RABBITS AND CHINCHILLAS AS EXPERIMENTAL MODELS

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Abstract: *In biomedical research, the rabbits are the most commonly used laboratory animal for the production of polyclonal antibodies, in toxicity and drugs safety studies. Rabbit offers many advantages over other species. The relatively large size gentle disposition and ease of maintenance in the laboratory condition are characteristics that ensure the continued use of rabbits as valuable and essential animal model for many diseases. Anatomical and functional particularities of rabbits are appropriate and sometimes unique to the study of human diseases. The main studies that rabbits are the model of choice are those related to atherosclerosis, Alzheimer's disease, osteoarthritis, tuberculosis and ophthalmic disease in addition to the familiar models of neoplastic investigations.*
Chinchillas are the traditional animal model in research related to the ear, especially hearing loss and otitis. Although some studies used mice and guinea pigs in the same area, due to special anatomical features the chinchillas remain the ideal model in this area. The chinchilla inner ear anatomy and ear physiology are similar with the humans. Other areas include the use of chinchillas to study renal anatomy, cardiovascular anatomy, arterial blood supply of the brain and ear, ovarian endocrine activity besides gastrointestinal motility studies, and respiratory infections.
This paper provides a review of the main areas in which those species are used as experimental model with emphasis of anatomical characteristics that make them compatible and suitable with specific areas of research.

Keywords: *rabbit, chinchilla, animal model*

INTRODUCTION

The key to using animal models is to select ones that have similarities to human anatomy and physiology or have the same disease states. Researchers should be guided by the ethical principles contained in the Guide for the Care and Use of Laboratory Animals () and the use of animals as models must be scientifically justified and approved by the local Institutional Bioethics Committee. Small animals have been extensively used in medical research. The choice of an animal model should take into consideration: 1) scientific hypothesis; 2) the degree of species similarity to the human anatomy; 3) the institutional capability to ensure the optimal and safety laboratory conditions to the chosen specie. Most of the proper animal models can be expensive to maintain and the funding must be appropriate to achieve the required number of animals which is need to accomplish a statistically significant result.

Rodents comprise the largest mammalian order with over 1800 species representing 40% of all mammals being found worldwide from the arctic to tropical and temperate regions. Lagomorphs differ from rodents in that they have four incisors and a different jaw structure, but otherwise they share many similar behavioural and anatomical characteristics. But the digestive strategy (non ruminant herbivores) of the species belonging the Rodent and Lagomorph orders is common (Stan, 2013 ,2014). Both rabbits and chinchillas possess anatomical particularities and similarities which makes them suitable and valuable models in biological research.

MATERIAL AND METHOD

A PubMed search using key search "rabbit" from 2010 to 2015 return over 27 500 items in which rabbits was not only species used in research but this demonstrate continued used of rabbits as experimental models. The main fields in which rabbit were used as animal models were atherosclerosis, ophthalmic disease, infectious disease, orthopedic procedures and neoplastic disease. Regarding the rodents, same method of PubMed search, using key word "rodent", return over 368000 articles demonstrating high use of rodents as experimental models, but only 459 items in which chinchillas were involved. The main field in which chinchillas have been used was ear related research. In this paper we will not summarize the 27500 or 368000 articles in which rabbits or rodents were used, instead we will provide an overview of the main fields of research in which rabbits and chinchillas are continuously used with emphasis of the anatomical particularities of the systems involved and due to which these small animals are experimental models of choice.

RESULTS AND DISCUSSIONS

Fundamental ethical concepts of using animals as experimental models

In this respect have appeared numerous directives for use of animal models in research, teaching and testing but the simplest with the greatest impact is the ethical concept "The 3Rs". This concept announced early in the 1956 by Russell and Burch is a call to apply, whenever is possible the alternatives of replacement of animals used in medical research, reduction of number of used animals in testing purposes and the refinement in procedures used on animals in research.

