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Challenges in creating diabetes mellitus animal model for physiological research

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Abstract

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from abnormalities in insulin secretion, insulin action, or both. Diabetes mellitus is one of the global health problems, and its prevalence continues to increase from year to year. Diabetes mellitus has several types, among which the most commonly encountered are type 1 diabetes and type 2 diabetes. Studies related to diabetes mellitus need to be conducted to understand preventive and curative measures that can be taken to address this disease. There are various methods that can be used to study diabetes mellitus, one of which is using experimental animals. This article briefly explains various methods that can be used in inducing diabetes in experimental animals, along with the advantages and disadvantages of these methods.

Keywords: Alloxan; Animal Model; Diabetes Mellitus; Streptozotocin

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from abnormalities in insulin secretion, insulin action, or both [1]. Diabetes is a global health problem, with the World Health Organization (WHO) noting that in 2019, it ranked as the ninth leading cause of global death among various diseases. The prevalence of diabetes has been increasing, from 4.7% in 1980 to 8.5% in 2014 [2]. In 2019, the number of people with diabetes rose to 463 million, approximately 9.3% of the population. It is estimated that by 2045, the prevalence of diabetes will reach 10.9%, affecting around 700 million people [3].

There are several types of diabetes mellitus, with the most common being type 1 and type 2. According to Indonesian Pediatric Society (Ikatan Dokter Anak Indonesia/IDAI) [4], type 1 diabetes is a systemic disorder resulting from glucose metabolism disturbances marked by chronic hyperglycemia. This condition is caused by damage to pancreatic β cells, either through autoimmune or idiopathic processes, leading to reduced or halted insulin production. Type 1 diabetes occurs in 5-10% of diabetes patients [5].

Type 2 diabetes, formerly known as non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes, encompasses 90-95% of all diabetes cases [6]. According to Indonesian Society of Endocrinology (Perkumpulan Endokrinologi Indonesia, Perkeni) [1], type 2 diabetes is characterized by peripheral insulin resistance and decreased insulin production, accompanied by chronic low-grade inflammation in peripheral tissues such as adipose tissue, liver, and muscles. Type 2 diabetes also includes individuals with insulin resistance and relative insulin deficiency.

Studies on diabetes mellitus are conducted with the aim of understanding preventive and curative efforts against diabetes mellitus. In practice, there are many methods that can be used to study diabetes mellitus, one of which is using

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induced diabetes mellitus animal models. This study will discuss various induction methods for creating experimental animal models of diabetes mellitus, covering the necessary treatment procedures, advantages, and disadvantages of the existing methods.

2. Types of Experimental Animal Model

There are several types of experimental animal models based on their induction mechanisms, including chemical induction, spontaneous autoimmune, and viral induction. Additionally, surgery can be performed to induce diabetes in experimental animals [7]. Furthermore, modifying the diet given to experimental animals has also been proven to induce diabetes in animal models [8].

Based on the type of animal used, various animals can serve as experimental models, including rats, mice, hamsters, tuco-tuco, silkworms, pigs, monkeys, and zebrafish [7]. Different induction methods are required for these animals to develop diabetes mellitus, such as chemical compound injections, genetic induction, surgical induced, and viral induced.

2.1. Chemical Induction

Chemical induction involves injecting a chemical compound into experimental animals. Several types of chemical compounds can induce diabetes mellitus in experimental animals, with two of the most commonly used being streptozotocin (STZ) and alloxan [7].

2.2. Streptozotocin

Streptozotocin (STZ) is a glucosamine-nitrosourea antibiotic that is toxic to pancreatic β cells. STZ has a structure similar to glucose and is transported to pancreatic β cells through the glucose transporter (GLUT2). This leads to necrosis in pancreatic β cells, causing a halt in insulin production, either entirely or partially [9]. Some experimental animals are known to be sensitive to STZ, such as rats, mice, and monkeys. On the other hand, there are experimental animals less sensitive to STZ injections, such as rabbits [10].

