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In-vivo Animal Models and *In-vitro* Techniques for Screening Antidiabetic Activity

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Abstract

Diabetes mellitus is a group of metabolic disorder, is characterized by absolute by lack of insulin and resulting in hyperglycemia. About 2.8% of global populations are affected by Diabetes mellitus. The search for new drug with new properties to treat the disease is still in progress. The current review have made an attempt to bring together all reported models and advanced techniques. Experimentally diabetes mellitus is generally induced in laboratory animals by several methods that include: chemical, surgical and genetic manipulations. The various *in vitro* techniques includes: *In-vitro* studies on insulin secretion, *In-vitro* studies on glucose uptake, Studies using isolated pancreatic islet cell lines, Assay of Amylase Inhibition and Inhibition of α-Glucosidase Activity. Experimental induction of diabetes mellitus in animal models and *in vitro* techniques are essentials for the advancement of our knowledge, clear understanding of pathogenesis and for finding new therapy. The animal models and *in vitro* techniques are essentials for developing a new drug for the treatment diabetes. More animal models, advanced techniques have to be developed for advances in diabetes research.

Keywords: Diabetes mellitus; Animal models; *In vitro* techniques

Introduction

Diabetes mellitus is a chronic metabolic disease, occurs when the pancreas is not producing insulin or produced insulin cannot be used by the body, these may lead to raise blood glucose levels. Hyperglycemia for the long-term are associated with damage to the various organs and tissues. The number of people living with diabetes is expected to rise from 366 million in 2011 to 552 million by 2030. IDF also estimates that as many as 183 million people are unaware that they have diabetes [1]. It can be predicted that by 2030, India, China and United States will have the largest number of diabetic patients [2]. There are two types diabetes: type 1 diabetes mellitus and type 2 diabetes mellitus. Despite the great interest in the development of new drugs to reduce the burden of this disease, the scientific community has raised interest to evaluate either raw or isolated natural products in experimental studies; few were tested clinically in humans [3].

Experimental studies of diabetes in animal models and advanced *in vitro* techniques are essential for the improvement of knowledge and clear understanding of the pathology and pathogenesis, and to find new therapy. Animal models of diabetes are therefore, greatly useful in biomedical studies because they offer the promise of new insights into human diabetes. Most of the available models are based on rodents because of their small size, shorter generation intervals and economic considerations. Experimental diabetes mellitus studied by several methods that include: chemical, surgical and genetic manipulations [4]. It is also very important to select appropriate animal model for the screening of new chemical entities (NCEs) and other therapeutic modalities for the treatment of diabetes [5]. The main aim of the present review is to being together all various *in vivo* animal models and *in vitro* techniques for carrying diabetes research.

Chemical Causes of Diabetes

Alloxan induced diabetes

Alloxan is most widely used in experimental diabetic research. Alloxan produces selective necrosis of the beta cells of pancreas. The alloxan is administered by various routes like intravenous, intraperitoneal and subcutaneous. Alloxan is used for induction of diabetes in experimental animals such as mice, rats, rabbits and dogs. The routes and dose of alloxan required may vary depending upon the animal species [6,7].

A First short lived hypoglycemic phase lasting for 30 min from the first minutes of alloxan administration. The hypoglycemic stage may be due to the stimulation of insulin release and high levels of plasma insulin levels. The mechanism at back of the hyperinsulinemia is due to the short term increase of ATP availability and glucokinase inhibition [8-10]. The second phase is the increase in the blood glucose levels one hour after administration of alloxan, the plasma insulin concentration decreases. The pronounced hyperglycemia lasts for 2-4 hours is due to decrease plasma insulin concentrations. This may be due to inhibition of insulin secretion and beta cell toxicity [11,12]. The third phase is hypoglycemic phase that long last for 4-8 hrs after administration of alloxan [13,14].

Alloxan treatment brings out a sudden rise in insulin secretion in the presence and absence of glucose. The insulin release occurs until the complete suppression of the islet response to glucose. Alloxan react with two sulfhydryl in the glucokinase resulting in disulfide bond and inactivation of the enzyme. The alloxan is reduced by GSH. Superoxide radicals liberate ferric ions from ferritin and reduce them to ferrous ions. Fe^{3+} can also be reduced by alloxan radicals [15-18]. Another mechanism reported is the fragmentation of DNA in the beta cells

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exposed to alloxan. The disruption in intracellular calcium levels also contribute for the diabetogenic action of alloxan [19-21].

