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Diabetogenic Activity of Streptozotocin on Kunming Strain Mice as Animal Model of Diabetes Mellitus

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Abstract: *Diabetes mellitus (DM), a chronic metabolic disorder, is increasing tremendously around the world. Assessment of interventions designed to prevent disease progression in humans takes years. On the other hand, the use of plants as diabetic agent needs preclinical test to obtain scientific evidence. Therefore, appropriate experimental animal model of diabetes mellitus is essential tools for understanding the pathogenesis of diabetes mellitus and effectiveness of diabetes phytotherapy. Streptozotocin-induced diabetes is a well-documented model of experimental diabetes. The present study is aimed to investigate the diabetogenic activity of streptozotocin influenced by difference of doses, sex, administration route, duration induction and injection frequency on Kunming strain mice. Induction of STZ on Kunming mice were done according to experimental design and fasting blood glucose level measured using automatic glucometer, in which blood glucose more than 11.1mmol/L is considered as diabetes mellitus. These findings suggest that mild diabetes refers to type 2 diabetes mellitus when fasting blood glucose level is between 11.1-24.9mmol/L. It can be obtained by inducing male Kunming mice using STZ administrated either intravenously at dose of 100 mg/kg or intraperitoneally at dose of 180 mg/kg, in single injection for duration of 3 and 7 days, respectively.*

Keywords :*diabetogenic, diabetes mellitus, Kunming mouse, streptozotocin*

I. Introduction

Diabetes mellitus is one of the common metabolic disorders with micro-and macro vascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world [1]. Diabetes mellitus is classified into two types, insulin dependent diabetes mellitus (IDDM, Type 1) and non-insulin-dependent diabetes mellitus (NIDDM, Type 2). Type I diabetes is an autoimmune disease characterized by a local inflammatory reaction in and around islets that is followed by selective destruction of insulin-secreting beta cells [2].Whereas, type II diabetes is characterized by peripheral insulin resistance and impaired insulin secretion [3].

Generally, current therapeutic strategies for diabetes are limited and usually have adverse side effect, decreased efficacy over time, ineffectiveness against some long-term diabetic complications and low cost-effectiveness [4]. Therefore, discovery and development of novel drugs for diabetes is widely still opened. Natural products showed a good bright future in the therapy of diabetes and its related complications. It is estimated that 1200 species of plants are used as folk medicines for diabetes therapy [5], but needed scientific evidence for efficacy.

DM can be studied in animal models of the disease, although these animals do not present a complete picture of DM in humans. These experimental models are useful for biochemical or anatomical studies that target the effects of hyperglycemia on diabetic complications including neuropathy, nephropathy and cardiovascular diseases [6]. The common diabetic rodent models are genetically engineered, or created by changes in diet or use of chemical agents. The most used substances to induce diabetes in rodents are alloxan or streptozotocin (STZ) [7].

Streptozotocin (STZ; N-nitro derivative of glucosamine) is a naturally occurring, broad-spectrum antibiotic and cytotoxic chemical that is particularly toxic to the pancreatic, insulin-producing beta cells in mammals [8]. The diabetogenic effect of STZ was first reported in 1963 by Rakieten et al. after injection of a single intravenous dose in rats and dogs [9]. Induction of diabetes in the mice using streptozotocin is very convenient and simple to use [10]. Streptozotocin injection leads to the degeneration of the Langerhans islets beta cells [11]. Additionally, Streptozotocin induced not only on animal models of IDDM [9], but also NIDDM with hypoinsulinemia by STZ administration to neonatus (1 or 2 days old of mice or rats) [12].

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It has been reported that STZ is capable of producing mild to severe type of diabetes according to the dose used when it is given to animals by either single intravenously or intraperitoneally [10]. Reports also indicate that the nature of diabetes development varies with the doses, administration route, and animal species [13]. Symptoms of diabetes are clearly seen in rats within 2-4 days following single intravenous or intraperitoneal injection of 60mg/kg STZ [14]. Therefore, this present study is aimed to investigate characteristics of diabetes mellitus on Kunming strain mice influenced by various factors, namely doses, administration route, sex difference, duration of induction and frequency of injection.

