Diabetes Mellitus Animal Models

Manisha Choudhary, Associate Professor

Department of Management Studies, Vivekananda Global University, Jaipur

Email Id- choudhary.manisha@vgu.ac.in

ABSTRACT: Diabetes research has made significant use of animal models. Early research on the function of the pancreas in glucose homeostasis relied on pancreatectomized dogs, which led to the discovery and purification of insulin. Animal testing has become controversial in today's society, with varying levels of legal and ethical restraint. Rodents are the primary experimental subjects, although some research is done on bigger animals as well. Streptozotocin and alloxan, among other toxins, cause hyperglycemia in rats and mice. It has been shown that selective inbreeding has generated a number of animal strains that are good representations of the phenotypes associated with type 1 and type 2 diabetes (as well as other metabolic disorders). The majority of novel therapies for diabetes, such as islet cell transplantation and preventive measures like insulin injections, are first tested in animals before moving on to humans. Many novel diabetic animal models have been developed recently using molecular biology methods, such as knock-in, generalized knock-out, and tissue specific knock-out mice.

KEYWORD: Diabetes, Mouse, Rat, Rodent, Transgenic.

1. INTRODUCTION

In the area of diabetes research, animal testing has a lengthy history. The purpose of this article is to examine the most frequently used animal models and to highlight the most current technical advancements in the field. The study is based on a thorough search of scientific journal databases such as MEDLINE using the keywords rodent, mouse, rat, animal model, transgenic, knockout, diabetes, and pathogenesis[1]. In addition, during the past 5 years, papers given at meetings of Diabetes UK, the European Association for the Study of Diabetes, and the American Diabetes Association were reviewed to obtain an understanding of recent and current research initiatives.

Because the subject is so broad, this review must be condensed in order to include both Type 1 and Type 2 diabetes models. Nonetheless, it should serve the goal of informing practicing physicians and others who are not presently involved in this kind of study about the current methods. It should also serve as a starting point for anybody interested in doing animal-based research. The many particular evaluations referenced throughout this page, as well as the outstanding book ‘Animal Models of Diabetes: A Primer,' are recommended to interested readers.

1.1 Issues of Ethics, Morality, And The Law:

To begin, it's essential to recognize that some individuals believe that animal research is an unethical way to learn more about human diabetes mellitus. There are some who believe that subjecting animals to experimental treatments that cause any degree of pain is ethically unacceptable since an animal is unable to give informed permission and does not profit directly or indirectly from the research[2]. A few medical scientists, on the other hand, believe that they should be allowed to conduct whatever studies they see fit with little or no outside intervention. A succession of Acts of Parliament have been enacted in the United Kingdom to restrict the use of animal research, mostly as a consequence of public concern over vivisection on non-anaesthetized animals. It's worth noting that legislative restrictions and the severity with which they're enforced differ dramatically across the globe, and even within Europe.

Animal research in the United Kingdom is presently regulated under the Animals (Scientific Procedures) Act 1986. Any live vertebrate (excluding man) and the common octopus are considered protected creatures (Octopus vulgaris). 'Regulated procedures' are any experimental or other scientific operations performed on a protected animal that may cause the animal pain, discomfort, suffering, or long-term damage, and are unlawful unless they are covered by proper licenses. It's worth noting that killing a protected species using specific techniques does not need a license, but it does necessitate proper training and competence. Individual researchers must get a Personal License (PIL) that allows them to conduct controlled operations on protected animals. The PIL is only given after thorough training and evaluation, and it specifies which regulated operations and endangered animals are covered by the license.

The PIL, on the other hand, may only be used with a Project License (PPL). The PPL allows particular labor for a defined purpose and requires each regulated operation to have a documented protocol[3]. The intensity of each operation must be determined. Furthermore, the study may only be conducted at a facility that has

been granted a 'Designation of Establishment,' which includes the appointment of a named animal care and welfare officer and a named veterinary surgeon. A Home Office Inspector oversees the whole system.

