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Review Article

Emerging paradigms in pre-clinical screening models for drugs acting against diabetes: Delving into complex approaches and advancements

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ABSTRACT

Animal models play a critical role in diabetes research, aiding in the comprehension of both type 1 and type 2 diabetes pathogenesis and the assessment of potential therapeutic interventions. These models offer numerous advantages, including the ability to induce stable hyperglycemia and hyperlipidemia, minimal variability, and the opportunity to study genetic mutations and single gene effects. However, they also present limitations such as lengthy induction periods, fluctuating glucose parameters, potential toxicity to other organs, and high costs. Various screening models, encompassing in vitro assays and *in vivo* models like Alloxan-induced and Streptozotocin-induced diabetes, serve as valuable tools for investigating diabetes and testing drug efficacy. The selection of an appropriate model requires careful consideration of specific research objectives. Despite their limitations, animal models continue to be indispensable for advancing our understanding of diabetes and developing effective treatments.

1. Introduction

Hyperglycemia, glycosuria, hyperlipidemia, a negative nitrogen balance and occasionally ketonemia are the hallmarks of this metabolic disease. Capillary basement membrane thickening, an increase in vessel wall matrix and cellular proliferation are common pathological changes that cause vascular complications lumen narrowing, early atherosclerosis, glomerular capillary sclerosis, retinopathy, neuropathy, and peripheral vascular insufficiency. The majority of cases of pancreatic islet cell death are autoimmune (type 1A), where antibodies that destroy cells are evident in blood, while some cases are idiopathic (type 1B), where no antibodies that destroy cells are found.[1] Circulating insulin levels are always low or very low in type 1 cases, and patients are more likely to experience ketosis. This variety has a lower prevalence and a weaker hereditary tendency.[1]

There is no loss or mild reduction in the number of cells, the level of insulin in the blood is low, normal, or even high, no anti-cell antibodies are visible, there is a high degree of hereditary predisposition, and the disease typically manifests itself later in life (past middle age). Type 2 diabetes accounts for more than 90% of cases.[2]

The advantages of using animal models in diabetes research include the induction of stable and selective loss of pancreatic beta cells, which results in hyperglycemia and hyperlipidemia that can be studied over a prolonged period without requiring insulin treatment.[2] The induction of diabetes can be achieved through various methods such as Streptozotocin-induced toxicity or diabetes-induced obesity due to over-nutrition. The results obtained from these animal models are more consistent, and the variability is minimum, requiring a small sample size.[2] These models resemble human type 2 diabetes as they exhibit reduced islets of beta cell mass, allowing for the

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investigation of complex genetic mutations and single gene effects on diabetes.[2] Additionally, the cytotoxic effects of diabetogenic chemicals on other organs can be avoided in these models.[3]

The development and use of animal models for studying diabetes have various limitations and drawbacks. The induction period is typically long, and hyperglycemia can develop upon dietary treatment in normal animals, making fluctuating glucose parameters unsuitable for antidiabetic drug testing.[3] The models are less stable and reversible due to the spontaneous regeneration of beta cells and may produce toxic effects on other body organs. Hyperglycemia primarily develops by direct cytotoxic action on beta cells and insulin deficiency rather than the consequence of insulin resistance.[4] The models are also comparatively costlier to develop, and mortality is relatively high. Handling virus-induced diabetes requires technical expertise, and symptoms of diabetes and obesity overlap but are not identical. Limited availability, expensive maintenance, occurrence of digestive problems due to excision of exocrine portion, deficiency of α-amylase, dissection of alpha cells, mortality observed due to ketosis, and the need for highly sophisticated and costly production and maintenance make these models challenging to use.[4]

2. Screening Models

2.1. In vitro models

2.1.1. Assay of α-α-amylase inhibition

By combining the test sample with starch solution and incubating while the test sample was permitted to react with the α-amylase enzyme, in vitro α-amylase inhibition can be evaluated. Dinitrosalicylic acid reagent was applied to both the control and the test following incubation. For a few minutes, place this mixture in a pan of boiling water.[5] Using a spectrophotometer, the absorbance at 540 nm was measured and the percentage of α-amylase enzyme inhibition was estimated.[5]

