International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 5 Issue 2, January-February 2021 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Transgenic Animals: A Better Approach towards Experimentation

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ABSTRACT

Animals play a crucial role in the development of medical products from medicines to various implants and major surgical procedures. They are not approved for human use until they qualify the safety parameters in animals. The animals play a pivotal role in drug discovery and development. One of the most challenging aspects of targeting drugs for any pathological condition is the development of animal models. Animal models are nothing but an approach to replicate the general symptoms of the diseased condition which occur in humans. The whole pathophysiology cannot be replicated but, the symptoms which are known and precipitate the most in any pathological condition are positively reproduced within the animal. With the increasing need in the medical field, the paradigm is now slowly shifting towards transgenic animals as they have given a new dimension to medical sciences to study the pathophysiology of the disease rather than focusing on the symptom-based analysis of the diseased condition. These genetically modified organisms have shown groundbreaking results not only in developing models for various diseases but also in developing biopharmaceuticals, receptor-selective drugs, and have a huge contribution to xenotransplantation and the agriculture sector. This review article vividly describes the newer approaches that are adopted to develop transgenic animal models and also highlights certain limitations to the older approach of developing the models.

KEYWORDS: *Transgenic animals, in vivo models, ex vivo models, transgenesis, xenotransplantation, disease resistant animals*

How to cite this paper: Swarnima Negi | Dr. Sachdev Yadav "Transgenic Animals: A Better Approach towards Experimentation" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-5 | Issue-2, February 2021, pp.13-17, URL: www.ijtsrd.com/papers/ijtsrd38268.pdf

IJTSRD38268

INTRODUCTION

Animal models are nothing but an approach to replicate the general symptoms of the diseased condition in animals which occur in humans. Over the decades with the refinement in technology, the animal models can be are prepared by using different methods that involve some genetic and non-genetic approaches of model development. The animals which are involved in the research are of two types:

1. Non-genetically modified animal model of human disease

The models developed can be in vivo, in vitro, or ex vivo. Usually, the study begins with the in vitro models later the data is extrapolated to in vivo and finally to humans.

In vitro models – These are developed by analyzing the action of the drug either on the tissue of the organ from the animal itself or on the tissue homogenate. These models usually opted for the screening of any new chemical entity. For example, the hepatoprotective screening studies are done on primary hepatocytes or fresh hepatocytes extracted from the animal. In some studies, direct human cells can be taken for analyzing the action of drugs.

Ex-vivo models- These models involve the organs extracted from the animal body and kept alive by giving almost same environment like inside the human body. For example heart is isolated from the animal body and action of different drugs are analysed. Similarly the liver or part of liver is also isolated to study the hepatoprotective actions of different drugs.

In-vivo models – In these models, there is the involvement of intact animals, which are produced by different methods.

Physical models – The induction of symptoms in these is done by physical factors such as electric shock to induce convulsions. Similarly, the pain is induced by radiant heat or cold treatment to study the analgesic effect of the drugs.

Chemical models – The symptoms are induced by exposing the animal to certain drugs, which alters the ongoing physiology of the animal and thus produces the symptoms of the disease. For example to study the hepatoprotective action of any chemical entity the animals are exposed to hepatotoxic agents such as carbon tetrachloride, ethanol, acetaminophen, thioacetamide, etc. Similarly, to develop the model for antipyretic agents the lipopolysaccharides and yeast are injected into the animal. Streptozotocin and alloxan, synergistically destroy the pancreatic beta cells and produce the hyperglycemic condition within the animal.

Surgical models – To develop certain symptoms the surgical interventions are adopted, one of the most common examples is pancreatectomy to induce diabetes-like condition in animals. Similarly to develop a model of

myocardial infraction either the coronary artery ligation is one of the methods which is opted and others may include thoracotomy. To produce renovascular hypertension the either one or both the kidneys are tied, to produce secondary hypertension within the rats.

