

A Comprehensive Review: The Use of Animal Models in Diabetes Research

Abstract

Diabetes, a lifelong disease for which there is no cure yet. It is caused by reduced production of insulin, or by decreased ability to use insulin. With high prevalence of diabetes worldwide, the disease constitutes a major health concern. Presently, it is an incurable metabolic disorder which affects about 2.8% of the global population. Fifty percent of all people with Type I diabetes are under the age of 20. Insulin-dependent diabetes accounts for 3% of all new cases of diabetes each year. Hence, the search for compounds with novel properties to deal with this disease condition is still in progress. Due to time constraints, the use of experimental models for the disease gives the necessary faster. The current review has attempted to bring together all the reported models, highlighted their short comings and drew the precautions required for each technique. In Type 1 or Diabetes mellitus, the body is unable to store and use glucose as an energy source effectively. Type 2 or diabetes insipidus is a heterogeneous disorder. In this review article we shall bring light as to how hyperglycemia, glucosuria and hyperlipidemia play an important role in the onset of diabetes.

Keywords: Diabetes mellitus; Hyperglycemia; Diabetes insipidus; insulin

Review Article

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Abbreviations: STZ: Streptozotocin; DNA: Deoxyribonucleic Acid; NAD: Nicotinamide Adenine Dinucleotide; GTG: Gold Thioglucose; DM: Diabetes Mellitus; KK: Kuo Kondo; ZF: Zucker Fatty; ZDF: Zucker Diabetic Fatty; T2D: Type 2 Diabetes; mZDF: Male Zucker Diabetic Fatty; TSOD: Tsumara Suzuki Obese Diabetes; NIDDM: Non Insulin Dependent Diabetes Mellitus; RNA: Ribonucleic Acid

Introduction

Diabetes is a chronic metabolic disorder which is illustrated by either the insufficient production or the lack of response to a key hormone of the body's metabolism, insulin. It can be categorized as Type-1 diabetes and Type-2 diabetes. Type 1 diabetes is a serious health condition that occurs when the pancreas makes little or no insulin. Without insulin, the body is incapable to take the glucose it gets from food into cells to fuel the body. Type 2 diabetes can occur when: the body develops insulin resistance and can't make good use of the insulin and the pancreas gradually loses its capacity to produce insulin. The disease is illustrated by hyperglycemia, hypercholesterolemia, and hypertriglyceridemia. These conditions are mainly caused due to defects in insulin secretion or reduced sensitivity of the tissue to insulin resistance. Insulin resistance arises when the body makes insulin but does not use it competently. To counterbalance for high blood sugar levels, the insulin-producing cells in the pancreas release more insulin, to try to keep blood sugar levels normal [1]. Slowly and progressively, these cells fail to keep up with the body's ever-increasing demand for insulin. This results in increase in blood sugar levels. Insulin resistance can lead to both pre-diabetes and type2 diabetes.

Animal models have a long history in the field of diabetes research. The aim of this review article is to give a summary about the commonly used animal models. Also, to discuss the recent technological advances those are being employed in the branch of learning. The review is based on an extensive literature search using the terms rodent (rat, mouse, hamsters, etc.), transgenics, diabetes and pathogenesis, in scientific journals. The topic is vast and this review is strictly limited in its depth, aiming to cover models of both Type 1 and Type 2 diabetes. Nevertheless, it should serve you the purpose of understanding the depth of the subject. It should also serve as a set-off point for those wishing to get on an animal-based research project.

The aim of the present review is to put together various experimental models, including Type-1, Type-2 diabetes. In this review article, we shall assess the merits and demerits of each model and highlight the precautions needed to avoid the necessary complications.

Animal Models for Type-1 & Type-2 Diabetes I

Chemically induced diabetes

Chemical agents that induce diabetes can be grouped into three categories. They include agents that: specifically target β -cells, cause temporary inhibition of insulin production and/ or secretion and reduce the metabolic efficacy of insulin in target tissue.

Streptozotocin (STZ) induced diabetes

STZ is a nitrosourea related antibiotic and antineoplastic

compound, which is produced by *Streptomyces achromogenes* and due to its alkylating properties, causes alkylation and thus fragmentation of DNA. It modifies biological macromolecules and finally destroys β -cells, causing insulin-dependent like diabetes. STZ is the most commonly used drug for induction of diabetes in rats [2]. Intra-venous injection of 60mg/kg dose of streptozotocin in adult wistar rats causes swelling of pancreas followed by degeneration of Langerhans islet beta cells and induces experimental diabetes mellitus in the 2-4 days. After three days of degeneration of beta cells, diabetes was induced in all animals. The Nicotinamide-adenine dinucleotide (NAD) in pancreas islet beta cells and causes histopathological effects in beta cells which leads to diabetes [3].