Streptozotocin induced diabetes

Streptozotocin (STZ) is a naturally occurring chemical it particularly produces toxic to the beta cells of the pancreas. It is used in medical research as an animal model for hyperglycemia [22,23]. STZ alters the blood insulin and glucose concentrations. Two hours after injection, the hyperglycemia is due to the decreased in blood insulin levels. Six hours later, hypoglycemia occurs due to the high levels of blood insulin. At last hyperglycemia develops and blood insulin levels drops. STZ impairs glucose oxidation [24] and decreases insulin synthesis and release. It was observed that STZ at first abolished the B cell response to glucose. STZ restricts GLUT2 expression. STZ changes the DNA in pancreatic B cells [25]. The B cell death is due to alkylation of DNA by STZ [26]. STZ-induced DNA damage activates poly ADPribosylation [27-29]. The activation of poly ADP-ribosylation is of greater importance for the diabetogenicity of STZ than generation of free radicals and DNA damage. Calcium, which may also induce necrosis [28-30].

Dithizone induced diabetes

Dithizone is an organosulfur compound, it have chelating property. Dithizone is used in induction of diabetes in experimental animals. In dithizonised diabetic animals, the levels of zinc, iron, and potassium in the blood were found to be higher than normal [31]. Dithizone has permeates membranes and complex zinc inside liposomes, then release of protons, this enhances diabetogenicity [32].

Monosodium glutamate induced diabetes

Monosodium glutamate (MSG) cause increase in plasma glutamate concentration. MSG stimulates insulin release. Administration of MSG in mice resulted in obesity associated with hyperinsulinemia. After 29 weeks level of blood glucose, total cholesterol and triglyceride levels were increased [33,34].

Insulin antibodies induced diabetes

The insulin antibodies have the affinity and capacity to bind insulin. Insulin deficiency mechanism may cause greater postprandial hyperglycemia because antibody-bound insulin is unavailable to tissues, but the prolongation of postprandial hyperinsulinemia may leads to hyperglycemia [35-37].

Ferric nitrilotriacetate induction of diabetes

In experimental animals parenteral administration on of large daily dose of ferric nitrilotriacetate for 60 days manifest diabetic symptoms such as hyperglycemia, glycosuria, ketonemia and ketonuria [38].

Goldthioglucose obese diabetic mouse model

Gold thioglucose (GTG) is a diabetogenic compound, which manifest obesity induced Type -2 diabetes. The intrapertonial administration GTG in experimental animal gradually develops obesity, hyperinsulinemia, hyperglycemia, insulin resistance for a period of 16- 20 weeks. The GTG is transported in particular to the cells and causes necrotic lesions, which is responsible for the development of hyperphagia and obesity. It also increases body lipid, hepatic lipogenesis and triglyceride secretion, increased adipose tissue lipogenesis and decreases glucose metabolism [39,40].

Virus Induced Diabetes

Viruses produce diabetes mellitus by destroying and infecting pancreatic beta cells. Various human viruses used for inducing diabetes include RNA picornoviruses, Coxackie B4, encephalomylocarditis (EMC-D and M variants), Mengo-2T, reovirus, and lymphocytic choriomeningitis [41,42].

D-Variant Encephalomyocarditis (EMC-D)

EMC- D virus can infect and destroy pancreatic beta cells in mice and produce insulin dependent hyperglycemia [43]. EMC-D virus known as NDK25. Intraperitoneal injection of NDK25 develops non-insulin dependent diabetes mellitus [44].

Coxsackie viruses

Coxsackie viruses also cause diabetes in mice; it can infect and destroy pancreatic acinar cells. Coxsackie B4 virus is strongly associated with the development of insulin-dependent diabetes mellitus in humans. Diabetes induced by Coxsackie virus infection release of sequestered islet antigen resulting in the re- stimulation of auto reactive T cells [45,46].

Hormone Induced Diabetes

Growth hormone induced diabetes

Repeated administration of growth hormone in higher experimental animals induces diabetes with ketonuria and ketonemia. Prolonged administration of growth hormone produced permanent diabetes; there was loss of pancreatic islets tissues and of beta cells [47].

Corticosteroid induced diabetes

Corticosteroid induces diabetes, which is called steroid diabetes. The prednisolone and dexamethasone, cause steroid diabetes. Glucocorticoids stimulate gluconeogenesis, in the liver, resulting in increase in hepatic glucose and induce insulin resistance and hyperglycemia [48].

