Current Trends in Biotechnology and Pharmacy
Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

An Approach Towards Method Development to Investigate the Anti-Diabetic Activity on Experimental Animals

Sasmita Dash[1], Gurudutta Pattnaik[1], Biswakanth Kar[2], Nityananda Sahoo[1], Sanjib Bhattacharya[3*]
[1]School of Pharmacy and Life Sciences, Centurion University of Technology and Management, Bhubaneswar 752050, Odisha, India
[2]School of Pharmaceutical Sciences, SOA University, J-15, Kalinganagar, Bhubaneswar 751003, Odisha, India
[3]West Bengal Medical Services Corporation Ltd., GN 29, Sector V, Salt Lake City, Kolkata 700091, West Bengal, India
Running title: Approach towards method development to investigate anti-diabetic activity
Corresponding author: sakkwai@yahoo.com

Abstract

lants are used predominantly as the source for diabetes treatment; some plants are authenticated scientifically worldwide for their active constituents. The occurrence of diabetes mellitus has been developed by establishing a large variety of experimental animal models. This development aims to analyze the available diabetic experimental animal models applied as tools to evaluate the mode of action of various drugs and their significant antidiabetic activity. Diabetes research is performed in rodents, even though some experiments are still carried out in larger animals. The current review outlines several methods of induction of diabetes in rodents and large animals and to establish the mechanism through which the available drugs show their potentiality against the development of diabetes. Preferably, we should study multiple animal models over the human diabetic person to signify the drugs' antidiabetic potential. Most of the plants prove their efficacy towards diabetic disorder conditions by different underlying mechanisms based on various experimental animal models. Presently, the process for evaluating the traditional antidiabetic medicines is hardly useful for testing raw plant materials that are used conventionally for the treatment of diabetes; and natural components, mostly isolated from plants, have been examined in chemically induced diabetic models. This review article proposes new approaches for developing novel antidiabetic drugs and treating this severe disease condition that signifies a worldwide public health issue.

Keywords: Diabetes mellitus, voluntary autoimmune model, genetically induced diabetes, virus-induced diabetes, type 2 diabetic models, alloxan, streptozotocin.

Introduction

Diabetes mellitus is a usual public health issue and a chronic disorder marked by the related or complete insulin deficiency that increases blood sugar. Subsequently, various complications like nephropathy, retinopathy, neuropathy, and improved heart disorder risk are mainly characterized by regular hyperglycemia. According to the current scenario, in the year 2008, approximately 347 million people are affected by diabetes. The disease's occurrence is 9.8% in men and 9.2% in women worldwide (1). Diabetes categorizes into different classifications, but type 1 diabetes and type 2 diabetes are the most common types.

The first type of diabetes is Type 1 diabetic condition described by the demolition of pancreatic beta cells' insulin. It is also called insulin-dependent diabetes mellitus or juvenile diabetes. It is mostly identified in young adults and children, and during the

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

diagnosis phase, the affected person has significantly less insulin production in the pancreas. Hence, in these conditions, regular monitoring of blood glucose levels must perform to prevent hypoglycemia. Also, injections have to be provided subcutaneously to replace the deficiency of insulin. Diabetes is developed under the influence of hereditary factors and many environmental causes. The literature suggests that this disease primarily occurs in about 27% of the case of identical twins (2). Depending upon the country, the disease development frequency, particularly type-1 diabetes, increases up to 100-fold, which is anticipated to be around 15 to 20 for each 100 000 within the United Kingdom (3).

Another kind of diabetes is Type 2; its incidence is approximately 4% in the United Kingdom, mostly found in adults middle-aged. Simultaneously, the disease's onset is mainly dependent upon falling with growing stages of obesity (4). The adult-onset diabetics are characterized on the defense to insulin, comparatively deficient in insulin production due to the lack of suitable compensation by the pancreatic beta cells. Hence, insulin resistance also can be enhanced by regular exercise patterns, change in lifestyle, and reducing weight. (5). A series of drugs are used for the treatment of type 2 diabetes (6).

- Drugs that mimic the production of insulin from pancreatic beta cells, i.e., sulphonylureas
- Drugs that decrease the production of hepatic glucose, i.e., biguanides
- Drugs those interrupt the uptake of carbohydrate in the digestive system, i.e., α-glucosidase inhibitors
- Drugs that increase insulin activity, i.e., thiazolidinediones
- Drugs directing the gut hormone, i.e., inhibitors of dipeptidyl peptidase-4

Based on the severity of diabetes and its features, a proper investigation should be done cautiously by choosing the experimental animal models. This review enlightens various experimental animal models for both type 1 and type 2 and the mechanism of action and pathophysiology of different chemically induced diabetes.

Type 1 diabetic experimental animal model

This type of diabetes is identified by the autoimmune demolition of the pancreas' beta cells that bring about insufficient insulin production. It is accomplished by various mechanisms like chemical excision of pancreatic beta cells in different experimental animal models that ultimately leads to the growth of the autoimmune diabetic disordered condition.

Chemically induced type 1 diabetic model

The type 1 diabetic model is of a specific kind where endogenous insulin production is reduced due to the destruction of a high proportion of pancreatic beta cells that finally leads to hyperglycemia and reduced weight. The chemically induced model can be applied to both rodents and higher animals as well as it is a simple and comparatively less expensive model (7). The two principal compounds used in this model for inducing diabetes are alloxan and streptozotocin (STZ). These are usually induced about 5–7 consecutive days before initiating the study to maintain the unchanged hyperglycemia. As glucose structure quietly resembles the form of both alloxan and streptozotocin (8), glucose can compete with them. Therefore, fasting animals are subjected to be highly sensitive. The chemicals, i.e., streptozotocin and alloxan, are relatively unstable, so new solutions should be preferably made before giving the injection to animals.

The chemically induced diabetic model is suitable for usage when testing medications or treatments. The critical mode of activity is dropping the blood sugar through a 'non-beta-cell-dependent' means to evaluate new

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

insulin preparations (9, 10). This model can be appropriately adapted to analyze therapies of transplantation where the ultimate goal is to lower the blood glucose (11-13). Finally, after completing the experiment, the experimental animals must be tested for kidney function and discontinuation to hyperglycemia adhered to eliminate the growth of endogenous cells of the pancreas (14,15). Additionally, the pancreas is separated and histologically tested in insulin-positive animals (15), even though this is to be emphasized that the existence of endogenous beta cells may not precisely be associated with their functioning (16).

The chemically induced diabetic model's main difficulties are the toxic effect of the chemicals on different organs. Correspondingly, the streptozotocin or alloxan administration causes variations in the isozymes P450 in the organs like the kidney, liver, lung, brain, testis, and intestines. Therefore, it should be carefully observed during the testing of drugs on experimental animal models (17).

Streptozotocin (STZ)

streptozotocin is chemically (2-deoxy-2-(3-(methyl-3-nitrosoureido)-Dglucopyranose) or 1-methyl-1-nitroso-3-((2R,3R,4R,5S,6R)-2,4.5-trihydroxy-6-(hydroxymethyl) oxan-3-yl) urea produced by *Streptomycetes achromogenes*. Upon intraperitoneal or intravenous administration, it enters between Glut-2 transporter, reaches the pancreatic beta-cell. There it performs the alkylation of DNA strand (18), which is followed by the consequent stimulation of PARP, which causes the reduction of NAD+, depletion of intracellular ATP, and decreased insulin production well (19). Streptozotocin is the origin of free radicals that can harm the DNA, followed by the death of cells. Streptozotocin can also be delivered even as multiple low doses as well as individual high doses.