Alloxan induced diabetes

Allantoin is a by product of uric acid excreted by the foetus in the allantois whereas oxaluric acid is derived from oxalic acid and urea that is found in urine. Dunn, Sheehan and McLetchi were the first amongst those who described the alloxan model of diabetes in 1943. Alloxan has been used to induce experimental diabetes due to the selective destruction of the insulin-producing pancreatic beta-islets. It induces a multiphasic blood glucose response when injected into to an animal, which is accompanied by inverse changes in the plasma insulin concentration. This is then followed by sequential ultrastructural beta cell changes ultimately leading to necrotic cell death. The dose of alloxan varies with different species of animals like rat 40-200mg/kg i.v or i.p., mice 50-200mg/kg i.v or i.p., rabbit 100-150mg/kg i.v. and for dogs it is 50-75 mg/kg i.v. [4]. Alloxan causes triphasic response in animals

- Phase I-early hyperglycemia of short duration (about 1-4hr) due to a sudden decrease or termination of insulin release and direct glycogenolytic effects on the liver.
- Phase II-hyperglycemia phase lasting up to 48 hrs and often resulting in convulsion.
- Phase III-chronic diabetic phase consequence of insulin lacks histologically only a few β - cells if any are detectable in animals with fully developed alloxan diabetes.

Gold thioglucose obese diabetic mouse model

Gold thioglucose is diabetogenic compound that induces hyperphagia and severe obesity induced Type -2 diabetes. Gold thioglucose produces obesity-induced diabetes in genetically normal mouse strains. Gold thioglucose treated C57BLKs, DBA/2 (Dilute Brown Non- Agouti), and BDF1 mice gained weight rapidly and significantly increased non fasting plasma glucose level within 8-12 weeks. These mice shows impaired insulin secretion, mainly in early phase after glucose load and reduced insulin content in pancreatic islets [5]. Type-2 diabetes with obesity can be induced in mice by intraperitoneal injection of gold thioglucose (GTG) in a dose of 150-350 or 200 mg/kg. The animal gradually develops obesity, hyperinsulinemia, hyperglycemia, insulin resistance over a period of 16-20 weeks after GTG injection. The GTG is transported to the cells of ventromedial hypothalamus and causes necrotic lesions that are responsible for the development of hyperphagia and obesity. It also increases hepatic lipogenesis, body lipid, triglyceride secretion and increased adipose tissue lipogenesis and decreases glucose metabolism in muscle. These

abnormalities are qualitatively similar to genetically obese mice (ob/ob). In addition, it exhibits many molecular defects in relation to insulin-signalling pathways [6,7].

Surgically Induced Diabetes

This method consists of complete or partial pancreatectomy in animals used for the induction of type-1 or type-2 diabetes respectively. Few researchers have worked this model to explore effects of natural products with animal species such as rats, pigs, dogs and primates [8,9]. However, partial pancreatectomy and/or combination methods on animals particularly non rodents are at times exploited in the diabetes investigation for some specific studies as described below.

Duodenal-jejunal bypass non-obese T-2 DM

In Goto-Kakizaki rats, this model has been shown to reverse type-2 diabetes (T-2 DM). After two weeks duodenal-jejunal bypass, oral glucose tolerance was measured and after three weeks insulin-induced signal transduction and glucose disposal was measured in skeletal muscle. The study proved that bypassing of the proximal small intestine does not increase skeletal muscle glucose disposal. The lack of skeletal muscle insulin resistance in Goto-Kakizaki rat's doubts whether this animal model is adequate to investigate the etiology and treatments for T- 2 DM. Additionally, bypassing of the foregut leads to different findings in other animal models of T-2 DM as well as in T-2 DM patients [10].

Non-obese partial pancreatectomized diabetic animals

It is an animal model, in which part of the pancreas is made diabetic due to almost total loss of insulin-secreting B cells. While the remainder of the gland remains normal. In rabbits, a vascular clamp is placed across the junction of the body and tail of the pancreas, thus occluding the circulation to the tail. Alloxan (200mg/kg) was injected i.v. and 4mins later dextrose (0.5g/kg) was given by same route. After 2 mins the clamp was removed. 50% of the animals died in the first week of complications. They also died of alloxan-induced toxicity to the liver and kidneys. The survivors were killed between 4 and 12 weeks after surgery and were found to be not metabolically diabetic. They had a complete absence of B cells but a normal population of A, D, and PP cells in the head and body of the pancreas. The islets in the tail of the pancreas appeared entirely normal. This model is considered appropriate for studying the effects of locally produced insulin on pancreatic exocrine function in metabolically normal animals [11]. Limitations to surgically induced diabetes include high level of technical expertise and adequate surgical room environment. Also, adequate post-operative analgesia and antibiotic administration, major surgery and high risk of animal infection, supplementation with pancreatic enzymes to prevent malabsorption and loss of pancreatic counter regulatory response to hypoglycemia.