Replacing defines total replacement of animals with computerized programs or relative replacements with animals that are lower on phylogenetic scale. But development of total replacement programs it is a slow process. In accordance with the topic of this paper is worth to be mentioned two areas where the use of rabbits has been replaced. One is eye toxicity (the Draize experiment) in which rabbits were replaced by the bovine corneal opacity and permeability test using a slaughtered cow eye or the isolated chicken eye test or a device the Cytosensor microphysiometer (Molecular Devices, Inc. USA). The Draize test was based on rabbit unique nasolacrimal anatomy namely the presence of a single lacrimal punctum and a tortuous nasolacrimal duct. The second domain is represented by the replacement of rabbits with the aquatic species (e.g horseshoe crabs) in tests which required injection of drugs, biologics or raw materials into rabbits to look for a febrile response as an indication of contamination with endotoxins.

Reduction involves a reduced number of animals used in research or obtaining maximum scientific information from a small number of animals. This approach is based on careful choice of experimental design, improved selection of an animal model including selection of animals with the most appropriate health and genetic status, use of new technologies, improved statistical design, and statistical analysis along a careful control of environment in order to obtain proper care conditions.

Refinement is related to the modifications and use of the least invasive experimental procedures in order to eliminate pain and distress which involved accurate recognition of pain and the use of analgesics and supportive care, enhanced housing and husbandry.

Another redoubtable concept enunciated in 1979 and resumed in 1993 is the concept of the 5 freedoms created by the United Kingdom Farm Animal Welfare Advisory Council. The principles of this concept are often coordinating rules in the enunciation of the ethical principles in medical research. The five freedoms (revised in 1993) include: 1) freedom from hunger and thirst by assuring ready access to fresh water and a sufficient diet to maintain full health and vigor; 2) freedom from discomfort, by providing an environment including shelter and a comfortable resting area; 3) freedom from pain, injury and disease by preventive means or rapid diagnosis and treatment; 4) freedom from fear and distress, by ensuring conditions to avoid mental suffering; 5) freedom to express normal behaviour, by providing sufficient space, proper facilities, and company of the animals own kind (Webster 2001).

Therefore, it is recognized that animal welfare is a core concern of veterinary ethics. This subject is of a major importance of society and the main professional category involved is veterinarians as the first line of care. In the European Union, the basis for the ethical rules of biomedical research on the protection of animals used for scientific purposes is "European Union Parliament and Council Directive 2010/63/EU, revised in 2010 (European Union 2010). One of the goals of this Directive, besides the statement of ethical guidelines in medical research is to minimize the discrepancies between member states which were present in 1986 Directive. In research often occur conflicts of interest among scientists, technicians, veterinarians and research institute on the one hand and on the other hand the nongovernmental companies that require compliance with animal rights. According to Tannenbaum, (1995) the simplest rules of ethics must follow the simplest principles of what is good and what is bad, what is right and what is wrong and looks for the correct norms for veterinary professional behaviour and attitude to respect the moral imperative to treat animals decently. The Guide for the Care and Use of Laboratory Animals (2011) states that "Humane care means those actions taken to assure that laboratory animals are treated according to high ethical and scientific standards".

Rabbits as experimental models

In biomedical research, the rabbits are experimental model of choice used in the production of monoclonal (Fortunato et al., 2014; Yam et al., 2014; Quin et al., 2015; Pan et al., 2015) and polyclonal antibodies (Hu et al., 2013; Nan et al 2015) and in recombinant protein production (Bősze et al 2003; Bősze and Houdebine 2006; Houdebine 2009). Comparing to monoclonal antibodies, the polyclonal antibodies are more heterogeneous having the ability to bind the specific antigen by binding several different epitopes. Rabbits have a high responsiveness to a great variety of antigens having only one immunoglobulin G isotype and combined with ready availability of secondary reagents these specie is the primary specie used in polyclonal antibody production. The procedure for the immunization of rabbits for polyclonal antibody production is dependent upon the immunogen, adjuvant and the purpose for which the antibodies are produce and are well documented (Lagatie et al 2015). Due to the relative large body size compare to the smallest rodents, easily access of the marginal ear vein rabbits remain the suitable species used in this immunologic research domain. Recently, in some studies, rabbits were largely replaced by some rodent with a short life span, a large number of offspring after a short gestation period, advanced and well documented genomics and proteomics and wide availability of reagents (Park et al 2015). Therefore, rabbits remain the linking model used in the early stages of research being an

important niche between laboratory mouse and domesticated animals having a major importance in preclinical translational research. This statement is supported by the low costs of purchasing, maintenance and regulation of research bioethics committees (Fisher 2010).