Single high-dose of streptozotocin

According to the various strain of mice (20), the single dose of STZ in case of mice varies from 100 to 200 mg/kg body weight (21, 22). For rats, the dose ranges from 35–65 mg/kg body weight (21) that brings about quick removal of pancreatic beta cells and increases the blood sugar level. Although it can regenerate the pancreatic islets naturally after injecting STZ in experimental animals, it does not influence blood sugar enhancement, which has been recommended by placing adequate controls (23). The chemically induced diabetic model, basically the high-dose induction of STZ, is very often useful in various transplantation type of models across which the pancreatic islets (13), assumed stem cells (24) and are relocated under the kidney capsules. According to the recent development, it has been found that STZ administration on experimental animals could inhibit the studies relating immune tolerance to transplants by causing lymphopenia and gradual development of suppressor T-cells (25).

Multiple less-dose of Streptozotocin

According to the type of experimental animal and their strain, the multiple doses of STZ vary as of 20 to 40 mg/kg body weight. That can be injected for five consecutive days in case of mice to persuade insulitis (26, 27) and rats as well (28), which is characterized by decreasing the islet number with islet volume visibly followed by reducing the capacity of insulin release (29). The islets of the pancreas can be permeated by macrophages, which is related to the formation of cytokines that influences the progress of diabetes (28). Diabetes can also progress without the presence of T and B cells; hence, not typically able to signify the human disorder comparably to various autoimmune models (30). The progress of diabetes can be decreased significantly by the process of directing cytokines (31), which brings about the destruction of pancreatic beta cells.

Alloxan

Alloxan is chemically (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil), or 1,3-diazinane-2,4,5,6-tetrone mostly injected

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

in experimental animals assigned to quickly absorbed through the pancreatic beta cells, thus forming free radicals, to which the beta cells produce deficient defense activity (32). Alloxan shows its activity by reducing form dialuric acid, which then undergoes re-oxidation to get alloxan. Thus, forming a redox cycle for generating superoxide radicals that dismutate to produce hydrogen peroxide with subsequently very active hydroxyl radicals. It initiates the destruction of the DNA strand of pancreatic beta-cell (18). Alloxan is absorbed through the liver; however, it protects the oxygen radicals a better way (33, 34), hence insensitive to destroy. The pancreatic beta-cell can also be damaged by alloxan by other mechanisms like oxidation of the vital sulphydryl group, particularly as for glucokinase enzyme (35) also interferes the intracellular calcium stability (36). According to the strain of experimental animal as well as the path via injection for s.c. and i.p. delivery, the doses in case of mice varies from 50 - 200 mg/kg body weight, also in case of rats from 40 - 200 mg/kg body weight, that needs up to three times more dose as i.v. route (18). An everlasting diabetic model in rabbits can be generated with a dose of 100 mg/kg body weight (37). As alloxan, a slight diabetogenic drug, it can produce a common toxic effect, particularly in the kidney, even in low overdose (18).

Type 1 diabetic voluntary autoimmune models

The NOD mouse or non-obese diabetic mouse and the BB rat or Biobreeding rat model (38) are the very frequently approved autoimmune type-1 diabetic model. Furthermore, the LEW.1AR1/Ztm-iddm rat model was also included as an autoimmune type 1 model of diabetes (39). The most widely used type 1 diabetic model is mentioned in (Table 1).

Non-obese diabetic (NOD) mouse

In 1974 in Osaka, Japan, the NOD mice were established in the Shionogi Research Labs (40) that grow insulitis around 3–4 weeks. Even though B lymphocytes and NK lymphocytes are existing in this hyperglycemia condition, the islets of the pancreas are permeated mostly by CD4+

Table 1 Outline of various type 1 diabetic experimental animal model

Mechanism of diabetes induction	Experimental animal model	Important characteristics
Chemically induced diabetes	• Large dose streptozotocin (Single) • Alloxan • Less dose streptozotocin (Multiple)	• Common model of diabetes with quick removal of pancreatic β- cells • Quickly absorbed through the pancreatic β-cells, thus forming free radicals • Act by decreasing the islet number with islet volume
Voluntary autoimmune	• NOD mouse model • BB rat model • LEW.1AR1/-iddm rat model	Demolition of β- cell because of the autoimmune mechanism
Genetically inbred diabetes	• AKITA mouse model	Deficiency of pancreatic β- cell (Insulin addicted)
Virus-induced diabetes	• Encephalomyocarditis virus • Coxsackie B virus	Demolition of β- cell may be either because of its uninterrupted infection

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

and CD8+ cells (41). The insulitis initiates the elimination of pancreatic beta cells, i.e., about 90% of abdominal insulin is destroyed at the age of 10–14 weeks, while diabetes can grow until 30 weeks. Diabetes is widespread in females with a frequency of 60% to 90% in female groups, while about 10% - 30% in male groups (40, 42). Diabetic mice are characterized by quick loss of body weight and hence need insulin therapy. The MHC class II molecules in NOD mice have a structural resemblance towards that in persons that develop resistance to the disease in both cases (43, 44). The mechanism used for type 1 diabetes can be expressed by the type 1 diabetic genes mainly available in both humans and NOD mice (38, 45). Therefore, The NOD mice can be used for treatments where an alteration in the autoimmune response has been selected. Also, it has been found the drugs that are active in NOD mice are exposed to be incapable in humans (46); when the drugs were administered in advance showed their efficacy in diabetes prevention in the case of young NOD mice (47). Alternatively, when the pancreas of NOD mice is extracted for testing after the study, the biomarkers are not found in the outer blood components in the case of humans, which can prove the benefits of this interference (48).

The NOD mice are much susceptible to the microorganisms while developing diabetes, so they must be stored in specific pathogen-free (SPF) environments to continue the diabetic occurrence. Because of the gender variances, randomness in the onset of disease, and the maintenance of SPF conditions, the NOD mice model for type 1 diabetes is quite expensive compared to the chemical-induced type 1 diabetic model.

On injecting cyclophosphamide in NOD mice, an enhanced onset of action can be attained (49); moreover, T-cells may be successfully carried from diabetic donor NOD mice into non-diabetic targeted mice by the adoptive transference mechanism and can grow diabetes in the receiver mice (50). The autoimmunity type of model's reoccurrence can be achieved by transferring isogenic islets in young non-hyperglycemic NOD mice to diabetic NOD mice (51). The transplant is demolished quickly through the autoimmune process.

Approaches to be considered to upgrade the NOD mice model comprise precise genetic management of NOD mice (52) and in making of improved mice models with elements of human defense approach (53, 54). Apart from the confines of this model, yet widely used because this signifies numerous features related to human disorders that have facilitated the identification of various genetic and signaling steps that promote the type 1 diabetic disorder.

Bio-breeding rats (BB rats)

Biobreeding rats came about consequently by crossbred Wistar rats. The autoimmune diabetic condition was primarily recognized in 1974 in one Canadian group. Subsequently, it brings the formation of two founder groups, out of which many substrains have obtained, an inbred (BBDP/Wor), as well as an outbred (BBdp) (55) and another group of BB rats of diabetic resistant, have been obtained that play as the control group.

The diabetes is typically established post-adolescence in BB rats and similarly occurred both in males and females, which is generally grown at the age of 8 to 16 weeks in rats of about 90%. As diabetes is rather acute; thus, insulin treatment is vital for existence. Even though the experimental animals possess insulitis due to the availability of T lymphocytes, B lymphocytes, macrophages, and NK lymphocytes, they become lymphopenic because of a critical reduction of CD4+ T lymphocytes and a non-appearance of CD8+ T lymphocytes (55). As type 1 diabetic disorder is not characterized by lymphopenia both in humans or of NOD mouse (55), thus using the BB rat model is found to be a limitation about type 1 diabetic disorder in humans. The BB rat model helps explain the genetics of juvenile diabetes (56), so it could be the better model for inducing the transfer of pancreatic islets (55).