In the United Kingdom, a researcher who wants to do diabetes research on animals must operate in a recognized facility and apply for both Project and Personal licenses. Prior to submission to the Home Office, applications are evaluated by a local animal research ethics committee, and final permission may take anywhere from 6 to 12 months. A high-profile group of UK academics has expressed worries that they are becoming unable to compete with research organizations based in nations with considerably less restrictions on animal testing.

Even if a certain experiment is lawful in the UK, administrative procedures and expenses may indicate that the research may be carried out more swiftly and inexpensively in another nation. Furthermore, certain diabetic organizations that depend significantly on public contributions may be pressured to refrain from funding animal studies[4]. Some donors, for example, may express a desire for guarantees that their funds would not be utilized for animal research.

1.2 Hyperglycemia Animal Models:

Researchers was researching on fat absorption from the gut in the 1880s when Minkowski recommended he remove a dog's pancreas. Polyuria and polydipsia occurred in the animal, and diabetes mellitus was discovered. Many studies on rabbits and dogs followed, but Marjorie, one of the dogs used by Banting and Best in their pioneering work on the separation and purification of insulin in the 1920s, has a unique place in history. Marjorie is the most renowned experimental animal in history, although she has since been surpassed by Dolly the Sheep.

The removal of the pancreas, either partly or completely, is one of the simplest methods of investigating the consequences of hyperglycemia in an animal. Several variables influence which animal species is chosen[5]. In general, the smaller the animal, the easier and less expensive the experiment will be. As a result, the most frequently utilized animals are pancreatectomized rats and mice. The use of the 'lowest' feasible animal is one of the guiding principles of animal research, and authorization to remove the pancreas of a dog would not be given unless a comparable experiment could not be done in a mouse. However, since rodents may not properly represent human situations, bigger animals such as cats, dogs, pigs, and primates are sometimes used as justification.

There are other non-surgical ways to cause hyperglycemia by injuring the pancreas. Toxins like streptozotocin and alloxan are used in these procedures. Surgical and toxin-mediated pancreatic injury are useful techniques in the research of hyperglycemia's effects.

For example, the onset of diabetes problems. They may be used to investigate the effects of gestational diabetes on the progeny when conducted on female animals. Experiments may be refined further by attempting to manage hyperglycemia with insulin or oral hypoglycemic medications. In pancreatectomized animals, however, restoring normal glycemia is almost difficult[6].

Much of the research on islet cell transplantation has depended on the use of diabetic rats created using the methods outlined above. The islets may then be transplanted and the results studied. Islets may be transplanted subcutaneously, beneath the renal capsule, or seeded into the liver through the portal vein, with or without capsules to prevent rejection. Anti-rejection treatments may then be administered to the animals. Even if human patients gave their informed permission, it's hard to see how such a vast number of experimental situations could be reproduced without utilising animal models. However, it is essential to note that what works in a rat may not work in a person, and that data from human trials will be used to determine the final success of islet cell transplantation programmes.

Diabetic rats are frequently employed in the evaluation of novel pharmacological compounds having a possible role in treating people with the illness, outside of the high-profile area of islet-cell transplantation. As a result, all novel orally active medicines will be evaluated in rodent models for effectiveness and safety[7]. This may lead to some intriguing and unexpected results, such as the possibility that PPAR agonists might help to maintain -cell mass. Occasionally, less desirable outcomes emerge, such as the formation of tumors in mice given an insulin analogue.

1.3 Glycosuria in Animals:

Dr. Phlorizin is a naturally occurring flavonoid that, when injected subcutaneously into an animal's body, attaches to and inhibits the resorption of glucose via the proximal renal tubule sodium/glucose transporter. The

injection of phlorizin to a diabetic mouse resulted in significant glycosuria and polyuria in the animal. In this model, the function of hyperglycaemia in the development of diabetic complications may be investigated. It can also be used to untangle the impact of glucose on diabetic complications from the effects of the multitude of other metabolic con- sequences that occur in experimental diabetes[8].