A potato starch solution including sodium phosphate buffer, sodium chloride, and was maintained in a batch of boiling water for a short period of time.[6] By combining α-amylase with the same buffer, the α-amylase solution was created. Equal parts of 3,5-dinitro salicylic acid (DNS) solution and sodium potassium tartrate tetra hydrate solution were combined to create the colorimetric reagent.[7] Test samples containing varying concentrations of acarbose were combined with starch solution, and α-amylase solution was added. The mixture was then incubated at 25°C to cause the starch solution to react.[7] The aforementioned solution was mixed with DNS reagent, and the mixture was then cooked over a boiling water bath for 15 minutes. Distilled water was used to create the final volume, and a spectrophotometer was used to measure the absorbance at 540nm. The IC_{50} value and % inhibition was determined.[7]

2.2. Inhibition of α-glucosidase activity

The α-glucosidase enzyme inhibition activity was carried out by incubating phosphate buffer containing test samples of various connections with α-glucosidase enzyme solution at 37°C for 1 hour in maltose solution.[8] The mixture for the reaction was briefly placed in boiling water before cooling. In order to determine how much glucose was released from maltose by the action of the enzyme α-glucosidase, glucose reagent was added, and its absorbance was measured at 540nm. The IC_{50} and the % of inhibition were computed.[8]

2.3. Studies using isolated pancreatic islet cell lines

Using isolated pancreatic cells from laboratory animals that have undergone a collagenase digestion procedure, sufficient separation, and transference to the proper culture medium can be used to study these pathways.[9] It is well known that the production of adenosine triphosphate (ATP) from adenosine diphosphate by pancreatic cells results in the release of insulin (ADP). The plasma membrane becomes depolarized as a result of the rise in the cytoplasmic ATP/ADP ratio, which blocks ATP-sensitive potassium channels and activates voltage-dependent Ca^{2+} channels.[9] As a result, the intracellular Ca^{2+} concentration rises, causing insulin production to be triggered.

3. In-vivo Models

3.1. Alloxan-induced diabetes

Alloxan's (dioxyuracil's) quick absorption by beta cells and the subsequent production of free radicals, against which beta cells have weak defenses, are primarily responsible for its ability to cause diabetes.[10] As Alloxan is converted to dialuric acid and subsequently reoxidized, a redox cycle is established for the production of superoxide radicals, which are highly reactive free radicals that cause beta DNA fragmentation.[10] Although Alloxan is similarly absorbed by the liver, it has superior resistance to reactive oxygen species and is less likely to cause harm.[10] Alloxan has the capacity to interact with the SH group, particularly membrane proteins on beta cells like glucokinase, ultimately leading to cell necrosis. Choose rabbits who weigh between 2 and 3kg while they are fasting. 150mg/kg of Alloxan is given for 10 minutes through the ear marginal vein. Animals develop hyperglycemic and uricosuric in about 70% of cases. The surviving creatures either perish or briefly experience hyperglycemia.[10]

Alloxan is administered intravenously in a beagle dog weighing 15 to 20kg at a dose of 60mg/kg. Alloxan has been administered intravenously to primates like monkeys at a dose of 65–200 mg/kg to cause diabetes.[10] All animals get Alloxan for a week along with regular insulin, and then a daily dose of 28IU of international units is given subcutaneously. Following were the triphasic alterations

that were seen: a rise at 2 hours, a hypoglycemia phase at 8 hours, and then the insulin depletion of the B-cells at 24 hours.[10] Recommended dosages (in milligrams per kilogram of body weight) of Alloxan for different species, including rats, mice, rabbits, and dogs. For rats, the suggested dosage for intravenous or intraperitoneal administration ranges from 40 to 200mg/kg, while for mice, it ranges from 50 to 200mg/kg.[10] For rabbits, the recommended intravenous dose is 100-150mg/kg, and for dogs, it is 50-75mg/kg via intravenous administration. Alloxan is a chemical compound often used in medical research to induce diabetes in animals, and the appropriate dose varies depending on the species and method of administration.[10]

The study induced diabetes in rats using a single dose of Alloxan monohydrate and confirmed diabetes after a week by measuring fasting blood glucose levels.[11] Only rats with blood glucose levels between 200-300mg/dL were included in the study and were divided into seven groups, including a nondiabetic control group and diabetic control group. Groups III-V was further divided into subgroups and received lower and higher doses of aqueous extract, ether extract, and methanolic extract once daily for four weeks.[11] Group VI was administered the hypoglycemic drug Glibenclamide once daily for four weeks, and group VII was given a daily dose of Glibenclamide and aqueous extract. Blood glucose levels were measured on various days using a glucometer.[11]

