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Animal Models in Diabetes Mellitus: An Overview

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ABSTRACT

Diabetes mellitus is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis dose not returns to normalcy and continues for a protracted period of time, it leads to hyperglycemia, which in due course turns into a syndrome called diabetes mellitus. Several animal models have been developed for studying diabetes mellitus or testing anti-diabetic agents. These models include chemical, surgical (pancreatectomy) and genetic manipulations in several animal species to induce diabetes mellitus. The diabetogenic drugs used include: Alloxan monohydrate, Streptozotocin with or without nicotinamide, Ferric nitrilotriacetate, Ditizona and Anti-insulin serum. The selection of these models to use for investigating the antidiabetic properties of a new compound may be a very difficult task especially for young researchers. The aim of the present review is give a brief idea about various experimental models developed for studying diabetes mellitus, assess the merits and demerits of each model and highlight the precautions needed to avoid erroneous results during the applications of these models.

Keywords: Diabetes Mellitus, Animal models, Alloxan, Streptozotocin.

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INTRODUCTION

Diabetes mellitus is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis dose not returns to normalcy and continues for a protracted period of time, it leads to hyperglycemia, which in due course turns into a syndrome called diabetes mellitus [1]. The long term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease [2]. There are two main categories of this disease - type 1 (insulin dependent diabetes mellitus, IDDM) and type 2 (non insulin dependent diabetes mellitus, NIDDM). Type 1 diabetes is typically a disease of juvenile onset and is associated with insulin deficiency, usually resulting from autoimmune destruction of pancreatic beta cells. Type 1 diabetic patients are prone to develop ketoacidosis and require insulin therapy to control their hyperglycemia. Type 2 diabetes is typically a disease of adult onset (although it is becoming increasingly recognized in children) and is caused by a combination of defective insulin secretion with reduced insulin sensitivity of the tissues. An increase in body fat is generally associated with an increase in risk of metabolic diseases such as type 2 diabetes mellitus, hypertension and dyslipidemia [3]. This impaired glucose tolerance is usually associated with obesity and physical inactivity. Hyperglycemia in type 2 diabetic patients can be often managed using a combination of dietary modification and oral hypoglycemic drugs. It has become increasingly recognized that there are some diabetic patients who develop diabetes as adults (usually older than 30 years of age), who are initially diagnosed as having type 2 diabetes but who are not overweight and who also show evidence of circulating autoantibodies. These patients are often managed initially using oral hypoglycemic drugs, but they usually progress to insulin therapy. This form of diabetes has now been classified as Latent Autoimmune Diabetes of Adults (LADA), sometimes referred to as type 1.5 diabetes. Juvenile onset type 1 diabetes appears to result from an overwhelming auto-immune response against pancreatic beta cells that rapidly leads to their total destruction. In contrast, LADA seems to result from a more slowly progressive autoimmune process and it takes several years before the beta cell mass has been reduced to an extent whereby normoglycemia cannot be maintained and clinical signs of diabetes become apparent [4]. Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with the onset or first recognition during pregnancy. The definition applies whether insulin or

only diet modification is used for treatment and whether or not the condition persists after pregnancy. It does not exclude the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with pregnancy. Approximately 7 per cent of all pregnancies are complicated by GDM, resulting in more than 200,000 cases annually. The prevalence may range from 1 to 14 per cent of all pregnancies, depending on the population studies and the diagnostic tests employed. Off springs of women with GDM are at an increased risk of obesity, glucose intolerance and diabetes in late adolescence and young childhood. Risk assessment for GDM should be undertaken at the first prenatal visit. Women with clinical characteristics consistent with high risk of GDM (marked obesity, personal history of diabetes) should undergo glucose testing. Women with GDM represent a high risk group for cardiovascular disease that can be identified years before the development of adverse events with the incidence of type 2 diabetes increasing at epidemic proportions [5].