1.4 The NOD Mouse:

In order to create the NOD mouse, researchers carefully bred offspring from a laboratory strain that was originally employed in the study of cataract formation. Insulitis appears in the mice when they are 4 – 5 weeks old, and it is followed by subclinical -cell death and a decrease in insulin concentrations in the bloodstream. Frank diabetes usually manifests itself between the ages of 12 and 30 weeks. Ketoacidosis, in contrast to Type 1 diabetes in humans, is a very benign condition in which afflicted animals may live for weeks without the injection of insulin. A further difference between men and females is seen in certain colonies, which is in contradiction to the results of most human research. In some colonies, 90 percent of females acquire diabetes, compared to just 60 percent of males[7].

In contrast to other animal models used in biomedical research, the NOD mouse seems to be especially similar to human Type 1 diabetes, and as a result, a large number of studies have been carried out in the 25 years since its creation. Several susceptibility genes, for example, have been identified via genetic research in which the MHC region, as in humans, has played a critical role. When it comes to human genetic linkage research, the necessity to gather a large number of families, worries about paternity, and the dependence on whatever pattern of mating has happened within the population are all obstacles. Animal studies avoid all of these issues since it is feasible to examine huge numbers of progeny from whatever mating arrangement the researcher decides to put up in the laboratory setting[9].

Inbred animals, such as the NOD mouse, offer further advantages for investigating various aspects of diabetes since genetic variability does not need to be taken into account as a confounding factor. As an example, researchers have attempted to put together the immunological cascade, which comprises T-helper type 2 (Th2) cells, effector cells (CD4+ and CD8+ cells), and the involvement of cytokines.

1.5 The BB Rat:

The Bio Breeding Laboratories, a commercial breeding business headquartered in Ottawa, was the first to identify the BB rat, which was discovered in 1974. At approximately 12 weeks of age in diabetic strains, symptoms such as weight loss, polyuria, polydipsia, hyperglycemia, and insulinopenia begin to manifest themselves, typically around the time of puberty. Exogenous insulin must be given in order to prevent ketoacidosis from becoming deadly, much as it is in humans with the illness. In the same way that the NOD mouse is exposed to an immunological assault, the pancreatic islets are recruited to the insulitis by T cells, B cells, macrophages, and natural killer cells. GAD and other auto-antibodies have been found in BB rats and NOD mice, however it is unclear which of them are primary autoantigens and which are secondary autoantibodies[10].

BB rat strains prone to diabetes usually exhibit significant T-cell lymphopenia in the circulating blood, with a deficiency in T cells that express the antigen ART2 being particularly prominent. It has been shown that the transfusion of histocompatible T lymphocytes expressing ART2 may prevent the development of spontaneous hyperglycemia in BB rats. Several susceptibility genes, including loci in the MHC region, have been discovered via genetic research.

1.6 Diabetes Prevention in The NOD Mouse and The BB Rat:

Several rodent models of Type 1 diabetes have been used in studies investigating the role of diet (for example, the consumption of cow's milk proteins) and a variety of viruses as environmental triggers for the disease. Examples include infection with the mouse hepatitis virus, which lowers the incidence of diabetes in NOD mice, and Kilham rat virus, which raises the risk of diabetes in BB rats. Combining immunosuppression with cyclosporin with other immunomodulatory compounds such as vitamin D has been shown to help prevent diabetes in both BB rats and NOD mice, according to the findings. In these mouse models, non-specific vaccination with Freund's adjuvant will also prevent diabetes from developing. In the hopes of inducing tolerance and reducing the immune attack on the pancreatic islets, insulin has been administered orally for several years. Some studies, but not all, have demonstrated that this strategy can prevent diabetes in the NOD mouse and the BB rat. Nicotinamide is well-known for its ability to protect beta-cells against toxins such as alloxan and for its ability to delay the development of diabetes in NOD mice. Because of these findings, many

clinical studies in people at high risk of developing diabetes have been conducted, including the use of oral insulin and nicotinamide, among other things. Unfortunately, the present outcomes are unsatisfactory in the short term.