3.2. Streptozotocin induced diabetes

A naturally occurring substance called Streptozotocin (STZ) is highly harmful to the beta cells in the pancreas.[12] It serves as an animal model for hyperglycemia in medical studies. The levels of insulin and glucose in the blood are changed by STZ. Hyperglycemia occurs two hours after injection because blood insulin levels have dropped.[12] Hypoglycemia develops six hours later as a result of the elevated blood insulin levels. Finally, hyperglycemia sets in, and blood insulin levels fall. STZ hinders the oxidation of glucose and lessens the production and release of insulin. We saw that STZ first eliminated the B cell response to glucose.[12] STZ limits the expression of GLUT2. The DNA of pancreatic B cells is altered by STZ. DNA is alkylated by STZ, which causes the demise of B cells. Poly ADPribosylation is activated by DNA damage brought on by STZ. For STZ to be diabetogenic, poly ADP-ribosylation activation is more crucial than free radical production and DNA damage. Also capable of causing necrosis is calcium.[12]

STZ (Streptozotocin) has replaced Alloxan as a preferred method for inducing diabetes in animal models due to its advantages.[12] STZ has a lower mortality rate and can induce irreversible diabetes, making it a more reliable method for research. Additionally, it has greater selectivity towards B cells, which are responsible for insulin production, making it a useful tool for studying diabetes. Moreover, guinea pigs and rabbits are resistant to the diabetogenic action of STZ, allowing for more targeted experimentation. Overall, STZ has become the preferred choice for inducing diabetes in animal models due to its advantages over Alloxan.[12]

The literature currently recognises odified streptozotocin models of type 2 diabetes.[13] The "Nicotinamidestreptozotocin induced" and "Fructose-fed, streptozotocin-injected" models were recently developed, although the effects of Nicotinamide in Streptozotocin-induced diabetes were first investigated.[13] The so-called "Fat-fed, Streptozotocin-treated" rat model was the first one described in the literature and was developed.[13] Nicotinamide was referred to as a medicines that changes Streptozotocin harmful effects, and also as a substance that can prevent or even reverse development of Streptozotocin diabetes.

3.3. Fat-fed, Streptozotocin-treated rat model of type 2 diabetes

This streptozotocin model of type 2 diabetes is the most popular and well-known. It was created with the intention of simulating the development and progressive alterations in metabolism that occur naturally in people with type 2 diabetes.[14]

The inspiration for this model came from research that showed a significant impact of a diet high in fat on insulin resistance, which was then followed by hyperinsulinemia[14] However, the percentage of hyperglycemic individuals was unsatisfactory because to hyperinsulinemia. To alter the current direction Sprague-Dawley rats aged 7 weeks were given Streptozotocin (50mg/kg) intravenously.[14] The rats had previously been fed a high-fat diet (40 percent of calories as fat) over a two-week period. This particular set of animals had continued to consume a high-fat diet following the Streptozotocin injection.[14] Several more modified variations have been created since this model was first launched. The lowering of a single dosage of Streptozotocin and several administrations of Streptozotocin at lower doses are two of the most significant adjustments.[14] These two models are thought to both be efficient at causing type 2 diabetes. In particular, altered the mode of delivery while also administering a single lower dose of Streptozotocin. A 35mg/kg intraperitoneal dose of Streptozotocin was administered. The high-fat diets recipe was also altered. A diet consisting of 58% fat, 17% carbohydrate and 25% protein was introduced to replace the previous one, which had 40% fat, 41% carbohydrate and 18% protein. As an alternative, Streptozotocin was given intraperitoneally in two smaller doses (each 30mg/kg). Also, the fat diet formula used in this study was distinct and included 22% fat, 48% carbohydrates, and 20% proteins. Prior to receiving the Streptozotocin injections, male Wistar rats were fed this diet for four weeks. Next, the same amount of Streptozotocin

was administered twice, the first time one week after the first injection. It's also vital to note that different animal species may employ the basic model or variations of it. As an illustration, this model was also applied to mice, often utilizing a high-fat diet with 60% of calories coming from fat and 40mg/kg streptozotocin injections.[14] Although the application of this approach to guinea pigs was also detailed, a change in food formulation was made by adding a high-fat, high-carbohydrate diet.[7,14]