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

LEW.1AR1/Ztm-iddm rats

The LEW.1AR1/Ztm-iddm rat model is included as an autoimmune type 1 diabetic model that appeared voluntarily in Lewis rats, comprising a distinct MHC haplotype (LEW.1AR1), produced in the Institute of Laboratory Animal Science of Hannover Medical School (Ztm). Moreover, Insulitis is exhibited by these rats, as well as diabetes establishes at the age of about 8 to 9 weeks. Initially, diabetes occurred around 20% (39); but afterward, the frequency of incidence of diabetes increased to 60% with subsequent inbreeding with an identical prevalence of both genders of diabetic rats (57). Just one week before becoming diabetic, the animals express a prediabetic phase with penetration of pancreatic islets. A successful study related to various stages of the immune cell's infiltration can be done (57). The LEW.1AR1/iddm rat does not show any more spontaneous disorder compared to the BB rat and NOD mouse, so it can be utilized to analyze various diabetic difficulties as they can persist effectively even after the appearance of diabetes (58).

Genetically inbred insulin-dependent diabetes

Akita mice

Akita mouse is associated with the models involving autoimmune type 1 diabetic models. The AKITA mouse, extracted in Akita, Japan out of the mouse C57BL/6NSlc by a mutation occurs spontaneously in the case of insulin 2 gene that prevents the proper functioning of proinsulin, which is ultimately characterized by hypoinsulinemia, polydipsia, polyuria and hyperglycemia as it leads to an acute IDDM initiated from the age of 3 - 4 weeks. But the non-diabetic homozygotes hardly persist up to more than 12 weeks of age. This particular model is identified by the deficiency of pancreatic beta-cell hence used as a substitute to streptozotocin-induced mice in the analysis of transplantation (59), also being accepted as one type of macrovascular disorder of type 1 diabetes (60). Additionally, it is frequently utilized for analyzing the potential relievers of ER stress, particularly within the pancreatic islets, along with the model for the therapies of type 2 diabetic disorders (61).

Virus-induced diabetic models

Viruses are involved in type 1 diabetic conditions (62) and hence are used to begin the destruction of the pancreatic beta-cell. The demolition of beta cells may be either because of their uninterrupted infection or by the induction of an autoimmune response in the case of the pancreatic beta-cell (63). The Viruses utilized for the induction of diabetes for animal models are encephalomyocarditis viruses (64-66) also coxsackie B viruses etc (67-69).

However, various stages of replication of the particular virus and the time limit of infection greatly influence the virus-induced diabetic model's complexity. It is apparent that as per the condition, viruses can encourage autoimmunity and can prevent it, too (70). Even though type 1 diabetes of human are some extent associated with viruses (62, 71), but still this is uncertain about mentioning their involvement in the occurrence as for type 1 diabetes.

Non-rodent type 1 diabetic models

Furthermore, various large animal models have been described along with the extensive study of the number of rodent diabetic models. Particularly in higher animals, the incidence of spontaneous kind of diabetes becomes comparatively infrequent; therefore, activated models for type 1 diabetes are very much needed, which may also be by streptozotocin or pancreatectomy.

Pancreatectomy

Pancreatectomy is a method applied within pigs (72,73) and dogs (74) to produce hyperglycemia. Pancreatectomy is a surgical method for induction of hyperglycemia performed by a proficient surgeon that includes the invasive surgery of the animal, which may

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

enhance the possibility of hypoglycemia and also cause the deficiency of pancreatic exocrine (75). But pancreatectomy is accompanied through autotransplantation of extracted pancreatic islets, a remarkably suitable method in humans (76).

Chemical excision of pancreatic beta cells in higher animals

Toxic effects of STZ (7, 77) and alloxan (78) on pancreatic beta-cell are found mostly among a variety of species due to variation in GLUT-2 expression (7). Studies have suggested that irretrievable diabetes can be produced in rats at a dose of 50 mg/kg body weight, whereas big animals like pigs need a larger dose of 150 mg/kg body weight. It is also found that the hyperglycemia is partially corrected in the case of pigs just four weeks later by the injection of STZ (7), but it may cause hepatic and renal toxicity in pigs when the dose increased to 200 mg/kg body weight. Thus combined models of limited pancreatectomy and STZ treatment are mostly used with a low amount of streptozotocin in the case of big animals (75, 79).

Type 2 diabetic animal models

Mainly, type 2 diabetic condition is represented by insulin resistance and the falling of pancreatic beta-cell for adequate compensation. Thus, type 2 diabetic animal models mostly comprise insulin-resistant models in addition to pancreatic beta-cell failure models. Most type 2 diabetic models are obese, considering the human status to which obesity is distinctly related to type 2 diabetic conditions.

Type 2 diabetic obese model

The recent type 2 diabetic animal models are mostly obese, as they are distinctly related to obesity. The obese condition can be occurred due to spontaneously happening mutations or rather by genetic disturbances and also by more intake of fat.

Obese models of monogenic type

Though human obesity is induced hardly by a monogenic transformation, the monogenic type of obesity is usually a part of type 2 diabetic study. The very commonly applied monogenic models are characterized by defective leptin signaling. As leptin brings about satiety; hence, a deficiency of functional leptin within animals gives rise to hyperphagia and obesity. The monogenic animal models mainly consist of Lepob/ob mice with leptin deficiency, whereas Leprdb/db mouse and the ZDF rat show deficiency in the leptin receptor. The above animal models are frequently used for the implication of new treatments for type 2 diabetic disorders. (80-82).

Lep$^{ob/ob}$ mouse

In the year 1949, it was found that the Lepob/ob mouse, the model of serious obesity which originates from a specific mutation identified within an outbred group in Jackson Laboratory where this phenotype developed to the group of C57BL/6 mice; however, the transformed proteins were not recognized as leptin till 1994 (83). The mice's body weight increases from the age of 2 weeks of age, followed by the development of hyperinsulinemia. By the period of 4 weeks, the hyperglycemia is evident with the gradual increase in blood sugar concentration. It reaches a maximum at the age of 3–5 months, which gradually decreases with the increasing age of the mouse, followed by other metabolic abnormalities like hyperlipidemia, temperature disturbances, and less physical action (84). The volume of the pancreatic islet is intensely enhanced in mice (85). Even though insulin is released abnormally (86), pancreatic islets balance the release of insulin. Due to the deficiency of complete failure of pancreatic beta-cell, the diabetes is not much severe and does not entirely represent type 2 diabetes.

Lepr$^{db/db}$ mouse

The Leprdb/db mice developed in the Jackson Laboratory (87) because of an autosomal regressive transformation in the leptin receptor (88). The characteristic features of this

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

animal model are the development of obesity, hyperinsulinemia, and hyperglycemia. Obesity becomes apparent around the age of 3–4 weeks and the clear growth of hyperinsulinemia at the age of 2 weeks, followed by hyperglycemia at around 4–8 weeks. A very frequently applied mouse strain is C57BLKS/J, in which ketosis is developed gradually with the age of a few months, and finally, the lifespan becomes shorter (21).

Zucker diabetic fatty rats

The strain of Zucker Fatty rats has mutated results in the formation of a new substrain with a diabetogenic type, i.e., the inherited Zucker Diabetic Fatty Rats (ZDF). The ZDF rats possess less obesity but show an acute resistance to insulin, which can't be compensated for the rise in apoptosis in pancreatic beta cells (89). Initially, hyperinsulinemia occurs at the age of about eight weeks, followed by the gradual lowering of insulin levels further (90); in males, diabetes is prominent at 8–10 weeks of age, whereas in females, diabetes does not grow (21). Generally, the symptoms of diabetic difficulties are noticed in ZDF rats. (90).

Pathophysiology of alloxan in diabetes

Alloxan can inhibit the enzyme glucokinase, therefore causes selective inhibition of glucose-induced insulin release, the glucose converter of the pancreatic beta-cell. By the capability of ROS development, it can initiate insulin-dependent diabetes and finally result in the selective destruction of pancreatic beta cells.

