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MOUSE MODELS OF DIABETES MELLITUS AND THEIR SIGNIFICANCE IN DIABETES RESEARCH

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ABSTRACT

Diabetes mellitus is a very rapidly increasing morbid syndrome that is getting highly prevalent day by day. It is necessary to find its causes, new and improved treatments, and discovering novel pharmaceutical and molecular medicine based therapeutics for this disease. Animal models are the most promising way to study disease mechanisms and therapeutic compounds for diabetes and for drug assessment before human trials.

Several strains of mice are currently being used in diabetes research, so having knowledge of the appropriate strain and model is crucial to get reliable results.

In the current review, we summarize a comprehensive knowledge about the most commonly used mouse strains in diabetes research. Moreover, we also comment on different mouse strains and models that could be better for any particular type of study.

Key words: diabetes mellitus, obesity, mouse model, knockout mice, pancreas, streptozotocin, alloxan,

INTRODUCTION

Diabetes prevalence and etiology

Diabetes mellitus is one of the highly morbid non-communicable diseases. Its prevalence is substantially high in both developed and developing countries. In a study conducted in 2009, it was reported that adult population affected with diabetes was 285 million in the year 2010 leading to the 439 million in 2030. An expected 69% increase for this disorder from 2010 to 2030 will be in the developing countries while in developed countries the estimated increase would be around 20% (Shaw *et al*., 2010). Diabetes is also quite prevalent in Pakistan and some studies have investigated the role of various factors in the pathogenesis of diabetes and its complications in Pakistani population (Hussain *et al*., 2010; Aslam *et al*., 2012; Hussain *et al*., 2012).

Type 1 diabetes (T1D) is comparatively less prevalent form of diabetes, as in overall population affected with this disease more than 90% patients have Type 2 diabetes (T2D) while less than 10% cases account for T1D. Both these types of diabetes have a common feature of chronically higher levels of blood glucose in the uncontrolled condition. T1D is mainly due to autoimmunity as the patient's own immune system considers β-cells as non-self and finally T-cells start to attack the β-cells of pancreas. On the other hand, T2D is mainly due to insulin resistance as the target cells become resistant to insulin even when insulin is present in the blood (Savage *et al*., 2005). However, at some stage of T2D, the β-cells of pancreas become dysfunctional; thus there is reduced or absent insulin availability. The pathobiology of T2D gets more complex than T1D but is relatively easy to manage specially in the early years when β-cells are producing enough insulin. In case of T1D, the administration of insulin is the only treatment option but T2D can be managed with diet modification and lifestyle interventions with requirement for medicines at the advance stage (Solomon *et al*., 1985, Krentz *et al*., 2008). In the latter stages of T2D, the β-cells are exhausted due to over production of insulin, which leads to the dysfunction and destruction of these cells. At such stage the clinical symptoms of both types of diabetes become almost same albeit different etiologies with exogenous insulin administration as the key treatment requirement.

Therefore, diabetes in spite of common clinical symptoms is indeed a very complex disorder due to the involvement of multiple pathways/mechanisms. This is why it is very important to choose a good and reliable animal model to study the disease mechanisms or evaluating novel experimental therapies.

Mouse models and their importance in research

Keeping in view the increasing rate of diabetes onset and associated pathologies, it is important to study the underlying causes of diabetes in its various types. This knowledge helps in identifying and establishing early markers of disease for its diagnosis, progression and monitoring response to therapies. Also, insights could be gained into evaluating new drug targets and developing specific drugs or molecular medicines. Moreover, action of traditional drugs from medicinal plants used in traditional or alternative medicines can be investigated on