3.4. Nicotinamide-Streptozotocin – induced rat model of type 2 diabetes

Streptozotocin and nicotinamide's connection has been studied for a long time, but their combination has only lately been made available as a model for type 2 diabetes.[15] Animals with streptozotocin-induced diabetes were initially used to test the protective effects of nicotinamide.[15] In fact, Nicotinamide's ability to provide protection is what led to its prolonged usage in the treatment of type 2 diabetes. The explanation for this concept is connected to the possibility that a certain type of diabetes may be related to the proportion of B cells mass reduction.[15] For instance, when type 1 diabetes is diagnosed, there is already a 60–80% decline in the number of functioning B cells. In this situation, Nicotinamide stops the major death of functioning B cells and causes mice to develop type 2 diabetes while still allowing them to make insulin. Nicotinamide's protective effect is accomplished by inhibiting PARP-1 activity.[16] The activity of this enzyme is greatly elevated in B cells in response to Streptozotocin-induced DNA damage. In the long run, increased activity of this enzyme promotes necrosis of B cells by reducing intracellular NAD(+) and ATP levels.[16]

Streptozotocin and Nicotinamide are administered concurrently in an enhanced model, though typically at different times. Although intravenous administration was also mentioned, both of these drugs can be delivered intraperitoneally.[17] The literature lists a number of various intervals between the administrations of these substances. For instance, the most common time to administer Streptozotocin is 15 minutes after Nicotinamide.[17] Moreover, this gap can be adjusted and can be either longer, such as 20 minutes between applications, or shorter, such as only 5 minutes.[17] The development of type 2 diabetes is unaffected by alterations in these intervals, and each strategy has been shown to be successful. Nicotinamide, on the other hand, might also be given following Streptozotocin. Similar to Streptozotocin, the concentration of nicotinamide varies often; it typically ranges from 90mg/kg to 120mg/kg.[17] However, the Nicotinamide-Streptozotocin model of type 2 diabetes also detailed the use of a substantially higher concentration of Nicotinamide (230mg/kg).[17] The Nicotinamide-Streptozotocin-induced diabetes model is utilized in a variety of additional animal species, in contrast to the previous two models of type 2 diabetes, which were initially developed and primarily used in rats. Similar to how it is used in rats, this model is utilized in mice to study type 2 diabetes, but it suffers from the same issues with irregular Nicotinamide and Streptozotocin doses and intervals between their administrations.[17]

3.5. Monosodium glutamate induced diabetes

Plasma glutamate content rises as a result of monosodium glutamate (MSG). MSG encouraged the secretion of insulin. MSG consumption in mice led to obesity and hyperinsulinemia. Blood glucose, total cholesterol, and triglyceride levels all rose after 29 weeks.[18]

4. Virus Induced Diabetes

Diabetes mellitus, an agent for the insulin-dependent diabetes mellitus, may be brought on by viruses. The virus can have systemic effects that do not directly affect B-cells, but can lead to autoimmune reactivity to B-cells. In some cases, the virus can infect and destroy B-cells in the pancreas. This can initiate the destruction of beta cells, either by directly infecting them or by triggering an autoimmune response against them. Overall, the virus can play a role in the destruction of beta cells and the development of autoimmune diseases.[18] Two double stranded RNA viruses, reovirus and lymphocytic choriomeningitis virus (LMCV, Armstrong variant), RNA picornaviruses, CoxsackieB4 (CB4), encephalo-myocarditis (EMC-D and M variations), and Mengo-2T are among the human viruses used to induce diabetes. These human pathogenic pathogens must be modified for growth either by injection into suckling mice or by passage in cultured mouse B-cells because their primary isolates are typically neither pancreatotrophic nor ilytic to mouse B-cells.[18]

5. Conclusion

In conclusion, animal models play a crucial role in diabetes research, particularly in understanding the pathogenesis of type 1 and type 2 diabetes and evaluating potential drug therapies. These models offer advantages such as stable induction of hyperglycemia and hyperlipidemia, minimal variability, and the ability to study genetic mutations and single gene effects. However, they also have limitations, including long induction periods, fluctuating glucose parameters, potential toxicity to other organs, and high costs. Various screening models, including in vitro assays and in vivo models like Alloxan-induced and Streptozotocin-induced diabetes, provide valuable tools for studying diabetes and testing drug efficacy. Each model has its own advantages and considerations, and researchers must carefully select the appropriate model based on their specific research objectives. Overall, the use of animal models, despite their limitations, remains essential in advancing

our understanding of diabetes and developing effective treatments.

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None.

7. Conflict of Interest

None.

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