Selectivity of pancreatic beta cell of alloxan

Alloxan has a structural similarity with glucose (Figure 1) and also chemically unstable (91). As both the compounds are hydrophilic cannot enter through the lipid bilayer of the plasma membrane. The structural similarity of alloxan to glucose is that they can be easily transported through GLUT2 to the plasma membrane of pancreatic beta-cell and finally reaches the cytosol. Because GLUT2 is not inhibited by alloxan (92), it can easily permeate the pancreatic beta cells without any difficulties (93). Due to the short half-life (91), alloxan voluntarily decomposes to form non-diabetogenic alloxanic acid with an aqueous solution within a short time (91). Thus, alloxan must be absorbed and accumulated rapidly in the pancreatic beta-cell (93) and found inactive when blood flow to the pancreas is disturbed initially after injection of alloxan (94). The derivative of alloxan, like butylalloxan with an extended carbon side chain, is chemically lipophilic (95), which destroys pancreatic beta cells (96). But due to the lipophilic nature, butylalloxan can permeate through plasma membranes without any expression of GLUT2 transporter (92). As a result, nephrotoxicity occurs due to the toxic effect of lipophilic compounds after systemic penetration (96), resulting in severe renal failure in animals prior to the development of diabetes (96).

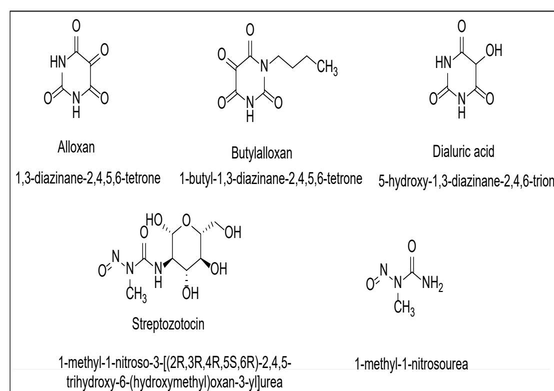

Fig. 1: Alloxan, butylalloxan, dialuric acid, streptozotocin and methylnitrosourea.

Inhibition of glucokinase

Alloxan consists of a vital 5-carbonyl functional group that interacts with the thiol groups. Glucokinase is also hexokinase IV, the essential thiol enzyme in the pancreatic beta-cell (97, 98), with a half-maximal inhibitory concentration (IC_{50}) level of 1–10 µmol/l range, as the concentration of alloxan increases inhibits several functionally active enzymes along with

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

many other proteins and cellular components (99). Glucokinase Inhibition causes a decrease in oxidation of glucose and ATP production (100), thus suppressing the ATP signaling that induces insulin release (97). So, glucokinase inhibition is attained within the period of 1 min upon exposure to alloxan. Due to the exposure, initially, the intake of ATP has reduced results from the closure of glucose phosphorylation induced by the enzyme glucokinase (97), which in turn causes the temporal rise in ATP within the pancreatic beta-cell and initiates the transient insulin secretion.

The inhibition of the enzyme glucokinase is protected by the group of compounds such as thiols, tripeptide glutathione (GSH), dithiothreitol, and cysteine against alloxan as alloxan is reduced to form dialuric acid, which does not react actively with thiol (91, 101, 102). But, only dithiothreitol (101, 102) can reverse the inhibition of glucokinase induced by alloxan because they can reduce the functionally require cysteine group of the enzyme glucokinase, which is oxidized by alloxan (101, 102).

Similarly, glucose saves the enzyme from inhibition induced by alloxan because of the ability to bind with the sugar-binding site of glucokinase that stops the oxidation of the thiol group. The analogue of glucose, i.e., 3-O-methylglucose, not an active substrate of the enzyme glucokinase, can block the inhibition by competitively blocking the absorption of alloxan to the pancreatic beta-cell through GLUT2.

Specific beta cell toxicity of alloxan

Alloxan can produce reactive oxygen species (ROS) by a cyclic mechanism with dialuric acid, the reduction product (Figure 2). The oxidation mechanism of dialuric acid is the primary pathway involving a series reaction depends on superoxide radicals, which becomes inhibited by the enzyme superoxide dismutase. The interaction between alloxan and dialuric acid is essential; an autocatalytic method occurs with SOD (103). In contrast, in any transition metal presence, another oxidation reaction occurs depending on hydrogen peroxide, which is inhibited by catalase, the inactivating enzyme of hydrogen peroxide (103). Another inactivating enzyme of hydrogen peroxide is glutathione peroxidase, performed by the same mechanism, but it needs the substrate GSH, which is oxidized during this reaction mechanism.

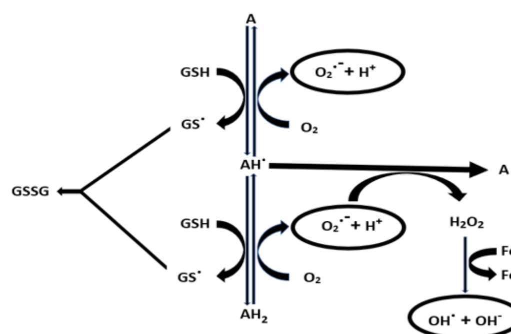

Fig. 2: Cyclic oxidation-reduction reactions among alloxan and dialuric acid. A- Alloxan, AH· **-**Alloxan free radical, AH_2-Dialuric acid, GS· **-**Glutathione free radical, GSSG-Glutathione after oxidation, OH·–Hydroxyl free radical, $O_2^{\cdot-}$**-**Superoxide free radical.

Alloxan in oxidized form cannot release ROS; hence, it does not cause cytotoxicity in the absence of thiols like GSH or when limited towards the extracellular region (104). Thiols present within the plasma membrane to which alloxan can interact and release ROS via a redox reaction are not present and also not available sufficiently to permit the ROS generation and impair the cells (104). It is known that superoxide radical species does not influence the toxicity of dialuric acid and alloxan, but the hydroxyl radical is responsible for it.

Firstly, the enzyme catalase, which inactivates hydrogen peroxide, can better protect the insulin making cells from the toxicity of dialuric acid and alloxan rather than the enzyme SOD. However, catalase cannot stop the redox reaction, hence forming superoxide radical species (103, 105).

Secondly, within liver cells, the

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

concentration level of intracellular GSH and insulin-making cells is in the equal millimolar range of concentration. The cell directs the glucose transporter, GLUT2. But the variation in the concentration of intracellular Glutathione may not be liable significantly for the toxic effect of alloxan on comparing the susceptibility of insulin-making cells to liver cells *in vivo* (17). Liver cells are better provided with catalase enzyme as compared to insulin- making cells (106, 107). The increased intracellular concentration levels of the enzyme catalase within insulin-making cells provide protection to the cells and upregulation of the gene meant for this enzyme (104).

Thirdly, hydroxyl radicals combine with the vital targets before the inactivation by hydroxyl radical scavengers. Similarly, it is complicated to capably suppress the formation of metal-catalyzed hydroxyl radical species by chelators (108, 109). As before the beginning of the formation of hydroxyl radicals, metal chelation must happen. With these chemical natures of both the radical scavengers and chelators, this may not be entirely achieved if not experimental states are improved. In such conditions, the toxic effect of dialuric acid and alloxan towards insulin forming cells in vitro is blocked by desferrioxamine, the iron chelator (104), that stops creating hydroxyl radicals during the Fenton reaction catalyzed by iron (103, 105).

As a whole, all these data deliver considerable information that the hydroxyl radical is the principal toxic ROS moiety instead of the superoxide radical, and also the formation of hydroxyl radical is blocked by the demolition of hydrogen peroxide via the enzyme catalase (103, 105).

Pathophysiology of streptozotocin in diabetes

Streptozotocin causes inhibition of insulin release resulting in insulin-dependent diabetes mellitus. Both effects are associated with the certain chemical nature of streptozotocin, i.e., its alkylate efficiency. Along with alloxan, the pancreatic beta-cell selectivity is mostly due to specific cellular absorption and growth.