Genetically induced diabetic animal model

Spontaneous diabetic animals of type-2 diabetes may be achieved, from the animals with one or several genetic mutations transmitted from generation to generation (e.g. db/db mice) or by selected from non-diabetic out bred animals by repeated breeding over several generation [BB rat, Tsumara Suzuki Obese

Diabetes mouse]. These animals generally inherit diabetes either as single or multigenic defects as seen in KK mouse, db/db mouse, or Zucker fatty rat. The metabolic peculiarities result from single gene defect (monogenic) which may be due to dominant gene (e.g., Yellow obese or KK/A mouse) or recessive gene (diabetic or db/db mouse, Zucker fatty rat) or it can be of polygenic origin [e.g., Kuo Kondo (KK) mouse, New Zealand obese mouse]. Type-2 diabetes occurring in majority of human beings is a result of interaction between environmental and multiple gene defects though certain subtype of diabetes do exist with well-defined cause [i.e., maturity onset diabetes of youth due to defect in glucokinase gene] and this single gene defects may cause type-2 diabetes only in few cases. Therefore, polygenic animals represent the human condition more closely when compared to monogenic animals [12,13].

Zucker diabetic fatty rat

The Zucker fatty (ZF) rat has a missense mutation (fatty, fa) in the leptin receptor gene (*Lepr*) and develops obesity without diabetes. Zucker diabetic fatty (ZDF) rats derived from the ZF strain displays obesity with diabetes and are extensively used for research on type 2 diabetes (T2D). The mutated animal is associated with disruption of normal islet architecture, β -cell degranulation, and increased β -cell death. In this strain all animals develop obesity, insulin resistance and evident NIDDM between 7 and 10 weeks of age, where by which time their average plasma glucose exceeds 22mM [14]. Another study has shown that the Male Zucker diabetic fatty (mZDF) rats spontaneously develop type-2 diabetes, whereas females become diabetic when fed with diabetogenic high-fat diet (HF-fZDF). Non-diabetic obese fZDF rats were compared with either mZDF or HF-fZDF for their hepatic molecular profiles, to single out those components that might be protective in the females. The work proved that the hepatic sex differences might contribute to the sex-based development of diabetes in ZDF rats [15].

Goto-kakizaki rat

The Goto-Kakizaki (GK) rat is a genetic model of type-2 diabetes and displays profoundly defective insulin secretion leading to basal hyperglycemia. It is widely used for studying type-2 diabetes. They are characterized by non-obesity, stable fasting hyperglycaemia, impaired glucose tolerance hypoinsulinaemia and normolipidaemia, which appear at 2 weeks of age in all animals and an early onset of diabetic complications. GK rats were killed at 7, 14, 21, and 35 weeks of age. Structural islet changes were not observed in 7 weeks old animals [16]. However, animals of 14 and 21 weeks age GK rats, displayed histopathological islet changes. The general shape of islets became irregular, and immunoreactions of β -cells against anti-insulin appeared diffusely weakened. Electron microscopy revealed that the number of so-called β -granules decreased and the number of immature granules increased. Various studies suggested that insulin deficiency in GK rats is not caused by simple dysfunction and/or degeneration of β -cells but rather by more complicated events within cells [17]. GK rat is one of the best characterized animal models used for studying the relation of changes in beta cell mass and occurrence of type 2 diabetes and diabetic complications.

LEW.1WR1 rats

Spontaneous diabetes in LEW.1WR1 rats (RT1u/u/a) occurs within age of 59 days. The disease exhibits hyperglycemia, glycosuria, ketonuria, and polyuria. The study shows that the islets of acutely diabetic rats lack β -cells, whereas α - and δ -cell populations are spared. The LEW.1WR1 rat is vulnerable to collagen-induced arthritis but is free of spontaneous thyroiditis. The animal provides a new model for studying autoimmune diabetes and arthritis in an animal with a genetic predisposition to both disorders that can be amplified by environmental perturbation [18].