II. Materials and Methods

2.1 Animal

Male and female Kunming (KM) mice (18-23 g) were obtained from the Laboratory Animal Center of Guangxi Medical University, China. The animals were housed in standard polypropylene cages (ten mice /cage) and maintained under controlled room temperature (22±2◦C) and humidity (55±5%) with 12:12 h light and dark cycle for 3 days before experiment. All the mice were provided normal pellet diet (NPD) containing 18.8% crude protein, 16.2% crude fat, 3.98% crude fiber, 5.2% crude ash, 1.24% calcium, 0.83% phosphorous and 45.2% nitrogen free extract, 1.38% lysine, 0.78% methionine and cysteine (served by Laboratory Animal Center, Guangxi Medical University, China) and water ad libitum. All procedures were carried out in accordance with the legislation on the use and care of laboratory animals.

2.2 Preparation of STZ

Streptozotocin (STZ) was purchased from Sigma Chemicals (St. Louis, MO, USA), containing 100 mg each vial and before used, it must be stored in -20°C. Streptozotocin was freshly dissolved in saline-1% tween 80 mixture in order to get concentration stoke of 10 mg/ml.

2.3 Induction of STZ

After 12 hours fasting, mice (male or female) were weighed using electrical balance (Clever), then induced by disposable syringe of 1 mL with maximal injected-volume was not more than 0.5 ml STZ dose, applied-administration route and duration of induction were based on experimental design. Five mice for each experiment was not injected by STZ and used only as health control group.

2.4 Experimental Design

This experiment was designed to investigate diabetogenic activity of STZ affected by different factors such as sex (male vs female mice), administration route (intravenously vs intraperitoneally), dose of STZ (120, 150, 180, 200 mg/kg body weight), duration of induction (3 vs 7 days) and frequency of injection (single vs double). Symptoms accompanying diabetes mellitus such as polydipsia, polyuria, and loss of body weight were observed every day. STZ-induced mice are considered as diabetes mellitus when fasting blood glucose level above 11.1mmol/L. Diabetes severity grade is classified into 3 types; severe, moderate and mild diabetes, based on fasting blood glucose level.

2.5 Determination of Blood Glucose Level

Before determination of blood glucose, mice were fasted overnight and weighed. Fasting blood glucose levels were determined using automatic glucometer (Accu-check Performa, Roche, Germany) from tail vein and noted as mmol/L. Furthermore, diabetic mice were classified into severe (> 33.3 mmol/L), moderate (24.9-33.3 mmol/L) and mild (11.1-24.9 mmol/L) diabetes mellitus.

2.6 Statistical Analysis

Results were expressed as percentage of diabetogenic activity formulated with equation = ($\Sigma R/\Sigma P$)x100, R= number of mice responding STZ diabetogenic activity; P= number of mice population. A qualitative analyses were carried out to determine diabetogenic activity of streptozotocin due to variation in doses, administration route, sex difference, induction duration, and frequency of injection. Semi-quantitative analyses were also carried out to characterize the symptoms of diabetes mellitus reflected with body weight change and water intake.

III. Results

3.1 Diabetogenic Activity of STZ Affected by Dose Difference

Dose differences-influenced diabetogenic activity showed that diabetogenic response was increased by dose-dependent manner. At a dose of 120 mg STZ /kg, diabetogenic activity causes mild diabetic mice as many as 7.5%, and percentage of response was increased up to 20% at dose of 150 mg /kg. While the induction of STZ at dose of 180 mg /kg, in addition to cause mild diabetic mice by 30%, was also found to induce moderate

diabetic mice by 5% (Table 1). And at dose of 200 mg STZ /kg, percentage of moderate diabetic mice was increased up to 53.6% and obtained died mice by 1.8%. Death of mice at dose of 200 mg /kg is possibly because of the toxic effects of STZ.

Table 1. Profile of STZ Diabetogenic Activity Affected by Dose Difference (Intraperitoneally)

Group	Number of mice with			
	Mild diabetes (11.1-24.9 mmol/L)	Moderate diabetes (24.9-33.3 mmol/L)	Severe diabetes (>33.3 mmol/L)	Death
120 mg/kg STZ	7.5%	0%	0%	0%
150 mg/kg STZ	20%	0%	0%	0%
180 mg/kg STZ	30%	5%	0%	0%
200 mg/kg STZ	23.2%	53.6%	0%	1.8%

One of the diabetes mellitus symptoms is polyuria, which is characterized by loss of body weight. Changes in body weight of mice affected by STZ dose difference during 7 days showed that body weight was decreased at dose of 180 mg /kg when compared to initial body weight (Fig. 1). Whereas at dose of 120 and 150 mg /kg, body weight were clear increased. Polydipsia is an increasing water intake as displaying excessive thirst, which is also a sign of the diabetes mellitus symptoms. In figure 2, all of treatment groups showed an increase in water intake on 1rd, 2rd and 3rd day. The highest water intake occurred at a dose of 200 mg /kg. It means that induction of STZ from 120 to 200 mg/kg on Kunming mice exactly can cause polydipsia signed by increasing in water intake.