Selectivity of pancreatic beta cell of streptozotocin

Streptozotocin is a derivative of nitrosourea; the toxicity of streptozotocin and the chemically associated alkylating agents need their absorption inside the cells. As nitrosoureas are lipophilic by nature, they can quickly absorb via the plasma membrane. Still, as a consequence of the substitution of hexose, streptozotocin is comparatively less lipophilic. Streptozotocin is accumulated mostly within the beta cells through the glucose transporter GLUT2 in the plasma membrane (110, 111). Hence, insulin-making cells that are restricted to express the GLUT2 become resistant to streptozotocin (112,113). It is also found that, streptozotocin shows the more toxic effect compared to N-methyl-N-nitrosourea within cells, which express the glucose transporter; however, both the substance can undergo alkylation for DNA in a similar manner (114, 115). Streptozotocin can damage various organs like the liver and kidney by expressing the glucose transporter, GLUT2 (116, 117).

Specific beta cell toxicity of streptozotocin

Usually, the toxic effect of streptozotocin depends on the specific DNA alkylating action of the methyl nitrosourea group (118). The shifting of methyl moiety from streptozotocin into the DNA structure results in damage, a well-defined sequence of the process (119), finally causes fragmentation of DNA structure (120). In beta-cell toxicity, glycosylation of protein is an added destructive factor (99). For the repairment of DNA, the poly(ADP-ribose) polymerase (PARP) can be overactivated. This reduces cellular coenzyme NAD+ and also ATP, the energy stores (19, 120), which eventually resulting in pancreatic beta-cell death. Even though streptozotocin can methylate proteins (115, 121), but methylation of DNA finally causes beta-cell necrosis, but this is reasonable that methylation of protein is responsible for the

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

functional deficiency of pancreatic beta cells upon contact with streptozotocin (Table 2).

The DNA methylation can be crushed by poly ADP-ribosylation inhibitors. Hence, prior to the intake of streptozotocin, the administration of nicotinamide and the PARP inhibitors parallelly can keep the beta cells safe from the toxic effect of streptozotocin can prevent the growth of any diabetic condition (122). The lack of PARP stops in reducing the coenzyme NAD+ subsequently ATP loss (119, 123, 124) and so cell necrosis.

Another theory describes the diabetogenic activity of streptozotocin, which is related to its tendency to perform as the donor of intracellular nitric oxide (NO) (125). Both the compound streptozotocin and N-methyl-N-nitrosourea carry a nitroso functional group and also can release NO. Streptozotocin increases the effect of the enzyme guanylyl cyclase, the development of cGMP, and the expected results of NO.

Finally, the dismutation of hydrogen peroxide during the metabolism of hypoxanthine causes the minor release of hydroxyl radicals and superoxide radicals (126), which may be associated with the activity of streptozotocin, that enhance the destruction of the pancreatic beta-cell, but ROS cannot perform a vital role for this.

Table 2 Description of the toxicity of streptozotocin and alloxan in beta cells producing chemical-induced diabetes.

Beta cell-toxic effect of glucose derivatives	Streptozotocin	Alloxan
Selective action of pancreatic β-cell	Specific absorption of β-cell through glucose transporter	Selective absorption of β-cell through the glucose transporter
Cell death mechanism	Toxicity of beta cell via alkylation	Toxicity of beta cell via ROS
Process of pancreatic β-cell death	Death of cell via necrosis	Cell death via necrosis
Result of pancreatic beta cell death	Insulin-dependent diabetes mellitus (IDDM)	Insulin-dependent diabetes mellitus (IDDM)
Type of chemical induced diabetes	Diabetes by streptozotocin	Diabetes by alloxan

Inhibition of secretion of insulin via streptozotocin

Streptozotocin can effectively influence glucose and insulin stability, which reflects the toxin-related disorders in the pancreatic beta-cell function. Primarily, streptozotocin affects the biosynthesis of insulin, glucose-mediated insulin secretion, and metabolism of glucose (oxidation of glucose, and also the use of oxygen) (126, 127). Alternatively, the transport of glucose (114) and phosphorylation of glucose-induced by the enzyme glucokinase cannot be inhibited directly by streptozotocin. But, at subsequent phases of pancreatic beta-cell loss, lacks in respect of the expression of gene and production of protein gives rise to the declination of equally glucose metabolism and transport (27).

Selecting the right animal model for diabetic research and method development

The purpose of studying several models for diabetic research might be applied to carry the pharmacological evaluation, genetics analysis, and understanding the mechanism related to various diseases. The selection of the model depends upon the intention of the study. In such circumstances as the pharmacological evaluation, the supposed mechanism for the specific drug being examined will help to select a proper animal experimental model. In type 1 diabetic research, the essential factor in selecting experimental animal models is whether an autoimmunity model is needed. The schedule, as well as the probability of onset, varies in several type 1 diabetic model.

Whereas in type 2 diabetic research, it is essential to study the basic mechanisms of diabetes and if the theory is appropriate for your research. The process may consist of resistance to insulin and the failure of pancreatic beta-cell. The intervention of a specific drug can enhance the symptoms of the animal model, which may depend upon the loss of beta-cell occurrences. Most type 2 diabetic models are of obese type, either by genetic or dietary methods. However, the mechanisms usually originate with various

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

related pathologies like atherosclerosis and dyslipidemia. Similarly, it must be considered that all diabetic animal models, as well as animal strains, cannot develop the complications of diabetes. So proper care must be taken while selecting an appropriate model, where the goal of the research is about investigating complications of diabetes like nephropathy and neuropathy.

Models can vary according to the physiological application. While selecting an animal model, either type 1 or type 2 diabetes, it is most desirable to use various models representing the multiplicity found in a human diabetic person.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ et al (2011). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. Lancet 378: 31–4.

2. Hyttinen V, Kaprio J, Kinnunen L, Koskenvuo M, Tuomilehto J (2003). Genetic liability of type 1 diabetes and the onset age among 22,650 young Finnish twin pairs: a nationwide follow-up study. Diabetes 52: 1052–1055.

3. Patterson CC, Dahlquist GG, Gyurus E, Green A, Soltesz G (2009). Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005-20: a multicentre prospective registration study. Lancet 373: 2027–2033.

4. Pinhas-Hamiel O, Zeitler P (2005). The global spread of type 2diabetes mellitus in children and adolescents. J Pediatr 146: 693–700.

5. Solomon TP, Sistrun SN, Krishnan RK, Del Aguila LF, Marchetti CM, O'Carroll SM et al. (2008). Exercise and diet enhance fat oxidation and reduce insulin resistance in older obese adults. J Appl Physiol 104: 1313–1319.

6. Krentz AJ, Patel MB, Bailey CJ (2008). New drugs for type 2 diabetes mellitus: what is their place in therapy? Drugs 68: 2131–2162.

7. Dufrane D, van Steenberghe M, Guiot Y, Goebbels RM, Saliez A, Gianello P (2006). Streptozotocin-induced diabetes in large animals (pigs/primates): role of GLUT2 transporter and beta-cell plasticity. Transplantation 81: 36–45.

8. Bansal R, Ahmad N, Kidwai JR (1980). Alloxan-glucose interaction: effect on incorporation of 14C-leucine into pancreatic islets of rat. Acta Diabetol Lat 17: 135–143.

9. Jederstrom G, Grasjo J, Nordin A, Sjoholm I, Andersson A (2005). Blood glucose-lowering activity of a hyaluronan-insulin complex after oral administration to rats with diabetes. Diabetes Technol Ther 7: 948–957.

10. Sheshala R, Peh KK, Darwis Y (2009). Preparation, characterization, and in vivo evaluation of insulin-loaded PLA-PEG microspheres for controlled parenteral drug delivery. Drug Dev Ind Pharm 35: 1364–1374.

11. Jonsson J, Carlsson L, Edlund T, Edlund H (1994). Insulin-promoter-factor 1 is required for pancreas development in mice. Nature 371: 606–609.

12. Makhlouf L, Duvivier-Kali VF, Bonner-Weir S, Dieperink H, Weir GC, Sayegh

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

M (2003). Importance of hyperglycemia on the primary function of allogeneic islet transplants. Transplantation 76: 657–664.