2. Genetically modified animal model of human disease. (Transgenic models)

Transgenic animals are the ones whose genome has been modified deliberately to achieve the desired genotype which resembles the diseased condition in human beings. Transgenic animals are considered the best models to work with because the researcher does not have to prepare the animal for a certain study which is usually done with normal animals a month or two months early before starting any drug therapy. Based on the increasing need for animals in medical sciences it has become more important to have animals that by birth show the characteristics of some of the diseases and disorders similar to that of human beings. The main advantage of this approach is it provides a method to rapidly introduce new genes into animals without cross-breeding.

Limitation of a non-genetically modified animal model of human disease

One of the major limitations of normal animals is they do not provide a solid basis for the pathogenesis of a disease. Thus, the research always revolves around the development of symptomatic treatment of the disease. This fact is not hidden that almost all the drugs which are available for the common aliments are based on giving symptomatic relief be it diabetes, Multiple sclerosis, Parkinson's disease, Alzheimer's disease, etc. These models are produced by giving the animals various environmental exposure, which is a tedious and time-consuming task. As animals have to be prepared for the induction of any drug therapy weeks and months prior. For example for induction of hypertension, the diet of the rat is modified, it has to be provided with an increased salt diet from a month earlier. Apart from this the methods which are adapted to induce the symptoms of any pathological condition are not always favorable. Sometimes because of the exposure of chemicals and surgery-induced models, the animals are known to produce symptoms other than the ones which are required, thus hampering the outcome of the final results. Although the researchers keep sham control for such purposes but cannot confidently rule out the possibility of surgery hampering the results.

Production of genetically modified animals or transgenic animals

The production of transgenic animals is the targeted mutation which is solely based on two approaches that are gene knock-in (insertion of any gene to the recipient animal) and gene knock-out (deletion of a gene from the animal). This addition and deletion are based on the altered genetic makeup in humans during any pathological condition.

The procedure involves the manipulation of endogenous genomic DNA by transfecting the desired set of genes from the donor animal. The process deals with the introduction of specific mRNA into the germ cells of the host by using recombinant DNA technology, with the thought that if germ cell lines are altered, the characters will be passed on to the forthcoming generation in normal reproduction. This whole process of transferring the manipulated gene or altering the DNA sequence is called transgenesis. Transpharmers is the name given to the transgenic animals which are involved in the production of pharmaceutical products. Table 1 shows the different methods for the production of transgenic animals.

Table 1 Methods of producing transgenic animals

S/N	Technique	Procedure	Limitations	Examples
	Gene knock-in			
1.	DNA microinjection (Pronucleus microinjection)	The eggs are obtained from a donor female and in vitro made to fuse with the sperms. The transgene is microinjected to the male pronucleus in the premature zygote. The zygote is then transplanted to the uterus of the pseudopregnant female.	The success rate of this method is very less, few offsprings produced are transgenic. The technique is not popular as it is tedious and very few cells can be handled at a time.	Muta mouse, big blue transgenic rats.
2.	Embryonic Stem cell-mediated gene transfer	The totipotent stem cells are isolated from the donor pregnant female. They are cultured invitro and later transfected with the desired gene. The pure population of transfected ES cells is microinjected to the blastocyst. The blastocyst is finally implanted into the uterus of the pseudopregnant recipient animal.	The progeny produce by this technique is chimeric i.e. they contain both normal cells and genetically modified cells.	Mice, rats
3.	Retrovirus mediated gene transfection	The vectors used for transfection is retrovirus which include lentivirus and adenovirus.These are modified by incorporating the desired gene into its genome. Later the recombinant virus is microinjected into the embryo of the animal and grown-up to blastocyst in invitro. Later the a blastocyst is implanted in the pseudopregnant female.	The progeny produced is chimeric in the F1 generation. The probability of offspring with transgene is very low.	Pigs,mice (Smian virus was introduced in mice for the first time)

