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EXPERIMENTAL MODELS OF DIABETES

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ABSTRACT
Insulin is a hormone secreted by the pancreas to secret the adequate amount of insulin, to transport glucose from blood into different cells of the body. If the pancreas does not produce enough insulin or the produced insulin does not work properly, the glucose cannot enter the body cells. So glucose stays in the blood cells, which makes the blood sugar level, become high causing diabetes. It is initially characterized by a loss of glucose homeostasis. Experimental models are conventions. The community of scientists designates, often informally, its subjects of study. Today scientists not only breed their experimental animals; they also exercise the power to add to, delete, exchange, or mutate the animal genes. Like all inventions, they may be judged by the way they serve our need.

KEYWORDS: Streptozotocin, Alloxan, Diabetes, Insulin

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INTRODUCTION

Diabetes is a chronic disorder of metabolism of carbohydrates, proteins, and fat due to absolute or relative deficiency of insulin secretion and with carrying degree of insulin resistance. Insulin is a hormone secreted by the pancreas to secret the adequate amount of insulin, to transport glucose from blood into different cells of the body. If the pancreas does not produce enough insulin or the produced insulin does not work properly, the glucose cannot enter the body cells. So glucose stays in the blood cells, which makes the blood sugar level, become high causing diabetes. It is initially characterized by a loss of glucose homeostasis[1]. The major effects of insulin on glucose, fatty acid, and amino acid metabolism, and on ion flux are initiated by the attachment of the insulin molecule to a *specific insulin receptor* on the cell surface. This hormone receptor interaction is reversible, and the insulin molecule is not chemically altered during this contact. The hormone receptor complex is then internalized by an endocytotic mechanism, with insulin molecule eventually being metabolized, and the insulin receptor is recycled to the membrane for use again.

Thus the body loses its main source of fuel for energy even though the blood contains high amount of glucose. This high-level blood glucose for longer periods causes many complications to the different systems of the body.

Insulin Dependent Diabetes Mellitus[2]

Insulin dependent diabetes mellitus is an autoimmune disease characterized by the destruction of the insulin–secreting β cells. Genetic factors are believed to be a major component for the development of TID. In addition, viral-induced model may more closely reflect acute-onset IDDM and not autoimmune-mediated juvenile-onset disease. Coxsackie-B4 (CVB) and encephalomyocarditis M (40) are two viruses that have been used to induce IDDM in rodent. Destruction of islet by macrophages can occur in the absence of detectable viral protein expression, suggesting that the development of IDDM is related to prolonged presence of viral RNA in the pancreas.

Non Insulin Dependent Diabetes Mellitus

Non-insulin diabetes is caused by the loss of functional β cells within the islets of langerhans in the pancreas, resulting in insulin deficiency and therefore hyperglycemia. Under normal condition, there is continual turnover of β cells with a proportion of cells undergoing apoptosis due to senescence and replacement of these dying cells by both β–cell replication and islet neogenesis, a process that involves differentiation of progenitor cells. (Bonner-weir, 2001, beta cell turnover: its assessment and implication. diabetes 50 (suppl. 1) 20-24) β cells have a limited capacity for replication; differentiation of progenitor cells also plays an important role in maintaining β-cells mass.

Experimental models are conventions. The community of scientists designates, often informally, its subjects of study. Today scientists not only breed their experimental animals; they also exercise the power to add to, delete, exchange, or mutate the animal genes. Like all inventions, they may be judged by the way they serve our need.[3]

The main drawback of experimental models is that the etiology and pathophysiology of experimental models are not same. Some chemicals which are toxic to the animals but they do not show any toxic effect on the human body. Example Streptozotocin induces diabetes in monkey[4], hamster, and guinea pig[5] but it has no diabetogenic action on human[6].

EXPERIMENTAL MODELS

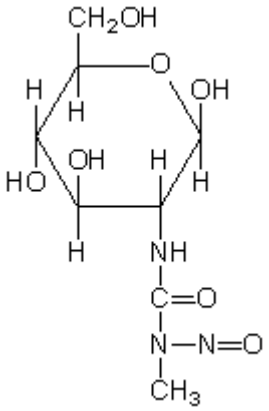
We may divide experimental models into two sorts: analogue models and intrimic models. Analogue models are useful as substitutes for some reality otherwise inaccessible to experimentation. A human disease, for example intrimic models, unlike analogue models, do not have to mimic reality, they fascinate on their own.