Rabbits have much closer phylogenetic features to primate compare to inbred rodent strains, providing a much broader genetic material. Moreover, the rapid advancement of genomics and proteomics knowledge of rabbits led to selection of specific lines of research available targets. But the most important aspect is related to the characteristics of the specific human diseases, which often is impossible to be replicated in other rodent experimental models. A topical example is seen in AIDS where infection with HIV-1 in rodent specie is not possible. Instead, rabbits seem to develop and replicate human disease pathogenesis like chimpanzees and gibbons ape, but not with the same consistent symptoms as in humans. It was suggested that rabbit CD4 (RaCD4) like receptor is the counterpart of human CD4 (HuCD4) the principal receptor of HIV-1 in human (Pan et al 2015; Hatziioannou 2012; Tervo et Kleper 2010)

Anatomical and functional particularities of rabbits are appropriate and sometimes unique to the study of human diseases. The main studies that rabbits are experimental model of choice are those related to atherosclerosis, Alzheimer's disease, osteoarthritis, tuberculosis and pharmacologic ocular research besides well known neoplastic models.

Although rabbits being herbivores do not spontaneously develop hypercholesterolemia (like man, several non human primate and pigs), which is considered the main factor to atherosclerosis developement, a diet rich in cholesterol lead to vascular lesions deposition of atheromatous plaques occurrence within a few weeks (Priyadharsin et al., 2015. Atherosclerosis is a chronic progressive inflammatory disease characterized by intimal arterial wall accumulation of lipids (especially LDL), enhancement of endothelial permeability and accumulation of inflammatory cells (monocytes, lymphocytes) leading to appearance of lesions and subsequent thickening of the arterial wall which compromise the residual lumen and ischemic event apparition (Konya 2008) The pathogenesis is complex and mediated by adhesion molecules, inflammatory cells, and smooth muscle cells. In the rabbit the atheromatous plaques are located in the vascular wall of the aortic arch and on the thoracic aorta at the origins of intercostal arteries with a less extent in abdominal aorta as in humans. The anatomical and functional explanation is the turbulent blood flow in these locations and a higher vascular permeability of LDL as a focal response of the artery wall to local vascular injury.

The model of diet-induced hypercholesterolemia in rabbits it is well known in the field. Hypercholesterolemia, in particular LDL fractions (low density lipoprotein) is a major risk to the occurrence of atherosclerotic lesions. Similar to the humans, rabbits show the same metabolism of the cholesterol fractions. In comparison, rodents show high levels of HDL (high density lipoprotein), which makes them unsuitable for this type of research. A natural and spontaneous occurrence of hyperlipidemia is found in Wantanabe heritable hyperlipidemic (WHHL) strain of rabbit which is the first animal model with endogenous hypercholesterolemia due to the lack of LDL receptors. (Wantanabe 1980). This inbreed strain develop more severe lesions located in the thoracia aorta, coronary arteries and abdominal aorta and adjacent branching vessels as humans. In this condition the WHHL rabbit inbred strain is the model of choice in atherosclerosis research. Another animal model

used in familial combined hyperlipidemia is the St. Thomas Hospital strain which show high level of VLDL, intermediate density lipoproteins-IDL, and high level of LDL (Taylor 1997).

Probably the only specie that could surpass the rabbit in those specified above in atherosclerosis experimental model is pigs**.** Alzheimer's disease is another condition in which due to the fact that its appearance is not spontaneous in animals (induction in animals is done either through diet, pharmacological or genetic manipulation) research was focused on developing the rabbit as experimental model due to spontaneous occurrence of atherosclerosis (Woodruff-Pak 2008). Furthermore, physiologically, at cellular and molecular level, rabbits show similarities with neuropathology of Alzheimer's disease in human**.** In rabbits affected by brain disease, the brain shows increased level of cholesterol and β amyloid, low levels of acetylcholine, Aß plaques in the extracellular space, increased permeability of the haematoencephalic barrier, with high population of glial cells and neural population decrease. Rabbits phylogenetic similarity with humans is reflected in a high percentage (97% of Aß protein amino acid sequence is conserved). Anatomically rabbits possess a suitably sized brain so the cognitive tests are relevant. From a cognitive point of view, rabbits show neurological deficiencies with the coming of age, similar to humans.

