuracy and reliability depends on many things, including the selection of test animals, research planning and how to extrapolate from animals to humans.

In extrapolating data from animals to humans, it must meet the requirements that the data taken from a study is adequate. These requirements include, among other things, the type and condition of the test animal according to what is needed, has a vulnerability similar to that of humans, the number of tested animals, the method of administration of the material under study and the physical and chemical properties of the material under study in accordance with the aims and objectives of the study The side effect of the material under study and the target organs must also be taken into account in preparing the research plan.

The most difficult problem in extrapolating data from animals to humans is to convert from one species to another. For almost all poisonous substances, the pathogenesis of poisoning between humans and mammals is almost the same so that the symptoms of poisoning are almost the same. Therefore, the different responses are more quantitative in nature. Humans can be more susceptible than some types of test animals but for some situations certain test animals are more susceptible than humans. An easy example of this is atropine. Mice are much more susceptible to atropine, cats are less susceptible while rabbits and dogs are less susceptible to atropine. Therefore, the last two test animals can tolerate atropine at a dose 100 times higher than the lethal dose in humans. In contrast, dogs are more sensitive to hydrocyanic acid than humans.

Differences in susceptibility between species that occur may be the result of differences in metabolism, particularly in the availability and ability of enzymes to detoxify toxins. In addition, these differences can also be caused by differences in the absorption, transport, distribution and elimination of these toxic substances. The existence of differences in characteristics and traits between species must always be considered in selecting test animals as models in research. If in a study the oral administration of the test material, the test animals used must have the same characteristics, characteristics and absorption patterns as humans, although there are still quantitative differences. Differences in absorption between species for some substances can also be caused by or closely related to different types of intestinal flora. Some experts claim the distribution and storage of certain compounds in humans is more consistent than in lower mammals, although in humans the binding of these substances by certain proteins is more pronounced. Urine excretion can also differ from one species to another and this is mostly due to dietary factors which can cause differences in urine pH and ionization rate of the studied compounds. Bile varies widely among the types of test animals and appears to be larger in mice and rabbits than in rats and humans. The difference in response that exists between the test animal and human species appears to be more closely related to the biotransformations which are generally more rapid in the lower test animals than humans.

One of the strong bladder carcinogens such as 2-*naphthaleneamine* or 2-*naphthylamine* can cause bladder cancer in dogs, squirrels and humans but not in white mice, rabbits or guinea pigs. The differences between species in cancer growth appear to be due to differences in metabolic patterns and abilities. In certain test animals, metabolites that are carcinogenic can be produced but not in other animals so that it can cause differences in the toxic effects of some toxic compounds.

If there is data or information on metabolism relating to the type of test animal and the compound under study, differences in absorption, distribution, biotransformation and elimination of toxic substances

in humans and test animals must be considered in selecting which test animals to use.

Differences in toxicity between species can also result in differences in cellular transport. As a carcinogen, aflatoxins are more toxic to mice than mice, are slower to enter the liver cells or are metabolized more slowly by mice than mice.

In determining the length of a study, it is necessary to compare the *life span* of tested animals with humans. The average length of life of the test animals is directly proportional to their body weight. With the law of the *body weight rule* and regression analysis, it can be shown that the life expectancy of a mammal that weighs the same as a human (70kg) is 15 years. From this assumption, the rat's life expectancy of about 2.5 years is equivalent to that of humans who are only valued at 15-17 years. From this it appears that these assumptions are not consistent. Therefore, in preparing a research plan or interpreting the results of research on animals, it is necessary to consider this long life expectancy factor.

Another problem in evaluating and estimating toxicity in humans from the results of animal studies is the difficulty in measuring the magnitude of the effect, how to make the conditions in the test animals suitable for humans (eg intelligence and changes in behavior). Likewise the effects of social factors that are very important in humans but cannot be replicated in test animals. Therefore, in extrapolating data from test animals to humans, it is necessary to make a conversion factor between species based on biological considerations and information about the tested animal.

Table 1. Size of the cage area for each type of test animal based on body weight.

Type of Test Animal	**Weight (gram)**	**Floor area for each tail (cm^2)**
Mice	10	39
	10-15	52
	16-25	80
	>25	100
Rat	100	200
	100-200	400
	200-300	600
	>300	600
Hamster	60	64.5
	60-80	83.9
	80-100	103.2
	>100	122.6
Guinea pig	250	277
	250-350	374
	>350	652
Rabbit	2000	1400
	4000	2800
	>4000	3800
Cat	4000	2800
	>4000	3700
Dog	15000	2800
	30000	3800

Table 2. Dose calculation conversions for various types (species) of test animals

	Mice 20g	**Mouse 200g**	**Guinea pig 400g**	**Rabbit 1.5kg**	**Cat 2kg**	**Monkey 4kg**	**Dog 12kg**	**Human 70kg**
Mice 20g	1.0	7.0	12.25	27.8	29.7	64.1	124.2	387.9
Mouse 200g	0.14	1.0	1,74	3.9	4.2	9.2	17.8	56.0
Guinea pig 400g	0.08	0.57	1.0	2.25	2.4	5.2	10.2	31.5
Rabbit 1.5kg	0.04	0.25	0.44	1.0	1.08	2.4	4.5	14.2
Cat 2kg	0.03	0.23	0.41	0.92	1.0	2.2	4.1	13.0
Monkey 4kg	0.016	0.11	0.19	0.42	0.45	1.0	1.9	6.1
Dog 12kg	0.008	0.06	0.10	0.22	0.24	0.52	1.0	3.1
Human 70kg	0.0026	0.018	0.31	0.07	0.075	0.16	0.32	1.0

Laurence and Bocharch, 1964

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