Diabetes caused by: [7]

- The removal of pancreas[8].
- By Diabetogens [9-10].
- By virus[11].
- By transcription factor[12].
- By HSPG[13].

Experiments in humans are limited; naturally occurring and especially genetic engineered rodent models have revolutionized research in diabetes[14].

Streptozotocin

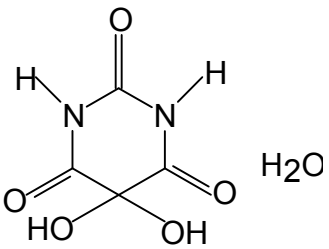


2-Deoxy-2- (3-methyl-3-nitrosoureido)-D-glucopyranose [15]

Streptozotocin is a broad-spectrum antibiotic isolated from *Streptomyces archromogenes* in 1959[16]. Rakieten and co-workers first reported its diabetic property[17]. The biological half-life was found to be 5 min. STZ induce diabetes in monkey, hamster and guinea pig but it has no diabetogenic action in human.
It is glucose linked to a reactive nitrosourea moiety, and as such it is internalized through the cells glucose transports. Once the molecule inside, the nitrosourea moiety is released and actively poisons the cells by cross –linking vital structure. Because the β cells of the pancreas are more active than other cells in taking up glucose (they continuously sample the blood glucose concentration), they are more sensitive than other cells to streptozotocin poisoning. Furthermore, it liberates toxic amount of nitric oxide that inhibits aconite activity and participates in[18]. DNA Alkylation. DNA damage induces activation of poly ADP – ribosylation leads to depletion of cellular NAD + and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibit aconitase activity and participates in DNA damage. As result of β cells undergo the destruction by necrosis[19].

STZ induces diabetes in almost all the species. Diabetogenic dose varies with the species and the optimal dose required to produce diabetes in various species was found to be, rats (50-60 mg/kg, IP or IV) [20], mice (175-200 mg/kg IP or IV) [21] and dogs (15 mg/kg, for 3 days) [22]. Diabetes is initiated in mice by three consecutive injection (IP or IV) of STZ (50 mg/ml); in citrate buffer (pH 4.8) during the fasting state (one intraperitoneal injection / day; day 1, 85mg/kg; day 2, 70 mg /kg; day 3, 55 mg/kg) [23]. STZ mice developed severe hyperglycemia without the weight loss characteristics[24]. Hyperglycemia was verified 1 week later by sampling from tail vein[25].
Regenerative success is noted to be inversely related to the duration of diabetes in rats. A blunted maturation of fibers occurs on early as 14 days after injury[26]. It is argued that STZ and subsequent hyperglycemia may retard normal growth rates of rats, resulting in deficient regeneration and delayed conduction velocity unrelated to hyperglycemia[27].

Alloxan



Alloxan is an oxidation product of uric acid .it is of pale reddish color, readily soluble in water or alcohol[28]. In vitro current studies suggest that Alloxan, which pharmacologically inhibits glucokinase activity at low doses[29-30] but induced cell death at higher concentration presumably through the production of reactive oxygen radicals[31-32].

Alloxan and the product of its reduction dealuric acid, established a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β cells[33].
Male albino rats are made diabetic by injection with a single I.P. Dose of alloxan (100mg/kg) [34] in 0.9% saline solution[35].
Alloxan can be administered by virtually through all routes i.e., IV [36], IM [37], IP [38], SC [39] and also by the oral route[40]. Owing to its short life (less than one minute) so it is best administered by infusion in the pancreatic artery[41].

By Transcription Factor

Dominant- negative suppression of HNF-1 (α) function markedly reduced cellular insulin, both at the m RNA p protein levels, by direct inhibitions of RIP1 activity [42-43] it has been demonstrated that the HNF-1 (α), regulates the PDX-1 promoter activity[44]. The altered expression of these genes may also be implicated in the progressive deterioration of β cell function and cell mass MODY3 patients[45].