Animal Models for Diabetes Mellitus [6]

The currently existing animal models include:

Chemically Induced Diabetes:

Chemically induced Type-I diabetes is the most commonly used animal model of diabetes. Chemical agents which produce diabetes can be classified into three categories, and include agents that:

- Specifically damage B-cell;
- Cause temporary inhibition of insulin production and or secretion, and
- Diminish the metabolic efficacy of insulin in target tissues.

In general, chemicals in the first category are of interest as they reproduce lesions resembling IDDM [6].

1. Alloxan-lnduced Diabetes

Alloxan, a cyclic urea analogue, was the first agent in this category, which was reported to produce permanent diabetes in animals.

How Alloxan induce diabetes in Experimental animals:

The mechanism by which it induces diabetes is not very clear. Alloxan is a highly reactive molecule that is readily reduced to diuleric acid, which is then auto-oxidized back to alloxan resulting in the production of free radicals. These free radicals damage the DNA of B-cells and cause cell death. Second mechanism proposed for alloxan is its ability to react with protein SH groups, especially the membrane proteins like glucokinase on the B-cells, finally resulting in cell necrosis. However, there are major species differences in response to alloxan [7].

General methodology to induce diabetes in Experimental animal: Rabbits weighing 2 to 3 kg are used. Alloxan is infused via the ear marginal vein at a dose of 150 mg/kg for 10 minutes. About 70% animals become hyper glycemic and uricosuric. The remaining animals either die or are temporarily hyperglycemic. When rats of Wistar or Sprague-Dawley strain each weighing 150 to 200 g are used, alloxan is injected s.c. in the dose of 100-175 mg/ kg. In male Beagle dogs weighing 15 to 20 kg, alloxan is injected i.v.at a dose of 60 mg/ kg. Alloxan has been given to nonhuman primates like monkeys and baboons in the dose of 65-200 mg/ kg i.v. to induce diabetes. All the animals, which are given alloxan, receive glucose and regular insulin for one week and food ad libitum. Thereafter, single daily dose of 28 IU insulin is administered s.c .The blood glucose level shows triphasic change, first a rise at 2 h, followed by hypoglycemic phase at 8 h and finally an increase at 24 h probably due to depletion of B-cells of insulin [8].

Modifications

Diabetes can be induced in neonatal Sprague-Dawley rats by intraperitoneal injection of 200 mg/ kg alloxan on days 2, 4, or 6.

Drawbacks -

- High mortality in rats.
- Causes ketosis in animals due to free fatty acid generation.
- Diabetes induced is reversible.
- Some species like guinea pigs are resistant to its diabetogenic action.
- Alloxan has been almost completely replaced by Streptozotocin (STZ) for inducing diabetes because of these drawbacks.

2. Streptozotocin- Induced Diabetes

STZ (2-deoxy-2-(3-methyl-3-nitrosourea) l-D glucopyranose] is a broad-spectrum antibiotic, which is produced from *Streptomyces achromogens*. Rakieten et al first described the diabetogenic property of STZ [9].

How Streptozotocin induce diabetes in animal:

- By process of methylation
- Free radical generation and
- Nitric oxide production.

General methodology to induce diabetes in animal: STZ induces diabetes in almost all species of animals. Diabeto genic dose varies with species and the optimal doses required in various species are: rats (50-60 mg/ kg, i.p. or i.v.), mice (175-200 mg/ kg i.p. or i.v.) and dogs (15 mg/ kg, for 3 days). The blood glucose level shows the same triphasic response as seen in the alloxan treated animals, with hyperglycemia at 1 h, followed by hypoglycemia, which lasts for 6 h, and stable hyperglycemia by 24-48 h after STZ administration [10].

Modifications:

Multiple low dose of STZ also induces diabetes by causing immune mediated pancreatic insulitis in rats. It has also been shown to have diabetogenic effect on the golden hamsters'. When given i.p at a dose of 50 mg/ kg. Cyclosporine-A when given with ST Z enhances its diabetogenic efficacy. STZ combined with complete Freund's adjuvant: Each of CFA, incomplete Freund's adjuvant, Mycobacterium butyricum (component of CFA), Listeria monocytogenes, or endotoxin administered 24 h prior to STZ (25 mg/ kg) and then repeated on the three subsequent weeks all produce hyper glycemia. Fasting for 48 h (24 h prior to and 24 h subsequent to the STZ injection) also produces hyperglycemia. Neither four administration of CFA nor of STZ alone result in persistent hyperglycemia [11].