4.	Transposons (Jumping gene)	These are the small DNA sequence that is used as a vector, which can jump from one place to another in a genome simply by cut and paste mechanism. Thus, recombinant transposons are microinjected into the embryo of the animal to have the desired character in the progeny.	-	Transgenic insects, zebra fishes, chicken cattle.
5.	Somatic cell Nuclear Transfer	The oocytes are arrested in meiosis II of the cell cycle, specifically at metaphase or telophase. These are reprogramed with the nucleus of the somatic cell (skin cell, liver, fat, or any other organ of the body is extracted). The cell is stimulated with an electric shock to begin the cell cycle.	-	Dolly sheep (mammary cell somatic cell was incorporated)
6.	Sperm Mediated Gene Transfer (SMGT)	Mature spermatozoa are allowed to bind to the exogenous transfected DNA which is then injected through ICSI (Intracytoplasmic Sperm Injection) to the cytoplasm of the oocyte.	The whole procedure is dependent upon the binding of the sperms to the DNA without losing its activity.	Cattle, Pigs
7.	Testis cell implantation	The testis is removed from the donor male and transfected with the desirable gene, the cells are then cultured and microinjected to the lumen of seminiferous tubules of the recipient animal. Later the recipient was followed to mate.	-	-
	Gene knock-out			
8.	RNA Interference (RNAi)	The siRNA has the complementary sequence which attaches covalently with the specific sequence on the principle mRNA strand. The powerful digesting enzyme argonaut destroys the complementary sequence on the principle mRNA strand and silences the particular gene.	-	Salt sensitive rats used in the antihypertensive study.

The superiority of transgenic models to the non-genetic animal model of human disease

The transgenic models are superior in every respect as compared to the other non genetically modified models of human disease. The transgenic animals have shown pioneering results in various fields. It has not only paved the path to developing new frontiers in medical sciences but also various other fields including agriculture. The scope of transgenic animals is not only limited to serve humanity in the medical area but also in various other sectors which is not seen with non genetically modified animals. The below mentioned are the areas where transgenic have shown their contribution more as compared to the non-genetic models which are restricted to only animal models of human disease.

Human disease models

The transgenic models are by far the best models to study the pathogenesis of the diseases. This technology has enabled the researcher to look beyond just symptoms and have focused the studies towards the curative aspect of treating the disease. The preference has now shifted towards the transgenic models in almost every research. This approach can decrease the number of higher animals to be used in the experimentation likes canines, nonhuman primates. A successful model can be produced by modifying the genomic sequence. This approach has very well defined the pathogenesis of human diseases and has lead to the birth of gene therapy. The advantage which comes with transgenic models is we are able to produce models for conditions like those whose models have not developed yet in normal animals. The transgenic animals are easy to work with because they do not require induction therapy.

For example, to study the basis of essential hypertension, no such model has been developed which could give an insight over the pathogenesis of the same. But transgenic animals have been produced known as Spontaneous Hypertensive Rats (SHR) and SHR-SP stroke-prone hypertensive rats, Dahl and Sabra rats which are salt-sensitive rats, and rats which already have highly activated RAAS. Thus the study becomes easy with the genetic models.

Production of Biopharmaceuticals

The transgenic animals are known as bioreactors when it comes to the production of biopharmaceuticals. The biopharmaceutical contains vaccines, blood products, gene therapies, etc. Several proteins like AT-III (antithrombin III), TPA (tissue plasminogen factor) are produced in the mammary glands of goats and sheep which are later extracted from them. Another blood product is produced in transgenic swine is hemoglobin that has the same oxygen-carrying capacity as that of human hemoglobin.

Certain enzymes are also produced by the use of transgenic animals, like the enzyme α-glucosidase which is required in

patients with Pompe disease thus the enzyme is produced in the milk of transgenic rabbits and later extracted and purified to be given to the patients. Similarly, the enzyme butyrylcholinesterase has been developed in the milk of goats and sheep which can reverse the poisoning of organophosphates.

The goat's mammary glands are transfected by the spider gene through which they produce milk which constant of strong strands known as Bio-Steel. The polymer is extracted from the milk and the threads are weaved and used in the preparation of military uniforms, medical sutures, and racket strings.