Cell models for MODY (Maturity–onset diabetes of the young) 1

MODY-1, caused by mutation in the HNF-4 α gene is much less prevented than other subtypes of MODY. Although it is less penetrate than MADY 3 and the average age of patient with MODY1 at diagnosis is slightly higher, clinical features of MODY 1 are very similar primary β cells dysfunction and leads to the development of a sever form of diabetes [46-47].
Two recent reports showed that a distant upstream promoter in the HNF-4 α gene P2 is specifically used in INS –1 and pancreatic β cells. The P2 promoter containing on HNF-1 α binding site is functional and is occupied by HNF-1 α selectively in pancreatic islets in vivo. Accordingly HNF-4 α transcription is severely decreased in islets, but not in liver, of HNF-1a. Sub-/-1 mice these studies thus indicate that HNF-1 α can also act upstream of HNF-4 α is β cells, revealing a complex transcription factor circuits operating to maintain normal β cell function Experimental models of MODY-4 PDX-1 is required for pancreatic development and for maintaining the β cell phenotype, homozygonity for a mutation in the PDX-1/IPF-1 gene results in failure of pancreas formation, whereas heterozygonity for an inactivating mutation and imissense mutation in this gene are associated with MODY4 and late onset type 2 diabetes in humans, respectively. The primary defect in MODY4 is impaired glucose-stimulated insulin secretion targeted disruption of PDX-1 is mice results in pancreatic aplasia, whereas mice with β cell – specific deletion of PDX-1 develop diabetes Brissova et al. Recently reported impaired glucose tolerance caused by defective glucose stimulated insulin secretion in the conventional heterozygones PDX-1 mutant mouse[48].

Virus Induced Diabetes

Viruses are one environmental factor that is implicated in the pathogenesis of type 1 diabetes. Molecular mimicry is based on two concepts involving pathogenic antigen and reactive lymphocytes[49]. The first concept is based on the observation that some pathogen proteins share sequence or structural homology with self-proteins. For example, the rubella virus capsid protein shares homology with a 52-kD pancreatic islet antigen[50], and the Coxsackie virus P2-C protein shares homology with another pancreatic antigen, glutamic acid decarboxylase (GAD). The second concept is based on the fact that not all self-reactive T (and B) lymphocytes are deleted from the repertoire. They may *a*) persist because of low affinity for antigen, *b*) exist at a very low frequency, or *c*) react with antigens that are normally sequestered in tissues[51].

Type 1 diabetes (T1D) results from the destruction of pancreatic beta cells. Genetic factors are believed to be a major component for the development of T1D[52]

Today, 14 different viruses have been reported to be associated with the development of T1D in humans and animal models. Viruses may be involved in the pathogenesis of T1D in at least two distinct ways: by inducing beta cell-specific autoimmunity, with or without infection of the beta cells, [e.g. Kilham rat virus (KRV)] and by cytolytic infection and destruction of the beta cells (e.g. encephalomyocarditis virus in mice)[53]. In addition, a review of transgenic animal models for virus-induced autoimmune diabetes is included, particularly with regard to lymphocytic choriomeningitis virus, influenza viral proteins and the Epstein-Barr viral receptor.[54]

A number of viruses have been linked to the onset of type 1 diabetes including rubella and mumps viruses[55], but the strongest association is with enteroviruses and, in particular, members of the group B coxsackieviruses (CVBs) [56-58]. The ability of coxsackieviruses to damage the pancreas and cause diabetes. Therefore, there is substantial evidence for the presence of coxsackieviruses but what is currently unclear is the underlying mechanism of pathogenesis.

Certain mouse strains are susceptible to virus-induced diabetes[59-60]. In individuals with a genetic predisposition to autoimmune reactions, direct killing of beta cells by virus, while limited, may be sufficient to stimulate pre-existing auto reactive T cells that may participate in islet destruction[61].

The D variant of encephalomyocarditis (EMC-D) virus causes diabetes in susceptible mice by direct cytolysis of pancreatic [β]-cells[62].

In the murine model of virus induced diabetes D-variant of Encephalomyocarditis (EMC-D). Virus infects and lags pancreatic β cells eventually leading to insulin insufficiency and diabetes[63]. Substantial data indicating the role of viruses in pathogenesis of (IDDM) has been reported[64-65].

D-variant of EMC virus (plaque-purified from mouse heart– passaged M-variant) was used in all the experiments[66]. Virus pools were prepared from L929 cells and the virus titre was determined by plaque assay on L929 cells.