The use of streptozotocin involves first dissolving STZ in a 50 mM sodium citrate buffer with a pH of 4.5. STZ is dissolved to achieve a final concentration of 4mg/ml. STZ must be used immediately within 5 minutes after dissolution because it can degrade within 15 to 20 minutes when dissolved in a citrate buffer [10]. Streptozotocin injection can be performed using various methods, with intraperitoneal and intravenous being two commonly used methods [7]. After STZ injection into experimental animals, there is a risk of sudden hypoglycemia. To prevent this, experimental animals are given a 10% dextrose solution for several days after injection [10, 11].

STZ injection can have an impact, resulting in either type 1 or type 2 diabetes, depending on the age and dose of STZ injected [9]. Various doses of STZ can be used to induce diabetes mellitus, and STZ administration is sometimes followed by dietary modifications. Below are some references for doses that can be used to induce experimental animals to develop diabetes mellitus.

Table 1 STZ Dose induction, administration route, type of animal model, and type of diabetes mellitus in animal model experiment using STZ

Reference	STZ Induction	Administration route	Type of animal model	Type of DM
Gunawan et al., 2020 [11]	50 mg/KgBW single dose	i.p	Wistar mice	-
Kottaisamy et al., 2021 [7]	100 – 200 mg/KgBW STZ single dose (for mice) 35 – 65 mg/KgBW STZ single dose (for rats) 20 – 40 mg/KgBB STZ for several days	i.p	-	-
Jakoviljevic et al., 2018 [12]	25 mg/KgBW STZ single dose followed by HFD	i.p	Wistar mice	-
Li et al., 2012 [13]	45 mg/KgBW STZ single dose	i.v	Wistar mice	-

Tate et al., 2019 [14]	55 mg/KgBW STZ injected 3 times followed by HFD for 18 weeks	i.p	FVB/N mice	Type 2 DM
De Blasio et al., 2020 [15]	55 mg/KgBW STZ injected 5 times	i.p	FVB/N mice	Type 1 DM
Zhang et al., 2018 [16]	40 mg/KgBW STZ injected 5 times after 4 weeks HFD	i.p	*Mus musculus c.*	-
Jia et al., 2018 [17]	55 mg/KgBW STZ single dose	i.p	Sprague Dawley mice	Type 1 DM
Marino et al., 2023 [9]	200 mg/KgBW STZ 40 mg/KgBW STZ 4 times followed by HFD for 4 weeks	-	C57BL/6J mice	Type 1 DM Type 2 DM

Diabetes mellitus animal models can be obtained using Wistar rats (Rattus norvegicus) injected with intraperitoneal streptozotocin (STZ) at a dose of 50 mg/kg body weight. It is known that within 2 days after injection, rats experience hyperglycemia, measured after a 6-hour fast [11].

A single-dose intravenous injection of STZ at a dose of 45 mg/kg body weight is also known to induce diabetes mellitus in Wistar rats. Intravenous injection is performed in the tail vein of the rat. This method is known to cause diabetes in rats 5 days after STZ injection [13].

In another type of rat, Sprague Dawley, STZ injection is also known to induce diabetes mellitus. Research by Jia et al [17] shows that an injection of 55 mg/kg body weight of STZ can induce diabetes mellitus in rats. Intraperitoneal injection is known to cause type 1 diabetes, as measured by blood sugar levels 3 days after STZ injection.

Induction of diabetes mellitus in experimental animals can also be done by administering multiple doses of STZ. Intraperitoneal injection of 55 mg/kg body weight of STZ for 5 days in FVB/N mice is known to induce diabetes mellitus in experimental animals. Diabetes occurring in experimental animals with this method is type 1 diabetes [15]. FVB/N mice are one of the transgenic rat species commonly used as experimental animal models [18].

A combination method of modifying diet patterns and STZ injection is also known to induce diabetes mellitus in rats. Research by Zhang et al [16] shows that a high-fat diet for 4 weeks followed by intraperitoneal injection of STZ at a dose of 40 mg/kg body weight for 5 days can induce diabetes mellitus in Mus musculus rats. The high-fat diet given consists of feed containing 10% sucrose, 10% yolk, 10% lard, 1.5% cholesterol, 0.5% bile salt, and 68% basic forage.