Organ transplantation (Xenotransplantation)

Transgenic animals have shown the best results when it comes to humanizing the animal organs which are successfully used for organ transplantation. The biggest challenge is to establish the compatibility between animal donors and human physiology, secondly, the aim is reducing the transmission of pathogens from donor to human. The two main approaches which are adopted to increase the compatibility are, the transgenic animals have been produced with human proteins on their surface, which has drastically reduced the incompatibility. The second approach is to knock out the genes which are responsible for the production of antigenic structures on the surface of the animal's organ. It was observed that the organ's survival time has increased to several months when the 1,3-αgalactosyltransferase gene was knocked out. The RNAi-mediated gene knockout is a successful tool to splice out the PERV (Porcine Endogenous Retrovirus) gene which has shown many complications in the human recipient for heart or valve transplantation. Thus, technology has made safe xenotransplantation from animals to humans. The animal which is widely used for this purpose is the transgenic pig as it shows a high level of anatomical and physiological similarities with humans. The transgenic animals are being used to transplant hearts, heart valves, skin (for burnt cases), kidneys. The xenotransplantation is not possible with normal animals as there is a risk of hyperacute and vascular rejection of animal organs in the human body.

Production of antibodies

The genetically modified animals are also used extensively to produce monoclonal antibodies. Usually, mammary glands are targeted for the production of antibodies. Mostly goat mammary glands are targeted for the production of the antibodies. The anibodies produced by transgenic animals give resistant against many disease as they are recombinant. Bovine offspring produced through transchromosomal techniques expressed human immunoglobulin in their blood.

Agriculture

Ranching is one of the fields in agriculture in which animals are reared for milk and meat purposes. The pigs are made transgenic to improve their fat-muscle ratio and growth rate by incorporating the porcine growth hormone gene with the human metallothionein as the promoter sequence, which enables the transcription. The sows are modified with the bovine lactalbumin gene to produce milk that is rich in lactose content and produces a higher yield as compared to the normal sows. Similarly, the transgenic sheep have been used for high-quality wool (clear fleece) production by knocking in the keratin-IGF-I gene into the sheep's genome. The bovine mammary glands are transfected with the human lysozyme gene, as human milk contains a higher amount of lysozyme and lactoferrin which are the potential antimicrobial and main source of iron in the milk respectively. Thus with this, the bovine milk quality is increased to many folds and more amount of lactoferrin is best suited for feeding infants. The transgenic cattle have been developed to produce superior quality milk that contains beta and kappa casein which in turn yield the high-quality production of milk-based products. Another modification that is done in a bovine genome is knocking out the B-lactoglobulin gene to produce lactose-free milk for lactose intolerant individuals. Thus, transgenic animals can be modified according to the need.

Improving reproductive performance and fertility

Some genes are known to increase the reproductive performance within the animals. Such as the gene ESR gene introduction within the swine genome can increase in the litter size. Similarly the another gene Booroola fecundity gene (FECB) when transfected in the sheep genome ovulation rate was increased.

Disease resistance

The animals which are either used for xenotransplantation or edible purposes possess a great threat of transmitting the infection which is prevalent in animals. Some disease does affect animals but shows detrimental effects when passed on to humans. Thus, transgenic animals have produced disease resistance by knocking out some specific genes from their genome. The prion protein is one of the biggest threats which could transmit from animals to human found in animals. In humans, it is known for causing fatal neurological disorders. Thus, the gene which encodes for this protein is knocked out from the transgenic animals. Thus these animals are much safer to use in any form as compared to normal animals. Similarly, pigs are prone to influenza infection thus the expression of the Mx1 gene is amplified in their genome as the gene is known to provide resistance against influenza.

The sheep are made resistant against the Maedi-Visna virus (MVV) as it can be fatal to human beings if the infected product is consumed. The cows have been made resistant to one of the common pathology that is mastitis by incorporating lysostaphin gene in their mammary gland.

Development of receptor-selective drug

The gene from the animal's genome is knocked out with encodes for the specific type of receptor subtype. Then the drug is given, to check if it is acting on other receptors other than the knockout one. These animals have helped in formulating the selective agonist and antagonist for particular receptor subtypes. In comparison to normal animals, these types of selective studies cannot be done precisely.

Conclusion

With the increase in medical advancement globally, the medical fraternity still lags to find the cure of very common diseases with normal animal models. Thus transgenic animals have now slowly taken over the animal model of human disease. This approach will change the fate of the medical sciences in near future.

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