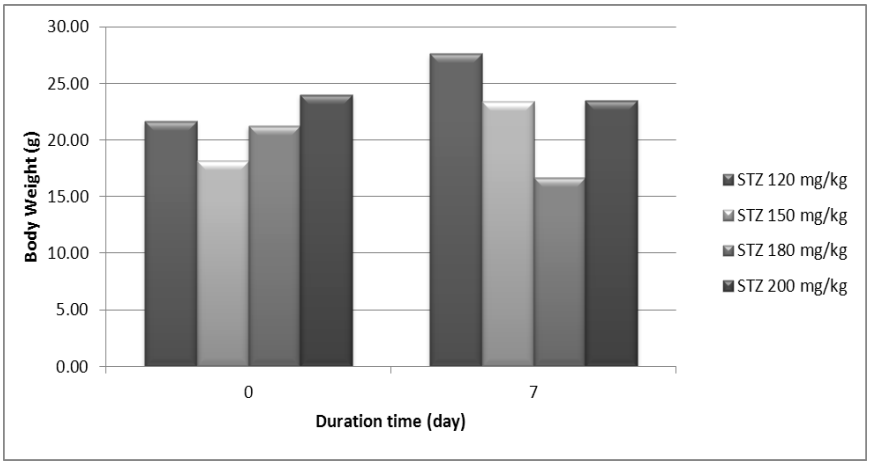

Figure 1. body weight change affected by dose difference

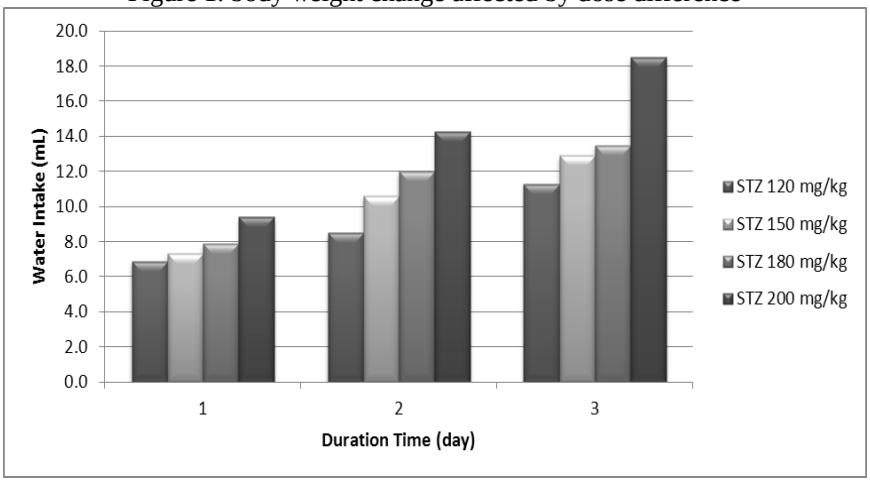

Figure 2. water intake affected by dose difference

3.2 Diabetogenic Activity of STZ Affected by Administration Route

Administration route-influenced diabetogenic activity was shown in Table 2. Intravenous injection at a dose of 150 mg/kg caused mild and moderate diabetic mice as many as 33.3% and 61.1%, respectively. Whereas the route of administration intraperitoneally caused the number of mild and moderate diabetic mice which were

smaller than intravenously, by 12.5% and 2.5%, respectively. Intravenous injection allowed STZ directly into the blood circulatory system without going through the stages of absorption as in the intraperitoneal administration. Absorption causes decrease in STZ concentration that affects the bioavailability in bloodstream.

Table 2. Profile of STZ Diabetogenic Activity Affected by Administration Route (150 mg/kg STZ)

Group	Number of mice with		
	Mild diabetes (11.1-24.9 mmol/L)	Moderate diabetes (24.9-33.3 mmol/L)	Severe diabetes (>33.3 mmol/L)
Intravenously	33.3%	61.1%	0%
Intraperitoneally	12.5%	2.5%	0%

3.3 Diabetogenic Activity of STZ Affected by Sex Difference

Sex differences allow distinction of STZ diabetogenic activity. As shown in Table 3, the induction of STZ at a dose of 100 mg /kg intravenously in male mice caused mild and moderate diabetes as many as 56.3% and 18.8%, respectively, whereas in female mice caused only mild diabetic as many as 16.7%. This was considered probably by hormonal differences between male and female mice. One of the dominant hormones in female mice affecting many metabolism systems is estrogen.