A study by Tate et al [14] also combines diet modification and STZ injection in rats. STZ administration of 55 mg/kg body weight intraperitoneally three times in FVB/N rats followed by a high-fat diet for 18 weeks is known to induce type 2 diabetes in rats. In this method, a high-fat diet using SF04-001 feed, known to be a high-fat rat feed, is used.

Another study by Jakoviljevic et al [12] shows that inducing a diabetes animal model is done by providing a high-fat diet to Wistar rats for 4 weeks. The high-fat diet given to rats contains 25% fat, 15% protein, 51% starch, and 5% fiber. Rats are then injected with a single intraperitoneal dose of 25 mg/kg body weight of STZ. After 72 hours post-STZ injection, rats experience hyperglycemia, as observed through the Oral Glucose Tolerance Test (OGTT).

Research by Marino et al [9] shows that inducing diabetes mellitus with a high dose of STZ, namely 200 mg/kg body weight, in C57BL/6J mice can induce type 1 diabetes in mice. In this method, STZ is given in a single dose. Meanwhile, the type 2 DM model can be induced by providing a high-fat diet for 4 weeks starting at the age of 6 weeks and injecting 4 mg/kg body weight of STZ into rats.

The use of streptozotocin as a method of inducing diabetes in experimental animals has its drawbacks. Streptozotocin is known to have cardiotoxic and nephrotoxic properties in experimental animals. This effect depends on the blood sugar levels of the experimental animals [19]. The toxic effect of streptozotocin can cause death in experimental animals. Premature death of experimental animals before the designated termination time or trial period can lead to differences in research results. One way to avoid the death of experimental animals due to the toxic effects of STZ is by using nicotinamide in experimental animals. Nicotinamide (NA, pyridine-3-carboxamide) is a form of amide of vitamin B3. NA has a protective effect on pancreatic β cells, while STZ has cytotoxic properties on pancreatic β cells. NA plays a role in

preventing the inhibition of glucose-stimulated insulin secretion caused by STZ. NA also reduces DNA damage caused by STZ [20].

The effects of inducing diabetes using STZ and NA depend on the doses of both compounds, the age of the experimental animals at induction, and the time of NA injection into the experimental animals. In rats induced with STZ at 65 mg/kg body weight and injected with NA at 100 mg/kg body weight 15 minutes before STZ injection, the blood sugar level in rats is 31.2 mmol/L. Meanwhile, with the same method using NA at 350 mg/kg body weight, the blood sugar level in rats is 6.7 mmol/L, slightly above the control group with a blood sugar level of 6.6 mmol/L (Szkudelski, 2012). Research by Cruz et al [21] also shows that administration of NA to rats induced with STZ causes a lower increase in blood sugar levels compared to the group that only received STZ injections. However, both groups, both STZ alone and STZ with NA, have higher blood sugar levels than the control group (STZ group 541.28 ± 18.68 mg/dl, STZ with NA 440.87 ± 20.96 mg/dl, control 110.00 ± 3.48, control with NA 108.50 ± 1.52 mg/dl).

NA is also known to increase the survival rate of rats induced with STZ. Within a period of 5 weeks, it is known that the group injected with only STZ experiences a significant number of deaths compared to the group given NA and STZ and compared to the control group, which has a 100% survival rate [21].

2.3. Alloxan

Alloxan (5,5-dihydroxyl pyrimidine-2,4,6-trione) is an organic compound, a derivative of urea, which is cytotoxic, carcinogenic, and analogous to glucose. Inducing diabetes mellitus using alloxan will lead to insulin-dependent diabetes, or type 1 diabetes [22]. Alloxan and streptozotocin have similarities in the mechanism of inducing diabetes but with different processes. Alloxan has a structural similarity to glucose, allowing it to enter pancreatic β cells through GLUT2. Alloxan will inhibit glucokinase and produce Reactive Oxygen Species, which will ultimately damage pancreatic β cells and cause diabetes [23].