Spontaneous Diabetic Obese Rodent Models

Ob/ob mouse

The ob/ob mouse strain, have leptin deficiency because of the mutation in leptin gene leading to severe insulin resistance [49,50]. The ob/ob mice exhibit rapid gain in body weight, insulin resistance and hyperinsulinemia occurs [51]. In the ob/ob model, hyperinsulinemia manifests at 3 to 4 weeks of age together with hyperphagia and insulin resistance. The symptom of Type 2 DM of ob/ob mice attenuates with age, continuous decline of plasma insulin levels in the second year of life, glucose tolerance and insulin resistance [52].

db/db mouse

The db gene mutation occurs spontaneously in the leptin-receptor-deficient C57BL/KsJ mice and is originally derived from mutation on chromosome 4 [53]. The db/db mouse becomes hyperphagic, hyperinsulinemic, and insulin resistant within 2 weeks of age, obesity at the age of 3 to 4 weeks. The hyperglycemia develops at the age of 4 to 8 weeks. At this age, the mouse exhibits ketosis and body weight loss occurs [54]. The db/db mouse was used to study renal and micro vascular diabetic complications [55,56].

Kuo Kondo mouse

The Kuo Kondo (KK) mouse is model of obesity and Type 2DM.

It has been crossed with the Bar Harbor C57BL/6J mouse [57]. KK mouse spontaneously exhibits distinct adiposity, hyperglycemia, and hyperinsulinemia [58]. At 2 months of age, the KK mouse manifested obesity due to hyperphagic, insulin resistance and compensatory hyperinsulinemia. The insulin resistance and hyperinsulinemia reached to the peak at 5 months [59].

Zucker Diabetic Fatty (ZDF) rat

The Zucker diabetic fatty (ZDF) rats are less obese, more insulin resistant, and rapidly progress to diabetes due to lack of sufficient insulin secretion [60]. The male ZDF rat becomes fully diabetic at 12 weeks. The serum insulin levels of male ZDF rat reach the peak at about 7 to 10 weeks, but cannot respond to glucose stimulus and the insulin levels drops [61].

New Zealand Obese (NZO) mouse

The New Zealand strain of obese mice, gains weight at 10 weeks of life as a result of hyperphagia, hyperglycemia and hyperinsulinemia [62]. NZO mouse manifests insulin resistance at an early age. With the growth of NZO mouse, hyperglycemia and glucose tolerance increase and the level of blood glucose reaches 300-400 mg/dL at the age of 20 to 24 weeks [63]. It is useful model for studying obesity and diabetes [64].

Otsuka Long-Evans Tokushima Fatty (OLETF) rat

The OLETF rat develops hyperglycemia at around 18 to 25 weeks age. OLETF rats exhibits obesity, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, hypercholesterolemia, and onset of diabetes similar to human Type 2DM. Many recessive genes on several chromosomes including the X chromosome are involved in the induction of diabetes in OLETF rats [65,66].

Nagoya-Shibata-Yasuda (NSY) mouse

The NSY mouse, imitates human Type 2DM with the characteristics are mild obesity, impaired insulin secretion and insulin resistance contributing to diabetes development in an age dependent manner. NSY mice, all males develop diabetes, while females is only about 30%. The NSY mouse is particularly useful for studying the age-related damages and phenotypes of Type 2 DM [67].

Tsumura Suzuki Obese Diabetes (TSOD) mouse

TSOD mouse, exhibits obesity and insulin resistant at 2 months old, which contributes for hyperinsulinemia and hyperglycemia [68]. In TSOD mouse, pancreatic islets are hypertrophic [69]. The impaired GLUT4 translocation in both skeletal muscle and adipocytes of TSOD mouse causes reduced insulin sensitivity and insulin resistance [70].

M16 mouse

M16 mice manifest obesity at all ages due to hyperphagia [71]. At 8 weeks of age, all M16 mice exhibit hyperglycemia, hyperinsulinemia, and hypercholesterolemia [72].

Spontaneous Diabetic Non Obese Rodent Models

Goto Kakizaki (GK) rat

The GK rat is a non-obese model of T2DM with hyperglycemia, hyperinsulinemia, and insulin resistance. In GK rats a stable fasting hyperglycemia was observed at the end of the first 2 weeks. After 8 weeks, hyperglycemia degenerates and insulin secretion of the islets stimulated by glucose. GK rats, develops complications of diabetes like peripheral neuropathy, and retinopathy [73-75].