13. Deeds MC, Anderson JM, Armstrong AS, Gastineau DA, Hiddinga HJ, Jahangir A et al. (2011). Single dose streptozotocin-induced diabetes: considerations for study design in islet transplantation models. Lab Anim 45: 131–140.

14. Baeyens L, De BS, Lardon J, Mfopou JK, Rooman I, Bouwens L (2005). In vitro generation of insulin-producing beta cells from adult exocrine pancreatic cells. Diabetologia 48: 49–57.

15. Rackham CL, Chagastelles PC, Nardi NB, Hauge-Evans AC, Jones PM, King AJ (2011). Co-transplantation of mesenchymal stem cells maintains islet organisation and morphology in mice. Diabetologia 54: 1127–1135.

16. Kargar C, Ktorza A (2008). Anatomical versus functional beta-cell mass in experimental diabetes. Diabetes ObesMetab 10 (Suppl. 4): 43–53.

17. Lee JH, Yang SH, Oh JM, Lee MG (2010). Pharmacokinetics of drugs in rats with diabetes mellitus induced by alloxan or streptozocin: comparison with those in patients with type I diabetes mellitus. J Pharm Pharmacol 62: 1–23.

18. Szkudelski T (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 50: 537–546.

19. Sandler S, Swenne I (1983). Streptozotocin, but not alloxan, induces DNA repair synthesis in mouse pancreatic islets in vitro. Diabetologia 25: 444–447.

20. Hayashi K, Kojima R, Ito M (2006). Strain differences in the diabetogenic activity of streptozotocin in mice. Biol Pharm Bull 29: 1110–1119.

21. Srinivasan K, Ramarao P (2007). Animal models in type 2 diabetes research: an overview. Indian J Med Res 125: 451–472.

22. Dekel Y, Glucksam Y, Elron-Gross I, Margalit R (2009). Insights into modeling streptozotocin-induced diabetes in ICR mice. Lab Anim (NY) 38: 55–60.

23. Grossman EJ, Lee DD, Tao J, Wilson RA, Park SY, Bell GI et al. (2010). Glycemic control promotes pancreatic beta-cell regeneration in streptozotocin-induced diabetic mice. PLoS ONE 5: e8749.

24. Song WJ, Shah R, Hussain MA (2009). The use of animal models to study stem cell therapies for diabetes mellitus. ILAR J 51: 74–81.

25. Muller YD, Golshayan D, Ehirchiou D, Wyss JC, Giovannoni L, Meier R et al. (2011). Immunosuppressive effects of streptozotocin-induced diabetes result in absolute lymphopenia and a relative increase of T-regulatory cells. Diabetes 60: 2331–2340.

26. Like AA, Rossini AA (1976). Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. Science 193: 415–417.

27. Wang Z, Gleichmann H (1998). GLUT2 in pancreatic islets: crucial target molecule in diabetes induced with multiple low doses of streptozotocin in mice. Diabetes 47: 50–56.

28. Lukic ML, Stosic-Grujicic S, Shahin A (1998). Effector mechanisms in low-dose streptozotocin-induced diabetes. Dev Immunol 6: 119–128.

29. Bonnevie-Nielsen V, Steffes MW, Lernmark A (1981). A major loss in islet mass and B-cell function precedes hyperglycemia in mice given multiple low doses of streptozotocin.

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

Diabetes 30: 424–429.

30. Reddy S, Wu D, Elliott RB (1995). Low dose streptozotocin causes diabetes in severe combined immunodeficient (SCID) mice without immune cell infiltration of the pancreatic islets. Autoimmunity 20: 83–92.

31. Sandberg JO, Andersson A, Eizirik DL, Sandler S (1994). Interleukin-1 receptor antagonist prevents low dose streptozotocin induced diabetes in mice. BiochemBiophys Res Commun 202: 543–548.

32. Nerup J, Mandrup-Poulsen T, Helqvist S, Andersen HU, Pociot F, Reimers JI et al. (1994). On the pathogenesis of IDDM. Diabetologia 37 (Suppl. 2): S82–S89.

33. Malaisse WJ, Malaisse-Lagae F, Sener A, Pipeleers DG (1982). Determinants of the selective toxicity of alloxan to the pancreatic B cell. Proc Natl Acad Sci U S A 79: 927–930.

34. Mathews CE, Leiter EH (1999). Constitutive differences in antioxidant defense status distinguish alloxan-resistant and alloxan-susceptible mice. Free Radic Biol Med 27: 449–455.

35. imWalde SS, Dohle C, Schott-Ohly P, Gleichmann H (2002). Molecular target structures in alloxan-induced diabetes in mice. Life Sci 71: 1681–1694.

36. Kim HR, Rho HW, Park BH, Park JW, Kim JS, Kim UH et al. (1994). Role of Ca2+ in alloxan-induced pancreatic beta-cell damage. BiochimBiophys Acta 1227: 87–91.

37. Wang J, Wan R, Mo Y, Zhang Q, Sherwood LC, Chien S (2010). Creating a long-term diabetic rabbit model. Exp Diabetes Res 2010: 289614.

38. Yang Y, Santamaria P (2006). Lessons on autoimmune diabetes from animal models. Clin Sci (Lond) 110: 627–639.

39. Lenzen S, Tiedge M, Elsner M, Lortz S, Weiss H, Jorns A et al. (2001). The LEW.1AR1/Ztm-iddm rat: a new model of spontaneous insulin-dependent diabetes mellitus. Diabetologia 44: 1189–1196.

40. Hanafusa T, Miyagawa J, Nakajima H, Tomita K, KuwajimaM,Matsuzawa Y et al. (1994). The NOD mouse. Diabetes Res Clin Pract 24 (Suppl.): S307–S311.

41. Yoon JW, Jun HS (2001). Cellular and molecular pathogenic mechanisms of insulin-dependent diabetes mellitus. Ann N Y Acad Sci 928: 200–211.

42. Pozzilli P, Signore A, Williams AJ, Beales PE (1993). NOD mouse colonies around the world – recent facts and figures. Immunol Today 14: 193–196.

43. Todd JA, Wicker LS (2001). Genetic protection from the inflammatory disease type 1 diabetes in humans and animal models. Immunity 15: 387–395.

44. Wicker LS, Clark J, Fraser HI, Garner VE, Gonzalez-Munoz A, Healy B et al. (2005). Type 1 diabetes genes and pathways shared by humans and NOD mice. J Autoimmun 25 (Suppl.): 29–33.

45. Driver JP, Serreze DV, Chen YG (2011). Mouse models for the study of autoimmune type 1 diabetes: a NOD to similarities and differences to human disease. Semin Immunopathol 33: 67–87.

46. von Herrath MG, Nepom GT (2009). Animal models of human type 1 diabetes. Nat Immunol 10: 129–132.

47. Roep BO (2007). Are insights gained from NOD mice sufficient to guide clinical translation? Another inconvenient truth.

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

Ann N Y Acad Sci 1103: 1–10.

48. von Herrath MG, Nepom GT (2005). Lost in translation: barriers to implementing clinical immune therapeutics for autoimmunity. J Exp Med 202: 1159–1162.

49. Caquard M, Ferret-Bernard S, Haurogne K, Ouary M, Allard M, Jegou D et al. (2010). Diabetes acceleration by cyclophosphamide in the non-obese diabetic mouse is associated with differentiation of immunosuppressive monocytes into immunostimulatory cells. Immunol Lett 129: 85–93.

50. Christianson SW, Shultz LD, Leiter EH (1993). Adoptive transfer of diabetes into immunodeficient NOD-scid/scid mice. Relative contributions of CD4+ and CD8+ T-cells from diabetic versus prediabetic NOD.NON-Thy-1a donors. Diabetes 42: 44–55.

51. Rydgren T, Vaarala O, Sandler S (2007). Simvastatin protects against multiple low-dose streptozotocin-induced type 1 diabetes in CD-1 mice and recurrence of disease in nonobese diabetic mice. J Pharmacol Exp Ther 323: 180–185.