The pharmacokinetic process of alloxan causing diabetes begins with the hydrophilic nature of alloxan and its analogy to glucose. The hydrophilic nature of alloxan prevents it from directly penetrating cell membranes, requiring a transporter to enter cells. Structural similarity to glucose allows alloxan to enter pancreatic β cells through GLUT2. Alloxan is known not to inhibit the performance of GLUT2. This leads to the accumulation of potentially toxic alloxan in cells [22]. There are differences in the glycemic effects of rats induced using alloxan and streptozotocin. Research by Rodrigues et al [23] shows that 50% of experimental animals injected with alloxan experience hyperglycemia, while the remaining 45% maintain normal blood sugar levels, and 5% die before the end of the experimental period. Meanwhile, 69% of experimental animals injected with STZ experience hyperglycemia, and the remaining 31% have normal blood sugar levels. Between the two types of diabetes mellitus induction, it is known that experimental animals with alloxan injections have higher blood sugar levels.

The use of alloxan to induce diabetes mellitus in experimental animals has limitations. One limitation is the instability and autoreversibility of blood sugar levels in animals induced with alloxan. There is inconsistent blood sugar levels in experimental animals after induction using alloxan. This makes alloxan less suitable for use as an induction method in experimental animals intended for the analysis of hypoglycemic effects or antidiabetic therapy [22].

2.4. Spontaneous Autoimmune

There are several types of experimental animals with spontaneous autoimmunity that can be used in studies related to diabetes mellitus. One commonly used species is the NOD mouse. NOD mice are relevant experimental animals in diabetes studies due to their genetic and environmental aspects that are relevant to humans. NOD mice have autoantibodies and increased circulating autoreactive T cells in their bodies. This autoimmune phenotype is accompanied by hyperglycemia due to a decrease in the number of pancreatic β cells in NOD mice [24].

The incidence of diabetes mellitus in NOD mice varies according to gender. At the age of over 30 weeks, it is known that the incidence of diabetes mellitus is 80% in female mice and less than 20% in male mice. Other studies mention that the early onset of diabetes in NOD mice occurs at the age of 18 weeks, but at the age of over 35 weeks, 60% of female mice have diabetes, and only 10% of male mice have diabetes. NOD mice are also known to have a spontaneous inflammatory phenotype in the lacrimal and salivary glands. This makes NOD mice suitable for being experimental animal models for Sjogren's syndrome [25].

Another type of experimental animal with spontaneous autoimmunity in diabetes is the BB rat. BB-DP rats, or Bio-Breeding Diabetic Prone, are a type of Wistar rat that undergoes spontaneous mutations in the Major Histocompatibility Complex (MHC). BB-DP rats exhibit a phenotype of diabetes mellitus with equal proportions between males and

females. This rat model is known to start developing diabetes between the ages of 50 to 90 days after birth. There are some limitations in using BB-DP rats as experimental animals, one of which is a decrease in T cell levels. This abnormality is not found in humans or other experimental animal models, raising questions about the validity of this experimental model [26]. T cell lymphopenia is caused by a frameshift mutation in the GIMAP5 (GTPase of the immune-associated protein 5) gene. This mutation leads to spontaneous selective apoptosis of T cells [27].

The LEW.1AR1-iddm rat model is also a type of experimental animal with spontaneous autoimmunity, representing type 1 diabetes. This rat model is considered suitable for use in diabetes experimental models due to several aspects, such as development, progressivity, and observation of type 1 diabetes therapy in these rats [28]. The LEW.1AR1-iddm rat model is obtained due to a point mutation in the Dock8 gene [29]. The LEW.1AR1-iddm rats are known to exhibit a pattern of autosomal recessive inheritance of diabetes traits [7].