Cohen diabetic rat

Cohen diabetic rat is a genetic model derived from diet-induced Type 2 DM model by placing the rat on a synthetic 72% sucrose-copper-poor diet for 2 months, manifest the human Type 2 DM. The manifestations include non-obesity, hyperinsulinemia, and insulin resistance. The Cohen diabetic rat expresses genetic susceptibility to a carbohydrate-rich diet, a feature of Type 2 DM in human [76].

Spontaneously Diabetic Torii (SDT) rat

SDT rat is a new spontaneously non-obese diabetic strain [77]. It has characteristics like glucose intolerance, hyperglycemia, hyperinsulinemia, and hypertriglyceridemia [78]. Because of the severe hyperglycemia, SDT rats develop diabetic retinopathy [79], diabetic neuropathy, and diabetic nephropathy. This model is suitable for studying complications of human T2DM [80,81].

Surgical Model of Diabetes Mellitus

Surgery technique used to induce diabetes, is complete removal of the pancreas [82]. Limitation to this technique include high level of technical expertise and adequate surgical room environment. Pancreatectomy has been employed; large resection is required to obtain mild to moderate hyperglycemia [83].

In vitro Techniques

Assay of amylase inhibition

In vitro amylase inhibition can be studied by adding the test sample was allowed to react with α- amylase enzyme and Incubated, add starch solution. After incubation dinitrosalicylic acid reagent was added to both control and test. Keep this mixture in boiling water bath for few minutes. The absorbance was taken at 540 nm using spectrophotometer and the percentage of inhibition of α-amylase enzyme was calculated [84].

A starch solution was prepared with potato in sodium phosphate buffer, sodium chloride and kept in a boiling water batch for few min. The α-amylase solution was prepared by mixing α-amylase in the same buffer. The colorimetric reagent was prepared by mixing equal volume of sodium potassium tartrate tetra hydrate solution and 3,5-dinitro salicylic acid (DNS) solution. Starch solution was mixed with test sample with various concentration or acarbose and α-amylase solution was added and incubated at 25°C to react with the starch solution. DNS reagent was added to the above solution, and the contents were heated for 15 min on a boiling water bath. The final volume was made up with distilled water, and the absorbance was measured at 540 nm using spectrophotometer. The percentage inhibition and IC50 value was calculated [85].

Inhibition of α-glucosidase activity

The α-glucosidase enzyme inhibition activity was performed by incubating α-glucosidase enzyme solution with phosphate buffer which contains test samples of different connections at 37°C for 1 hr in maltose solution. The reaction mixture was kept in boiling water few min and cooled. Glucose reagent was added and its absorbance was measured at 540 nm to estimate the amount of liberated glucose from maltose by the action of α-glucosidase enzyme. The percentage of inhibition and IC50 was calculated [86].

In-vitro studies on insulin secretion and glucose uptake

The oral antidiabetic agents can affect several pathways of glucose metabolism such as insulin secretion, glucose uptake by target organs

as well as nutrient absorption. Incretins and transcription factors such as peroxisome proliferator activated receptors-PPAR are targets of modern therapy. Insulin receptor, glucose transporters, has not been focused for antidiabetic therapy [87,88].

Adipose tissue is considered to have a link between obesity and Type 2 diabetes, elevated intracellular lipid concentrations and insulin resistance [89]. Insulin resistance either at the adipocyte or skeletal muscle levels contribute to hyperglycemia. Pathways related to insulin resistance may be studied in cell lines of adipocytes such as marine 3T3-L1 cells [90] and rat L6 muscle engineered to over-express GLUT4 [91].

Studies using isolated pancreatic islet cell lines

These pathways can be studied with isolated pancreatic cells from experimental animals that can be obtained by collagenase digestion technique, followed by adequate separation and transference to appropriated culture medium [91]. It is known that insulin secretion occurs when pancreatic cells utilize glucose to generate adenosine triphosphate (ATP) from adenosine diphosphate (ADP). The resulting increase in cytoplasmic ATP/ADP ratio closes ATP-sensitive potassium channels, causing depolarization of the plasma membrane, which activates voltage dependent Ca^{2+} channels. This results in elevation of the intracellular Ca^{2+} concentration which triggers insulin secretion.

Conclusion

In this review many of the animal models and *in vitro* techniques has been described which share the animal model shave similar characteristics and features similar to human diabetics. Various experimental animal models have been used in diabetic research. There is no single species or animal model which may mimic the human diabetes. Each model is essentials tools for investigating endocrine, metabolic, genetic changes and underlying mechanism of human diabetes. The animal models and *in vitro* techniques are essentials for developing a new drug for the treatment diabetes. More animal models, software, advanced techniques have to be developed for advances in diabetes research.

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