Using rabbits as experimental model in ophthalmologic research is due to an anatomical characteristic: the eye has an optimal size related to body size, which allows maneuvers of intraocular injection, histopathological examination. Moreover because of the physiological similarities of the visual apparatus, the rabbit is often the model of choice in this type of research (Gwon 2008). The main areas in which the rabbit is used as experimental model are related to eye surgery: remedying cataract (Woodruff-Pak 2008), corneal transplantation, insertion of intraocular lenses, procedures for refractive laser (Kang and Grossniklaus 2011), implantation of shunts in glaucoma (Albrecht 2008), retinal detachment and vitreous transport of medication. The most suitable strains of transgenic rabbits that are appropriate for ophthalmic research are the New Zealand White Rabbit (NZWR) breed (Kondo 2009). The use of young rabbits in pediatric ophthalmology research is justified by prompt and strong inflammatory response after surgery, similar to that seen in children.

Another condition that is idiopathic or post traumatic in humans, in which rabbits are the ideal model, is osteoarthritis, which leads to tissue changes in joint cartilage, subchondral changes of the bone and synovial ligaments and subordinated muscles (Poole 2010). Although several species of mammals have been used (mice, guinea pigs, dogs and even horses), none of them offer specific advantages of rabbit as an experimental model. An example is the morphology of the femoral-tibial-patellar joint (knee) of rabbits being very similar to that of humans. Moreover, rabbit joints are sufficient size for collecting histopathological samples, compared to small animals (Laverty et al. 2010). Another aspect is related to the skeletal ossification that in humans, like in rabbits, at adult ages is complete, which does not happen for small rodents, hence the difficulty of extrapolating translational research findings from these species.

In research related to the pathogenesis of human tuberculosis there are three species which are commonly used: rabbits, guinea pigs and mice because all can be infected by inhalation, develop an antibody response and in general have a mechanism to control the disease before being fatal, like humans (Dharmadhikari and Nardelli 2008). However, only rabbits, like humans, develop tuberculosis cavities and may develop a localized infection.

Curious, it is that man can present spontaneous reactivation of the disease, while rabbits require immunosuppression for the disease to be reactivated.

The use of rabbits in cancer research is essential in finding and understanding of fundamental mechanisms of malignancies and metastasis process in order to improve prevention, obtaining an accurate and early diagnosis and to develop advanced treatments. Even the use of rabbits as models in mammary carcinomas is low (Stan 2014) the following cancers are studied using rabbits as experimental model: VX-2 tumors, endometrial adenocarcinoma, monoclonal gammopaties, nephroblastoma, lymphoblastic leukemia and malignant fibroma. The Shope papilloma virus which was first isolated in cottontail rabbits show substantial sequence similarities to HPV1a and due to this feature it has been used as a model to human papillomaviruses (Willer et.al., 1999). This finding has a great importance in HPV vaccine producing and establishing new antiviral therapies. The VX-2 tumor induced anaplastic squamos cell carcinoma played a crucial role in using rabbits as cancer models of liver, pancreas, lung, kidney, urinary bladder, uterus, bone and even brain (Gaba et al.,2012).

As a general rule for all studies involving animals used as experimental model in cancer research it should be apply ethical principles of biomedical research. The main considerations that must be fulfilled are: a careful choice of study design and statistical methods; correct choice of tumour models - genetically modified, metastatic, orthotopic; the possibility of applying the therapy and stopping the experiment – human endpoints- if the cancer affects multiple systems.

Chinchillas as experimental models

Chinchillas are the traditional model of research related to the ear canal. Although some studies used mice and guinea pigs in the same area (Salvi and Boettcher 2008), due to special anatomical features, chinchillas remain the ideal model in this field of research.