52. Yang Y, Santamaria P (2003). Dissecting autoimmune diabetes through genetic manipulation of non-obese diabetic mice. Diabetologia 46: 1447–1464.

53. King M, Pearson T, Rossini AA, Shultz LD, Greiner DL (2008). Humanized mice for the study of type 1 diabetes and beta cell function. Ann N Y Acad Sci 1150: 46–53.

54. Niens M, Grier AE, Marron M, Kay TW, Greiner DL, Serreze DV(2011). Prevention of ‘Humanized’ diabetogenic CD8 T-cell responses in HLA-transgenic NOD mice by a multipeptide coupled-cell approach. Diabetes 60: 1229–1236.

55. Mordes JP, Bortell R, Blankenhorn EP, Rossini AA, Greiner DL (2004). Rat models of type 1 diabetes: genetics, environment, and autoimmunity. ILAR J 45: 278–291.

56. Wallis RH, Wang K, Marandi L, Hsieh E, Ning T, Chao GY et al. (2009). Type 1 diabetes in the BB rat: a polygenic disease. Diabetes 58: 1007–1017.

57. Jorns A, Gunther A, Hedrich HJ, Wedekind D, Tiedge M, Lenzen S (2005). Immune cell infiltration, cytokine expression, and beta-cell apoptosis during the development of type 1 diabetes in the spontaneously diabetic LEW.1AR1/Ztm-iddm rat. Diabetes 54: 2041–2052.

58. Mathews CE (2005). Utility of murine models for the study of spontaneous autoimmune type 1 diabetes. Pediatr Diabetes 6: 165–177.

59. Mathews CE, Langley SH, Leiter EH (2002). New mouse model to study islet transplantation in insulin-dependent diabetes mellitus. Transplantation 73: 1333–1336.

60. Zhou C, Pridgen B, King N, Xu J, Breslow JL (2011). Hyperglycemic Ins2AkitaLdlr-/- mice show severely elevated lipid levels and increased atherosclerosis: a model of type 1 diabetic macrovascular disease. J Lipid Res 52: 1483–1493.

61. Chen H, Zheng C, Zhang X, Li J, Li J, Zheng L et al. (2011). Apelin alleviates diabetes-associated endoplasmic reticulum stress in the pancreas of Akita mice. Peptides 32: 1634–1639.

62. van der Werf N, Kroese FG, Rozing J, Hillebrands JL (2007). Viral infections as potential triggers of type 1 diabetes. Diabetes Metab Res Rev 23: 169–183.

63. Jun HS, Yoon JW (2003). A new look at

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

viruses in type 1 diabetes. Diabetes Metab Res Rev 19: 8–31.

64. Craighead JE, McLane MF (1968). Diabetes mellitus: induction in mice by encephalomyocarditis virus. Science 162: 913–914.

65. Baek HS, Yoon JW (1991). Direct involvement of macrophages in destruction of beta-cells leading to development of diabetes in virus-infected mice. Diabetes 40: 1586–1597.

66. Shimada A, Maruyama T (2004). Encephalomyocarditis-virusinduced diabetes model resembles 'fulminant' type 1 diabetes in humans. Diabetologia 47: 1854–1855.

67. Yoon JW, London WT, Curfman BL, Brown RL, Notkins AL (1986). Coxsackie virus B4 produces transient diabetes in nonhuman primates. Diabetes 35: 712–716.

68. Kang Y, Chatterjee NK, Nodwell MJ, Yoon JW (1994). Complete nucleotide sequence of a strain of coxsackie B4 virus of human origin that induces diabetes in mice and its comparison with nondiabetogenic coxsackie B4 JBV strain. J Med Virol 44: 353–361.

69 Jaidane H, Sane F, Gharbi J, Aouni M, Romond MB, Hober D (2009). Coxsackievirus B4 and type 1 diabetes pathogenesis: contribution of animal models. Diabetes Metab Res Rev 25: 591–603.

70. von Herrath MG, Filippi C, Coppieters K (2011). How viral infections enhance or prevent type 1 diabetes-from mouse to man. J Med Virol 83: 1672.

71. Richardson SJ, Willcox A, Bone AJ, Foulis AK, Morgan NG (2009). The prevalence of enteroviral capsid protein vp1 immunostaining in pancreatic islets in human type 1 diabetes. Diabetologia 52: 1143–1151.

72. Morel P, Kaufmann DB, Matas AJ, Tzardis P, Field MJ, Lloveras JK et al. (1991). Total pancreatectomy in the pig for islet transplantation. Technical alternatives. Transplantation 52: 11–15.

73. Mellert J, Hering BJ, Liu X, Brandhorst D, Brandhorst H, Brendel M et al. (1998). Successful islet auto- and allotransplantation in diabetic pigs. Transplantation 66: 200–204.

74. Fisher SJ, Shi ZQ, Lickley HL, Efendic S, Vranic M, Giacca A (2001). Low-dose IGF-I has no selective advantage over insulin in regulating glucose metabolism in hyperglycemic depancreatized dogs. J Endocrinol 168: 49–58.

75. He S, Chen Y, Wei L, Jin X, Zeng L, Ren Y et al. (2011). Treatment and risk factor analysis of hypoglycemia in diabetic rhesus monkeys. Exp Biol Med (Maywood) 236: 212–218.

76. Matsumoto S (2011). Autologous islet cell transplantation to prevent surgical diabetes. J Diabetes 3: 328–336.

77. Eizirik DL, Pipeleers DG, Ling Z, Welsh N, Hellerstrom C, Andersson A (1994). Major species differences between humans and rodents in the susceptibility to pancreatic beta-cell injury. Proc Natl Acad Sci U S A 91: 9253–9256.

78. Tyrberg B, Andersson A, Borg LA (2001). Species differences in susceptibility of transplanted and cultured pancreatic islets to the beta-cell toxin alloxan. Gen Comp Endocrinol 122: 238–251.

79. Wise MH, Gordon C, Johnson RW (1985). Intraportal autotransplantation of

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

cryopreserved porcine islets of Langerhans. Cryobiology 22: 359–366.

80. Yoshida S, Tanaka H, Oshima H, Yamazaki T, Yonetoku Y, Ohishi T et al. (2010). AS1907417, a novel GPR119 agonist, as an insulinotropic and beta-cell preservative agent for the treatment of type 2 diabetes. BiochemBiophys Res Commun 400: 745–751.

81. Gault VA, Kerr BD, Harriott P, Flatt PR (2011). Administration of an acylated GLP-1 and GIP preparation provides added beneficial glucose-lowering and insulinotropic actions over single incretins in mice with Type 2 diabetes and obesity. Clin Sci (Lond) 121: 107–117.

82. Park JS, Rhee SD, Kang NS, Jung WH, Kim HY, Kim JH et al. (2011). Anti-diabetic and anti-adipogenic effects of a novel selective 11beta-hydroxysteroid dehydrogenase type 1 inhibitor, 2-(3-benzoyl)-4-hydroxy-1,1-dioxo-2H-1,2-benzothiazine-2-yl-1- phenylethano ne (KR-66344). BiochemPharmacol 81:1028–1035.

83. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994). Positional cloning of the mouse obese gene and its human homologue. Nature 372: 425–432.

84. Lindstrom P (2007). The physiology of obese-hyperglycemic mice (ob/ob mice). Scientificworldjournal 7: 666–685.

85. Bock T, Pakkenberg B, Buschard K (2003). Increased islet volume but unchanged islet number in ob/ob mice. Diabetes 52: 1716–1722.

86. Lavine RL, Voyles N, Perrino PV, Recant L (1977). Functional abnormalities of islets of Langerhans of obese hyperglycemic mouse. Am J Physiol 233: E86–E90.

87. Hummel KP, Dickie MM, Coleman DL (1966). Diabetes, a new mutation in the mouse. Science 153: 1127–1128.

88. Chen H, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ et al. (1996). Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. Cell 84: 491–495.