STZ has almost completely replaced alloxan for inducing diabetes because of:

- Greater selectivity towards B-cells
- Lower mortality rate and
- Longer or irreversible diabetes induction

- However, guinea pigs and rabbits are resistant to its diabetogenic action.

3. Hormone-induced Diabetes Mellitus

Dexamethasone, a long-acting glucocorticoid, is used to produce NIDDM. NIDDM form of diabetes is produced when dexamethasone is administered at a dose of 2-5 mg/ kg i.p. twice daily over a number of days in rats [12-15].

4. Insulin Antibodies-induced Diabetes

Giving bovine insulin along with CFA to guinea pigs produces anti-insulin antibodies. Intravenous injection of 0.25-1.0 ml guinea pig anti-insulin serum to rats induces a dose dependent increase in blood glucose levels up to 300 mg%. This unique effect to guinea pig anti- insulin serum is due to neutralization of endogenous insulin by the insulin antibodies. It persists as long as the antibodies are capable of reacting with insulin remaining in the circulation. Slow i.v .infusion or i.p. injection prolongs the effect for more than a few hours. However, large doses and prolonged administration are accompanied by ketonemia, ketonuria, glycosuria and acidosis and are fatal to the animals. After lower doses, the diabetic syndrome is reversible after a few hours [16].

5. Diabetes induced by Viral Agents

Viruses are thought to be one of the etiologic agents for IDDM; Viruses may produce diabetes mellitus by:

- lnfecting and destroying of B-cells in pancreas,
- A less infecting or cytologic variant producing a comparable damage by eliciting immune auto reactivity to the B-cells,
- Viruses producing systemic effect, not directly affecting the B-cells.

Various human viruses used for inducing diabetes include RNA picornoviruses, CoxsackieB4 (CB4), encephalo-mylocarditis (EMC-D and M variants), Mengo-2T, as well as two other double stranded RNA viruses, reovirus and lymphocytic choriomeningitis virus (LMCV, Armstrong variant) (Table 1). Primary isolates of these human pathogenic agents are generally not pancreatotrophic or ilytic to mouse B-cells and must be adapted for growth either by inoculation into suckling mice or by passage in cultured mouse B-cells [17-18].

Table 1: susceptible mouse strain [17-18]

Viruses	Susceptible mouse strain
EMC-D or M variant Mengo-2T CB4 Reo	SJL/J ,SWR/J ,DBA/1J SJL ,C57BL/6L ,CBA/J SJL/J SJLj

6. Surgically Induced Diabetes

Induction of diabetes mellitus can be achieved through the surgical removal of all or part of the pancreas. In partial pancreatectomy more than 90% of the organ must be removed to produce diabetes. Depending on the amount of intact pancreatic cells, diabetes may range in duration from a few days to several months. Total removal of the pancreas results in an insulin-dependent form of diabetes, and insulin therapy is required to maintain experimental animals. The portion of the pancreas usually left intact following a subtotal pancreatic resection is typically the anterior lobe or a portion thereof.

Disadvantages

1. Surgical removal of pancreas results in loss of α and β-cells in addition to β-cells. This causes loss of counter-regulatory hormones, glucagon and somatostatin.

2. There is a loss of the pancreatic enzymes necessary for proper digestion; therefore, the diet for pancreatectomised animals must be supplemented with these pancreatic enzymes.

3. The total resection of the pancreas in rat is very difficult to achieve and the development and severity of the diabetic state appear to be strain specific.

The use of pancreatectomy in combination with chemical agents, such as alloxan and STZ, produces a stable form of diabetes mellitus in animals, such as cats and dogs that does not occur when each procedure is applied independently. The combination therapy reduces the organ damage associated with chemical induction and minimizes the intervention, such as enzyme supplementation, necessary to maintain a pancreatectomised animal [19-20].