These considerations are based on anatomical similarities of the inner ear – including the cochlea with that of humans. Chinchilla ear has three rounds of cochlea and the tympanum similar to that as described in humans. Easily accessible cochlea, large tympanic membrane together with an easy approach of the middle ear through a large and thin tympanic bullae are just some substantial and consistent anatomical attributes that support the above statements (Songer et al., 2007, Lim et al., 2005). The main diseases of the ear for which chinchillas are used as an experimental model are: hearing loss (Dun et al 1991; Salvi et al. 2008), otitis media (Lim et al., 2002, 2005; Giebink, 1999), tinnitus, perforation of the tympanic membrane (Downey and colab.2003), cochlea implants (McFaden et al. 2002; Naito et al. 2004), perilymph fistulas (Wall and Casselbrandt 1992), cholesteatoma (Masaki et al. 1989), implantation of electrodes, superior canal dehiscence (Songer and al. 2007).

Another anatomical feature on which numerous studies on drug ototoxicity are based, is the thinness and smoothness of the round window of the ear (10-14μm) - compared to humans (40-70μm) - that allows easy penetration of drugs (Daniel et al. 2008). Also, on this anatomical characteristic are based pharmacokinetic studies of otic antibiotics administration. The average lifespan of chinchillas -15 years- compared to mice -2 years - offers safety research without interference of senescence phenomenon of auditory system during the study period (Giebink 1982; Bakaletz 2009). The audiogram conducted on chinchillas is closer to that of humans than the ones achieved in other rodents given that the frequency ranges from 20 MHz to 30 MHz (Miller 1970; Giebink 1999; Heffner and Heffner 1991).

Immunologically and genetically chinchillas have many advantages as an experimental model. Mucous membranes (digestive, respiratory, genital, urinary) are the most vulnerable structures, their main characteristic being the prompt answer on contact with antigens. These structures represent not only an anatomical entity, but also an immunologically functional one, with protective role against infections coming through the mucosa. The mucosal structures most involved are the respiratory and digestive mucosa. In the structures above mentioned the immune system is represented anatomically and functionally by: BALT (lymphoid tissue Associated bronchus) and GALT (gut lymphoid tissue Associated). In most rodents the BALT system is well developed compared to humans in which BALT structures are not developed being replaced by palatine tonsils and those nasopharyngeal (Waldeyer ring) representing the NALT barrier (Associated nasal lymphoid tissue). NALT equivalence is well developed in chinchillas compared to other rodents, dogs, cats and rabbits in which the presence of nasal branched turbinates are not suitable for research in this direction (Barone 1996; Quesenberry and Carpenter 2012; McGilvary et al.2008). Anatomically, chinchillas are the ideal candidates in the studies on transport and responsiveness of nasally administered vaccines and the possibility of establishing therapy in this way for otitis of the median ear (Bakaletz 2009).

Chinchilla's immune response can be easily studied because they can use human-based reagents based on the fact that the cytokines involved in the immune response cross-react (Giebink 1999; Sato and colab.1999). Human cytokines stimulate maturation of dendritic cells derived from bone marrow and such human immunoglobulin antibodies cross-react with immunoglobulins of chinchillas. There are no studies at the molecular level due to a lack of a synthesized anti-chinchilla serum, as is done with mice and other laboratory rodents (Lim colab.2005). However, there is sufficient similarity at the genetic level between the genes encoding the formation of mucin, the effectors of innate immunity and epithelial cell receptor (Bakaletz 2009). Therefore immunology is another area of wide interest where chinchilla can be used as an experimental model due to diversity of immune response.

Other areas of study where chinchillas can be used as an experimental model include renal micro-anatomy, cardiovascular anatomy, arterial blood supply of the brain and ovarian endocrine activity besides gastrointestinal motility studies, and respiratory system diseases.

CONCLUSIONS

Animal models are and will remain necessary in both basic and applied research projects. The value of these models lies in proper knowledge of the anatomy of the specie involved and the ability to manipulate anatomical systems and apply rigorous scientific methods to discover the underlying mechanism of the disease and to develop the most advanced treatments.

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