89. Pick A, Clark J, Kubstrup C, Levisetti M, Pugh W, Bonner-Weir S et al. (1998). Role of apoptosis in failure of beta-cell mass compensation for insulin resistance and beta-cell defects in the male Zucker diabetic fatty rat. Diabetes 47: 358–364.

90. Shibata T, Takeuchi S, Yokota S, Kakimoto K, Yonemori F, Wakitani K (2000). Effects of peroxisome proliferator-activated receptor-alpha and -gamma agonist, JTT-501, on diabetic complications in Zucker diabetic fatty rats. Br J Pharmacol 130: 495–504.

91. Lenzen S, Munday R (1991) Thiol-group reactivity, hydrophilicity and stability of alloxan, its reduction products and its Nmethyl derivatives and a comparison with ninhydrin. BiochemPharmacol 42:1385–1391.

92. Elsner M, Tiedge M, Guldbakke B, Munday R, Lenzen S (2002) Importance of the GLUT2 glucose transporter for pancreatic beta cell toxicity of alloxan. Diabetologia 45:1542–1549.

93. Hammarström L, Hellman B, Ullberg S (1967) On the accumulation of alloxan in the pancreatic beta-cells. Diabetologia 3:340–344.

94. Gomori G, Goldner MG (1945) Acute nature of alloxan damage. Proc Soc Exp Biol (NY) 58:232–233.

95. Munday R, Ludwig K, Lenzen S (1993) The relationship between the physicochemical

properties and the biological effects of alloxan and several N-alkyl substituted alloxan derivatives. J Endocrinol 139:153–163.

96. Brückmann G, Wertheimer E (1947) Alloxan studies: the action of alloxan homologues and related compounds. J Biol Chem 168:241–256.

97. Lenzen S, Panten U (1988) Alloxan: history and mechanism of action. Diabetologia 31:337–342.

98. Tiedge M, Richter T, Lenzen S (2000) Importance of cysteine residues for the stability and catalytic activity of human pancreatic beta cell glucokinase. Arch BiochemBiophys 375:251–260.

99. Konrad RJ, Kudlow JE (2002) The role of O-linked protein glycosylation in beta-cell dysfunction. Int J Mol Med 10:535–539.

100. Gunnarsson R, Hellerström C (1973) Acute effects of alloxan on the metabolism and insulin secretion of the pancreatic B-cell. HormMetab Res 5:404–409.

101. Lenzen S, Freytag S, Panten U (1988) Inhibition of glucokinase by alloxan through interaction with SH groups in the sugarbinding site of the enzyme. Mol Pharmacol 34:395–400.

102. Lenzen S, Mirzaie-Petri M (1991) Inhibition of glucokinase and hexokinase from pancreatic B-cells and liver by alloxan, alloxantin, dialuric acid, and t-butylhydroperoxide. Biomed Res 12:297–307.

103. Munday R (1988) Dialuric acid autoxidation. Effects of transition metals on the reaction rate and on the generation of 'active oxygen' species. BiochemPharmacol 37:409–413.

104. Elsner M, Gurgul-Convey E, Lenzen S (2006) Relative importance of cellular uptake and reactive oxygen species for the toxicity of alloxan and dialuric acid to insulin-producing cells. Free Radic Biol Med 41:825–834.

105. Winterbourn CC, Munday R (1989) Glutathione-mediated redox cycling of alloxan. Mechanisms of superoxide dismutase inhibition and of metal-catalyzed OH formation. BiochemPharmacol 38:271–277.

106. Grankvist K, Marklund S, Sehlin J, Taljedal IB (1979) Superoxide dismutase, catalase and scavengers of hydroxyl radical protect against the toxic action of alloxan on pancreatic islet cells in vitro. Biochem J 182:17–25.

107. Tiedge M, Lortz S, Drinkgern J, Lenzen S (1997) Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. Diabetes 46:1733–1742.

108. Fischer LJ, Harman AW (1982) Oxygen free radicals and the diabetogenic action of alloxan. In: Autor AP (ed) Pathology of oxygen. Academic, New York, pp 261–275.

109. Heikkila RE, Cabbat FS (1982) The prevention of alloxaninduced diabetes in mice by the iron-chelator detapac: suggestion of a role for iron in the cytotoxic process. Experientia 38: 378–379.

110. Tjälve H, Wilander E, Johansson EB (1976) Distribution of labelled streptozotocin in mice: uptake and retention in pancreatic islets. J Endocrinol 69:455–456.

111. Karunanayake EH, Baker JR, Christian RA, Hearse DJ, Mellows G (1976) Autoradiographic study of the distribution and cellular uptake of (14C)-streptozotocin in the rat. Diabetologia 12:123–128.

Vol. 15 (3) 330-348, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.34

112. Ledoux SP, Wilson GL (1984) Effects of streptozotocin on a clonal isolate of rat insulinoma cells. BiochimBiophys Acta 804:387–392.

113. Schnedl WJ, Ferber S, Johnson JH, Newgard CB (1994) STZ transport and cytotoxicity. Specific enhancement in GLUT2- expressing cells. Diabetes 43:1326–1333.

114. Elsner M, Guldbakke B, Tiedge M, Munday R, Lenzen S (2000) Relative importance of transport and alkylation for pancreatic beta-cell toxicity of streptozotocin. Diabetologia 43:1528–1533.

115. Wilson GL, Hartig PC, Patton NJ, LeDoux SP (1988) Mechanisms of nitrosourea-induced beta-cell damage. Activation of poly(ADP-ribose) synthetase and cellular distribution. Diabetes 37:213–216.

116. Rerup CC (1970) Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol Rev 22:485–518.

117. Weiss RB (1982) Streptozocin: a review of its pharmacology, efficacy, and toxicity. Cancer Treat Rep 66:427–438.

118. Ledoux SP, Woodley SE, Patton NJ, Wilson GL (1986) Mechanisms of nitrosourea-induced beta-cell damage. Alterations in DNA. Diabetes 35:866–872.

119. Pieper AA, Verma A, Zhang J, Snyder SH (1999) Poly (ADPribose) polymerase, nitric oxide and cell death. Trends Pharmacol Sci 20:171–181.

120. Yamamoto H, Uchigata Y, Okamoto H (1981) Streptozotocin and alloxan induce DNA strand breaks and poly(ADP-ribose) synthetase in pancreatic islets. Nature 294:284–286.

121. Bennett RA, Pegg AE (1981) Alkylation of DNA in rat tissues following administration of streptozotocin. Cancer Res 41: 2786–2790.

122. Schein PS, Cooney DA, Vernon ML (1967) The use of nicotinamide to modify the toxicity of streptozotocin diabetes without loss of antitumor activity. Cancer Res 27:2324–2332.

123. Pieper AA, Brat DJ, Krug DK et al (1999) Poly(ADP-ribose) polymerase-deficient mice are protected from streptozotocininduced diabetes. Proc Natl Acad Sci U S A 96:3059–3064.

124. Masutani M, Suzuki H, Kamada N et al (1999) Poly(ADP-ribose) polymerase gene disruption conferred mice resistant to streptozotocin induced diabetes. Proc Natl Acad Sci U S A 96:2301–2304.

125. Turk J, Corbett JA, Ramanadham S, Bohrer A, McDaniel ML (1993) Biochemical evidence for nitric oxide formation from streptozotocin in isolated pancreatic islets. BiochemBiophys Res Commun 197:1458–1464.

126. Nukatsuka M, Yoshimura Y, Nishida M, Kawada J (1990) Allopurinol protects pancreatic beta cells from the cytotoxic effect of streptozotocin: in vitro study. J Pharmacobiodyn 13:259–262.

127. Bedoya FJ, Solano F, Lucas M (1996) N-Monomethyl-arginine and nicotinamide prevent streptozotocin-induced double strand DNA break formation in pancreatic rat islets. Experientia 52:344–347.