Genetic Models

1. The NOD Mouse

Non-obese Diabetic (NOD) mice are an inbred strain of albino mice developed by Makino and co-workers, in Japan. It is derived from breeding JCL: ICR (Swiss mice) progenitors and NOD mice represent the product of over 80 generations of sib matings. Over the first 20 generations of sib matings, the strain was being maintained as a normoglycemic control line to match with another line being selected for impaired glucose tolerance (NON strain). Once spontaneous development of lDDM was observed in a female of the control NOD strain at F20, development of frank hyper-glycemia and glycosuria rather than non-glycemia it became the selected phenotype [21].

2. The BB Rat

Spontaneous diabetes in the BB Wistar rat was initially diagnosed in 1974 by the Chapel brothers at the Biobreeding Laboratories commercial breeding facility in Ottawa, Ontario, Canada in a non inbred but closed outbred colony of Wistar rats. It was decided to name this syndrome BB after the initials of the breeding lab. The clinical presentation of diabetes or the BB rat is similar to that of its human counterpart. Marked hyperglycemia, glycosuria, and weight loss occur within a day of onset and are associated with decreased plasma insulin that if untreated will result in ketoacidosis within several days. Like the NOD mouse, the BB rat is one of the few rodent models in which significant ketosis occurs in the absence of obesity. Unlike most NOD mouse colonies, both sexes of BB rats are equally affected [22].

MODELS FOR NIDDM

Chemically Induced Diabetes:

1. Neonatal STZ Model of NIDDM

Neonatal rats of Wistar or Sprague-Dawley strain are treated with STZ (80 to 100 mg/kg i.p.) at birth or within the first 5 days following birth. There is severe pancreatic β-cell destruction, accompanied by a decrease in pancreatic insulin stores and a rise in plasma glucose levels. However, in contrast to adult rats treated with STZ, the B-cells of the treated neonates partially regenerate. Following an initial spike in plasma glucose the STZ heated neonatal rat becomes normoglycemic by 3 weeks of age. In the next few weeks, the

B-cell number increases mainly from the proliferation of cells derived from ducts, the extent, depending upon both the age at which the animal is treated with STZ and the species of the treated rat [23-24].

OTHER CHEMICALLY INDUCED NIDDM MODELS

Agents used for induction of NIDDM in rabbits include adrenaline (0.1 mg/kg s.c.). The peak hyperglycemic effect is noticed at 1 h and lasts up to 4 h. The increase in blood sugar levels is found to be 120-150 mg/ 100 ml. Oral hypoglycemic agents can be screened by this method. Diabetes can also be induced in animals with chelating agent's 8-hydroxy quinoline and biphenyl thiocarbazine. EDTA has been reported to be diabetogenic in partially depancreatized rats. Injection of an antiserum produced against ox insulin in guinea pigs or sheep causes diabetes in mice. Diabetes is associated with acute insulin deficiency and the animals exhibit marked hyperglycemia and ketonuria. It is, however, temporary in nature. This model does not have serious toxic side effects like other models, but induces mild pancreatitis in rats. Administration of thiazides, chlorthiazide, hydrochlorothiazide, diazoxide and furosemide produced hyperglycemia and glycosuria in experimental animals, including rabbits, rats and mice. Diazoxide is found to be effective either alone or in combination with other drugs [16].

CONCLUSION

Hyperglycemia is presumed to be a primary factor in the onset of diabetes, although hyperlipidemia also plays a role. The major organs active in the regulation of blood glucose are the pancreas, liver, skeletal muscle, adipose tissue, intestine, and kidney. In this overview, we laid emphasis upon number of experimental animal models used in diabetes research. It is important to emphasize that various experimental animal models are critical for developing new anti-diabetic drugs and explaining the activity of any agent before human clinical trials are to be conducted. With the advances in research, more therapeutic options will become available in the treatment of Diabetes Mellitus. These models explained help in the screening of the anti-diabetic activity of various promising molecules.

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