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scientific principles. Such research could be conducted at various levels to study the disease biology and therapeutic options. For example using cell culture models, animal models, biofluids, tissue biopsies and at human or population levels using various biochemical, molecular, cell biology and genetics approaches. All these approaches have their pros and cons for diabetes research but in this paper we highlight the importance of animal models for diabetes research particularly in the perspective of finding out new therapeutics and drugs for this metabolic disorders. In vitro cell culture models of disease have their own strengths; but lack the true nature of a particular disease due to the absence of a holistic or system level biology approach. Although they are good for mechanistic studies yet animal models present a more universal scenario. A more reliable way to study the true natural history of a disease is through in vivo systems. For in vivo studies, the key requirement is the availability or development of an animal model of disease under study. Animal models used in biomedical research may have an existing, inbred or induced disease or injury that mimics a human disease condition. Animals with such experimental conditions are called as animal models of disease (Kari *et al*., 2007). Several animal species can be used for such studies but animals which are used for this purpose should have a taxonomic equivalence with human. To study mechanisms of disease or pharmacological interventions rodents particularly mice have been used most often because of their small size, short life span, fast breeding, easy availability and their economic value (Srinivasan and Ramarao, 2007).

Currently available models

There are several types of mouse models e.g., genetically induced, Chemical/drug induced and diet/nutrition induced (King, 2012). Genetically induced mouse models are of two types generated by knocking in or out a particular gene, or by breeding different strains. Most commonly used rodent models for diabetes are ob/ob mice, NOD (non-obese diabetic mice), Zucker fatty rats (ZFR), KK mouse and corpulent (cp) rats etc. (Plum *et al*., 2005; Srinivasan and Ramarao, 2007; Lee and Cox, 2011).

There is a long list of mouse strains which are generated by outbreeding and inbreeding of already available different strains (Clee and Attie, 2007). Particularly for diabetes and obesity related research there is an overview of strains which are preferable over others for some reason.

Mouse models for Type 1 diabetes

Type 1 diabetes is attributed to the autoimmune destruction of beta cells by T-cells. In type1 diabetes the immune system mediated cytokines like IL-1β, TNF-α and IFN-γ provoke the β-cell apoptosis via NF- kβ activation. That leads to the production of nitric oxide and chemokines while it also depletes endoplasmic reticulum calcium. Type2 diabetes mechanism is nitric oxide and NF-kβ independent, in this case high nutrients levels trigger the β-cell death in a different way (Cnop *et al*., 2005).

The animal model or particularly mouse models to study the lack of insulin are produced by using following approaches.

1) Drug-induced T1D: a. Single high dose of Alloxan/ STZ (Szkudelski, 2001), b. Multiple low doses of STZ. To analyze the therapeutic effect of different drugs and plants extracts most commonly used animal models are Alloxan or Streptozotocin induced diabetic models. Alloxan and STZ both are drugs that ultimately damage the β-cells but with different mechanism.

2) Spontaneous autoimmune: NOD (Non Obese Diabetic) mice are the most dominant examples of this type of model, as these mice are helpful in studying the parallel genetic behavior of human and mice in T1D (Yang and Santamaria, 2006; Driver *et al*., 2011).

3) Genetically induced: AKITA mice are the most important example of genetically induced mice, these mice are derived from C57BL/6NSlc mouse by inducing a spontaneous mutation in the insulin 2 gene. This mutation prevents the proper processing of pro-insulin (Mathews *et al*., 2002).

4) Pathogen induced: Since, viral infections have been implicated in the onset of T1D, pathogen induced mouse models are available to study the etiology of T1D. These pathogens either directly destroy the β-cells or induce the autoimmune reaction against these cells (Jun and Yoon, 2003).

Mouse models for Type 2 diabetes

Type 1 diabetes mouse model, it is fairly simple to generate such models. Because in such cases β-cells depleted animals are relatively easy to generate and maintain. But in the case of Type 2 diabetes mostly High Fat Diet fed approach is used to generate an appropriate model. Although it is quite effective way to mimic the condition of disease caused by consistent higher levels of glucose in blood, yet it is not the complete picture. In case of Type 2 diabetes the mechanism of β-cell death is different from Type 1 diabetes. It is therefore, necessary to choose the animal model carefully to conduct the appropriate research.