2.5. Surgically Induction

Pancreatectomy is one of the procedures that can be performed to induce diabetes in experimental animals. Pancreatectomy can be carried out in various types of experimental animals, such as dogs, cats, rats, mice, chickens, sheep, ducks, guinea pigs, and pigs. In the dog experimental animal model, pancreatectomy is known to be the only induction method that yields sufficiently significant diabetes results compared to other methods [30].

There are several advantages to using the pancreatectomy method in inducing diabetes mellitus in experimental animals. This model represents the isolated effect of beta cell reduction, especially immediately after pancreatectomy in experimental animals. The effects such as insulin deficiency and hyperglycemia can be observed immediately after surgery. This allows researchers to have full control over the onset of diabetes in experimental animals [19].

Inducing diabetes with pancreatectomy can be done by cutting 60% of the rat's pancreas, specifically by cutting the corpus pancreas and cauda pancreas. This method shows that in the first week after pancreatectomy, rats still experience normoglycemia, with the blood glucose levels of rats being <10 mmol/L. Meanwhile, a 90% pancreatectomy, leaving only pancreatic tissue between the common bile duct and the duodenum, shows hyperglycemia from the first week after the procedure. In the 90% pancreatectomy model, it was found that 50% of rats still experienced hyperglycemia while the rest had normoglycemia according to their random blood glucose levels. Similar results were also seen in OGTT, where 50% of the 90% pancreatectomy rat model experienced hyperglycemia at 15-120 minutes of OGTT [19].

The pancreatectomy procedure can be combined with the administration of low-dose intravenous STZ. Research by Jin et al [31] combined partial pancreatectomy in male rhesus monkeys followed by low-dose STZ injection, namely 15 mg/kgBW 1-5 times every 4 days intravenously. Pancreatectomy was performed by leaving the head of the pancreas and the uncinate process. The blood glucose levels of monkeys after pancreatectomy fluctuated between 3.3 and 21.6 mmol/L and returned to normoglycemia on the third day post-pancreatectomy. After five injections of STZ at a dose of 15 mg/kgBW, the fasting blood glucose levels of rhesus monkeys were found to increase to 19.53 + 2.72 mmol/L. This method successfully induced hyperglycemia in rhesus monkeys similar to induction with high-dose STZ (80, 100, and 120 mg/kgBW) intravenously."

2.6. Induction with Viruses

Diabetes can be induced using viruses through the degradation and infection of β pancreas cells [7]. One common type of virus used in the induction of experimental animal diabetes is coxsackievirus B. Coxsackievirus B (CVB) is a single-stranded positive-sense RNA virus that belongs to the picornaviridae genus. Persistent CVB infection observed in vitro and in vivo is known to induce structural and functional changes in the pancreas. CVB infection can also lead to alterations in immune cells and the development of autoimmunity against β pancreas cells [32].

CVB infects β cell pancreas by binding to the coxsackie and adenovirus receptor (CAR), which is highly expressed on insulin-secreting β cells. Variations in CAR expression are associated with an increased incidence of type 1 diabetes mellitus [33].

Infection with CVB can be identified by examining enterovirus components, including IgM, IgG, IgA anti-enterovirus, CVB capsid protein, and RNA in serum, monocytes, and gastrointestinal mucosa. These components are generally detected more in type 1 diabetes patients compared to healthy individuals. Acute CVB infection in β pancreas cells can lead to changes in the golgi apparatus, decreased insulin secretion, increased expression of interferon-stimulated genes, and cell death. Persistent CVB infection can be classified into two types: stable phase infection and carrier phase infection. Stable phase infection is characterized by a significant proportion of infected cells without viral replication

cycle lysis. Meanwhile, in the carrier phase, only a small fraction of cells are infected, but there is a production of virus particles with high titers [32].

3. Conclusion

There are various methods to induce animal models to develop diabetes mellitus, such as chemical induction, spontaneous autoimmune induction, surgically induced induction, and viral induction. Each method has its own advantages and disadvantages in inducing hyperglycemia in various animal models. The selection of an animal model must be based on necessity.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest in this article.

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