2. DISCUSSION

The use of molecular biological methods has resulted in a significant increase in the number of animal models utilized for the study of diabetes. In genetic engineering, gene targeting refers to the method by which a single gene may be damaged in an embryonic stem cell and subsequently propagated down the germ cell line. This results in the production of 'knockout' animals. Transgenic is the term used to describe the introduction of changed genes (transgenes) into the pronuclear of a zygote throughout the development of the organism. The trans-gene is randomly integrated into the host genome, and some progeny will display the changed gene as a result of this integration. This kind of approach, according to theory, allows for the selective disruption of a single element of a complex system. For example, rats that over- or under-express proteins believed to play a critical role in glucose metabolism may be created and used in research.

Briefly stated, it is feasible to super ovulate a female mouse or rat and then enable her to mate with another female mouse or rat. The next day, the single cell zygotes are collected and kept in culture for a few hours until they are ready to be used. A genetic construct (for example, the insulin gene) can then be injected into the pronuclear of the zygote, resulting in the development of the embryo. The construct will integrate itself into the genome of the zygote, and if this occurs at an appropriate location in the host DNA, the result will be an embryo that will overexpress insulin in adulthood. This is known as the insulin overexpression syndrome. It is feasible to exert some control over the expression of the transgene in adult life by adding particular promoter regions in the genetic construct. For example, by including the metallothionein promoter in the inserted gene, the inserted gene will be 'turned on' when heavy metals are administered to the adult in the laboratory.

When studying the offspring of these transgenic experiments, it is necessary to take a number of factors into consideration. Because the transgenes are incorporated into the host genome at random, the impact seen in progeny will vary depending on where the integration has happened and how many copies of the transgene have successfully integrated into the host genome. In other instances, the transgenic causes a disruption in a native gene near the location of its integration into the genome, making it even more difficult to interpret the phenotype that results as a consequence of the transgene. Furthermore, since the genetic background of the mice has a significant impact on the effect of transgenic expression, the findings of experiments may differ depending on which strain is used.

Knockout animals are created via the use of a genetic construct that disrupts the function of a normal gene. Typically, a construct is created that includes DNA sequences that are homologous to the target gene but that have been disrupted or have a deletion in the middle. If you inject one of these into an embryonic stem (ES) cell, the cells will undergo recombination with their normal genes, which will result in the normal gene being "knocked out." An antibiotic resistance sequence is usually included in the construct, which allows the ES cells that have successfully incorporated the DNA to be isolated and studied further. In the following step, embryonic stem cells are injected into pre-implantation mouse embryos and transferred to the oviducts of pseudo-pregnant mice, where they are allowed to develop until they are born. As soon as the ES cells have contributed to the germ line of the offspring, selective breeding can be used to produce mice that are either heterozygous or homozygous for the knockout.

3. CONCLUSION

Some animal models of diabetes, without a doubt, have provided invaluable insight into the pathogenesis of the human disease, which is beyond dispute. The use of animals in the development of insulin and the evaluation of other therapies has resulted in immediate benefits for patients. On the other hand, there have been a number of 'blind-alleys' in research as well, such as a lack of repeatable paradigms for human diabetic complications and the disappointing outcomes of Type 1 diabetes prevention trials based on methods that have been effective in rats.

The three Rs—reduction, refinement, and replacement—are the guiding principles of animal research in the United Kingdom. Extensive efforts should be undertaken to minimize the number of animals needed for each given research, experimental design should be improved such that it gives the animals the least amount of pain, and, eventually, animal models should be removed entirely. When comparing findings from seemingly

comparable studies conducted at various institutions, as well as when extrapolating results from animal trials to the human scenario, extreme caution should be used. Despite these limitations, substantial breakthroughs in knowledge have occurred in recent years, particularly with the introduction of transgenic and the development of new technologies. It is probable that animal models will play a significant role in the ultimate treatment of human diabetes mellitus in the foreseeable future.

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