Obese models of type 2 diabetes: T2D is closely associated with obesity and weight gain, so monogenic obesity related models are available, although in humans, obesity is rarely induced by one gene. But these models are used to test the therapeutic agents (Gault *et al*., 2011). Leptin deficient (*Ob/Ob*) and leptin receptor (db/db) mice are other obesity and T2D models made by genetic modifications (Hummel *et al*., 1966).

High fat feeding: High fat or high carbohydrate feeding mice can lead to the hyperglycemia, obesity and hyperinsulinimia and serve best purpose to study the mechanisms involved in the T2D and also to test the therapeutic agents for the disease.

Non-obese models of type 2 diabetes: Although T2D is mostly related to the obesity yet there are many cases of non-obese T2D. To understand the differences, mechanisms and pathways involved in the lean T2D there are lean animal models available like hIAPP mice.

While working on the analysis of some new compounds assumed to have therapeutic potential for diabetes, it is very difficult to choose the right type of mouse models.

In this regard, it is very important to have the basic knowledge about mouse strains and their genetic background. Some of the commonly used strains are A/J (Classic diabetes resistant strain), CAST/Ei (Used for obesity and atherosclerosis) Balb/C, C57BL/6, C56BLKs/J, NOD (Non-Obese Diabetic) and NON (Non-Obese Non-diabetic) mice, New Zealand obese (NZO), KK mice (closely related to NZO), M16: created through long term ICR (Imprinting Control Region) outbred mice for body weight gain (Clee and Attie, 2007).

Generally most of the rodent s have tendency to be obese after feeding on high-fat diets, but the response from every strain could be different. Some strains show more susceptibility to obesity and insulin resistance when fed high-fat diets such as the C57Bl6 or AKR mice. C57Bl6 and AKR being fed on same percent high fat content may have similar degrees of weight gain but C57Bl6 mice are more glucose intolerant as compared to AKR (Rossmeisl *et al*., 2003).

Other strains of mice like SWR/J and A/J mice have more resistance for obesity (Surwit *et al*., 1995; Prpic *et al*., 2002).

We observed in our experiments Balb/C mice take a longer time to be diabetic by drug induced diabetes (un-published data), while in other experiments with C57Bl/6 showed a clear susceptibility for β-cell dysfunction and depletion (Wang *et al*., 2011).

When going to choose the right drug for the induction of diabetes STZ has several merits over Alloxan. Although Alloxan is cheaper than STZ but Alloxan has higher mortality rate in rodents while in the case of STZ there is no such report. Hence, STZ generates a suitable model for diabetes.

Low cost animal models for resource limited laboratories

Transgenic mice are made by over expressing the gene of interest to analyze the effect of that gene in metabolic pathways, while knockout mice have a functional gene removed or blocked from their genome. The genetically created knockout or transgenic mice are very sophisticated and expensive, as the cost associated for generating or purchase of these animals as well as their maintenance is quite high, which is usually a limiting factor for the research laboratories in developing countries; thus, working with such animal models present real obstacles for experimental diabetes research in low-income countries. In such situations which are predominant in the developing countries cost effective animal models are required to investigate and test remedies for diabetes research in scientific way. So that there is opportunity for cost effective models, which can be developed for diabetes research e.g. drug induced diabetic mice and diet induced models. Drug induced diabetes mouse models are most commonly made by diabetogenic drugs: alloxan and STZ. Alloxan and STZ are two drugs with different mechanisms of action but ultimately they cause the same effect i.e. pancreatic β-cell destruction (Rex, 1988; Szkudelski, 2001). Such drug induced models can be used to study the drug interventions, potential of stem cell based therapies and studies of pancreas regeneration biology or islet transplantation biology.