Tsumara suzuki obese diabetes mice

TSOD mouse is polygenic in origin and characterized by polydipsia and polyuria at about 2 months old. In male mice, the conditions are followed by hyperglycaemia and hyperinsulinemia too [19]. The TSOD mouse has been established as an inbred strain with spontaneous development of diabetes mellitus as the first clinical signs of diabetes. Following these symptoms, obesity gradually develops until about 12 months old. When seen at the histopathological examination of the pancreas, severe hypertrophy of pancreatic islets was observed due to proliferation and swelling of B cells. It has been shown that the TSOD mouse similar to NIDDM in humans, the TSOD mouse should be a useful model for the pathogenic study of diabetic complications, especially of peripheral neuropathy [20].

C57BL/6J mice

One can induce Type-2 diabetes by simply feeding high fat feed to non-obese, non-diabetic C57BL/6J mouse strain. It is characterized by increased obesity, hyperinsulinemia, insulin resistance and glucose intolerance [21]. In addition to all these traits, they exhibit marked fasting as well as basal hyperglycaemia in contrast to normal basal glucose level seen in C57BL/6J (ob/ob) mice. C57BL/6J (B6) mice develop severe obesity and diabetes if weaned onto high-fat diets, whereas A/J mice tend to be obese and diabetes-resistant. The severity of diabetes is a direct proportional to obesity and diabetes and is completely reversible by reducing the intake of dietary fat [22]. Further, its usefulness for drug testing has been reported in the literature as these mice treated with orally active inhibitor of dipeptidyl peptidase-IV (LAF237) are shown to have normalized glucose tolerance in association with augmented insulin secretion [23].

db/db mice

The db/db mouse also known as *lepr^{db}* is originally derived from an autosomal recessive mutation on chromosome 4 in mice of C57BL/KsJ strain. The mutation in this diabetic animal was traced to db gene that translates for the leptin receptors. These mice are insulin over-secretors becoming obese, hyperglycaemic, hyperinsulinemia and insulin resistant within 30 days of age and develop hypoinsulinaemia, hyperglycaemia later with a peak between 3-4 months of age [24]. These db/db mice have been extensively used for the investigation of type-2 diabetes and for screening of agents such as insulin mimetic and insulin sensitizers [25].

Obese rhesus monkey (*Macaca mullata*)

Obese rhesus monkey is an excellent non-rodent model, which close. It relates to humans, develops obesity, hyperinsulinemia and insulin resistance when maintained on ad libitum laboratory diet. It gradually progresses to necrosis of beta cells, severe fall in insulin levels and overt hyperglycaemia over a period of several years. Unlike conventional rodent models, the final secretion loss is interestingly associated with deposition of amylin or amyloid in beta cells and the development of diabetic complications similar to human type-2 diabetes. Pioglitazone has been demonstrated to improve insulin resistance in obese rhesus monkeys [26,27].

Virus Induced Diabetic Animal Model

Viruses produce diabetes mellitus by destroying and infecting pancreatic beta cells. A less infecting or cytotoxic variant produces a comparable damage by eliciting immune auto reactivity to the β -cells. There is evidence from animal models, which the hypothesis that viruses induce disease via mechanisms linked with innate immune up regulation. Various human viruses used for inducing diabetes include Coxsackie B4, encephalomyocarditis (EMC-D and M variants), RNA picornaviruses, lymphocytic choriomeningitis and, Mengo-2T, reovirus [28,29]. Information retrieved from retrospective and prospective epidemiological studies strongly suggests that enteroviruses, such as coxsackie virus B4 (CV-B4), may be associated with the development of T-1D. It has also been shown that enterovirus infections are significantly more prevalent in at-risk individuals such as the siblings of diabetic patients [30]. The oral disposition index, which is recently introduced marker that integrates insulin secretion and insulin sensitivity, raises growing interest, more particularly for the prediction of type-2 diabetes [31].

Conclusion

The animal models of diabetes mellitus are considered as very useful means for studying the pathophysiology and the clinical phases of the disease. In fact, they are always used as the initial step for examining a new therapy. Although they have many differences from the human condition and are usually characterised by many limitations such as animal size, availability and cost, investigators continue to rely on animal models due to the fact that they can be readily available, tested, biopsied and autopsied. Also, their genetic and environmental background is already known. Therefore, the continuing effort for inventing new models has always positive critics and animal models will continue to have a major and meaningful place in diabetes research. However, every researcher should always keep in mind the ethical limits in the use of these animal models for their experiments. One should only utilize animals only when they are indispensable for a study and avoid causing them pain, distress, suffering and lasting harm.

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