EMC-D virus was injected ip into mice with 0.2% ml/mice containing a range of plaque forming units (PF4) from 100 to 10,000 Pfu /ml [67].

A single injection of 100 to 5000 Pfu ml EMC-D viruses can induce diabetes in 5 weeks after virus inoculation the incidence of diabetes increased with the higher dose of the virus[68.] Higher doses of virus always led to high mortality within a fortnight after virus inoculation whereas as lower doses left. Survival and even exhibited spontaneous recovery and reversal from diabetic state in 28 to 36.3% of the total mice injected with EMC-D virus[69].

Heparan Sulfate Proteoglycan in Experimental Diabetes

Proteoglycans are ubiquitous extra cellular proteins that serve a variety of functions throughout the organism. Proteoglycans are classified based on the structure of the glycosaminoglycan carbohydrate chains, not the core proteins. Perlecan, a member of the heparin sulfate Proteoglycans (HSPG) family, has been implicated in many complications of diabetes. Decreased levels of Perlecan have been observed in the kidney and in other organs, both in patients with diabetes and in animal models. Perlecan has an important role in the maintenance of the glomerular filtration barrier. Decreased Perlecan in the glomerular basement membrane has a central role in the development of diabetic albuminuria. The involvement of this proteoglycan in diabetic complications and the possible mechanisms underlying such

a role has been addressed using a variety of models. Due to the importance of nephropathy among diabetic patients most of the studies conducted so far relate to diabetes effects on Perlecan in different types of kidney cells. The various diabetic models used have provided information on some of the mechanisms underlying pelican's role in diabetes as well as on possible factors affecting its regulation. However, many other aspects of Perlecan metabolism still await full elucidation. The present review provides a description of the models that have been used to study HSPG and in particular perlecan metabolism in diabetes and some of the factors that have been found to be important in the regulation of perlecan[70].

Immune Regulation

One of the research efforts supported by the Diabetes national research group is to identifying antigen that might be involved in the onset of diabetes. Antigen are highly active compounds actually destroy the T cells.

Juvenile diabetes is attack of insulin producing cells in the pancreas by the body's own T cells.

Scientists are now examining a prominent member of antigen –presenting cells, glutamic acid decarboxylase GAD. A large majority of individuals who will develop diabetes have antibodies to GAD in their serum long before the symptoms are visible.

A severe form of immune deficiency characterizes the bio-breeding rat, so it has little in common with most humans who have diabetes. The nonobese diabetic –mouse model seems to mimic much more faithfully the autoimmunity associated with type 1 diabetes in many humans. Indeed, recent clinical trials of specific immune modulation of the diabetogenic process are based on studies of the nonobese diabetic model[71].

Other studies

In another current study, glucagons the hormone that raises blood sugar is being tested against "libraries" to find a possible inhibitors. Future research of glucagons will focus on the use of an inhibitor in additional experimental models for diabetes[72].

The other agents used for induction of NIDDM include adrenaline (0.1 mg/kg. sc) administered in rabbits. The peak hyperglycemic effect was observed at 1 hrs and lasted up to 4 hrs[73]. Diabetes can also be induced in animals with the chelating agents 8-hydroxy quinoline and diphenylthiocarbazine. EDTA has been reported to be diabetogenic in partially depancreatized rats. Administration of thiazides, chlorthiazides, hydrochlorthiazides, and dizoxide and furosimide produced hyperglycemia and glycosuria in experimental animals: including rabbits, rats and mice[74].

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Table 1: Other Diabetogenic Agents

Name of drug	Dose	Animal	Reference
Lithium	4m Eq/kg IV infusion	Rats	64
Cyclosporine A (CsA)	40mg/kg for 7 days orally	Wister rats	65
Dehydroascorbic acid	650mg/kg 3days	Rats	66
Dehydroisoascorbic acid	1.5mg/kg	Rats	67
Dehydroglucoascorbic acid	3.5-3.9mg/kg	Rats	68
Methyl Alloxan	53 mg/kg	Rats	69, 70
Ethyl Alloxan	50-130mg/kg	Rats	69, 70
Oxine and Dithizone	50mg/kg	Rabbits	71
Sodium diethyl Dithiocarbonate	0.5-1gm/kg	Rabbits	72
Potassium Xanthate	200-350mg/kg	Rabbits	72
Uric acid	1gm/kg	Rabbits	73, 74.

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