Another approach to make animal models of diabetes is through dietary changes (Winzell and Ahrén, 2004). Higher concentration of carbohydrates (e.g. sucrose) in diet or drinking water can also be used for the development of an animal model that can be used for the study of diabetes. Studies of drug-induced diabetes due to damage to pancreas are mainly focused on insulin dependent or T1D. However, T2D is generally non-insulin dependent at least in the early years and its etiology is very complex due to insulin resistance in the target tissues. So to study this form of diabetes in a more closely resembling fashion to humans, it is preferable to investigate diabetes in a diet-induced animal model.

Our Observations

A simple hyperglycemic model was established in our lab by high glucose consumption in the drinking water (20% sucrose) for 5 months. Changes in body weight and blood glucose levels were observed.

Body weight and fasting blood glucose levels of mice

Body weight was measured before killing animals for harvesting tissues. There was a significant increase in the body weight of treated mice as compared to control (Control =26.1 ± 4.7 vs Treated = 30.8 ± 6.7 g mean ± sd; p = 0.0343) using two-tail unpaired t test with Welch correction using GraphPad Instat (V3.05). Fasting blood glucose was also measured for both groups and it was significantly high in the treated group (Control = 76.4 ± 12.1 vs Treated = 85±8.5 mg/dl mean ± sd; p = 0.0263), using two-tail unpaired t test with Welch correction using GraphPad Instat (V3.05).

Pancreas histology

We have been working on the mouse models of diabetes both Type 1 and Type 2. For Type 1 diabetes the animals were treated with single dose of Alloxan (160mg/Kg) and for Type 2 diabetes the mice were kept on drinking 20% (W/V) sucrose water for 5 months. In both cases animals exhibited higher levels of blood glucose levels as compared to control group. Mouse pancreata were dissected and preserved in 10% formalin for further use for paraffin sectioning. Paraffin sections of mice pancreata were subjected to Hematoxylin and Eosin staining to observe the change in the histology of pancreas following the development of hyperglycaemia condition in the treated group. Control islet is significantly smaller than the treated one, and treated isl*et al*so showing amyloid formation (Fig. 1).

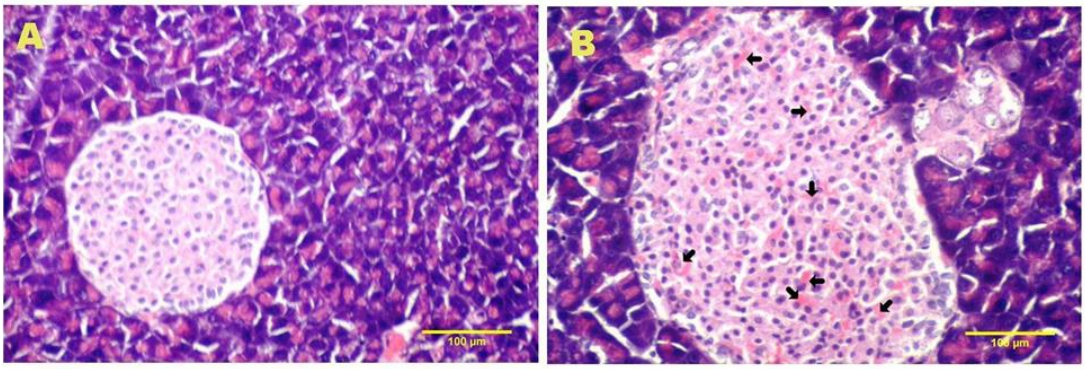

Fig.1. Histological comparison of pancreas in the control (A) and treated (B) mice.

Conclusion

In animal model studies it is very crucial to select right type of strain and mode of disease induction. In case of diabetic mouse models, knowledge of more susceptible strains for glucose intolerance is very important. Mode of diabetes induction should be chosen according to study design like the one drug-induced diabetic model is good to study the Type 1diabetes while diet induced diabetic mouse model is frequently used as Type 2 diabetes. In recent years a more Type 2 diabetes mimicking model has been developed by multiple low doses of streptozotocin and high fat diet (Srinivasan *et al*., 2005).

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