Table 3. Profile of STZ Diabetogenic Activity Affected by Sex Difference (100 mg/kg STZ, Intravenously)

Group	Number of mice with		
	Mild diabetes (11.1-24.9 mmol/L)	Moderate diabetes (24.9-33.3 mmol/L)	Severe diabetes (>33.3 mmol/L)
Male mice	56.3%	18.8%	0%
Female mice	16.7%	0%	0%

3.4 Diabetogenic Activity of STZ Affected by Difference of Induction Duration

As shown in Table 4, diabetogenic activity of STZ was influenced by duration of induction. Kunming mice injected intraperitoneally by STZ at dose of 150 mg/kg for 7 days showed the percentage of mild diabetes mice was increased up to 20% when compared to induction for 3 days (12.5%). Accumulation of STZ in pancreatic β-cells via GLUT 2 transporter uptake depends on duration time for induction. Prolonged-induction duration causes increasing of diabetes response.

Table 4. Profile of STZ Diabetogenic Activity Affected by Difference of Induction Duration (150 mg/kg STZ, Intraperitoneally)

Group	Number of mice with		
	Mild diabetes (11.1-24.9 mmol/L)	Moderate diabetes (24.9-33.3 mmol/L)	Severe diabetes (>33.3 mmol/L)
Duration of 3 days	12.5%	2.5%	0%
Duration of 7 days	20%	0%	0%

3.5 Diabetogenic Activity of STZ Affected by Difference of Injection Frequency

Frequency of injection also affects number of diabetes in Kunming mice as shown in Table 5. After single intraperitoneal injection at dose of 180 mg/kg for 3 days induction, there were 30% of mice considered as mild diabetes, whereas number of moderate diabetes was 5%. For double injection, there was increase in number of mild and moderate diabetes up to 31.8% and 50%, respectively. Additionally, double injection caused death in mice by 4.6%.

Table 5. Profile of STZ Diabetogenic Activity Affected by Frequency of Injection (180 mg/kg STZ, Intraperitoneally)

Group	Number of mice with			
	Mild diabetes (11.1-24.9 mmol/L)	Moderate diabetes (24.9-33.3 mmol/L)	Severe diabetes (>33.3 mmol/L)	Death
Single injection	30%	5%	0%	0%
Double injection	31.8%	50%	4.6%	4.6%

IV. Discussion

The use of plants as diabetic agent needs previously preclinical test to obtain evidences of scientific effectiveness, but assessment of interventions designed in humans to prevent progression of this disease takes years. So, experimental induction of diabetes mellitus in animal models is essential for the advancement of knowledge and understanding of the various aspects of its pathogenesis and ultimately finding new therapies and cure [15].

Several methods have been used to induce diabetes mellitus in laboratory animals, such as pharmacological, surgical and genetic manipulation [16], with variable success and many difficulties. Surgical removal of the pancreas is effective method; however, to induce diabetes, at least 90-95% of the pancreas has to be removed [17]. Injection of anterior hypophysis extract has been used to induce diabetes with less reliable results [18], whereas genetic models of diabetes are high costs [17]. Another method which is more uniformly effective and widely used is pharmacological method by injection of streptozotocin (STZ). In comparison to alloxan as diabetogenic agent, STZ is more frequently used as many as 69% when alloxan is only 31% [16].

STZ (2-deoxy-2-(3-methyl-3-nitrosourea)-1-D-glucopyranose) is a broad-spectrum antibiotic which is produced by Streptomyces achromogenes [19]. Streptozotocin is a nitrosourea analogue in which the N-methyl-N-nitrosourea (MNU) moiety (Fig. 3) is linked to the carbon-2 of a hexose that is accumulated preferentially in pancreatic β-cells via GLUT 2 transporter uptake in the plasma membrane [20]. STZ taken up by pancreatic β-cells via the GLUT 2 transporter causes β-cell death by DNA fragmentation due to the nitrosourea moiety [21], especially at the O^6 position of guanine [22]. The transfer of the methyl group from streptozotocin to the DNA molecule causes damage, which along a defined chain of events. Protein glycosylation may be an additional damaging factor [23] (Konrad and Kudlow, 2002).

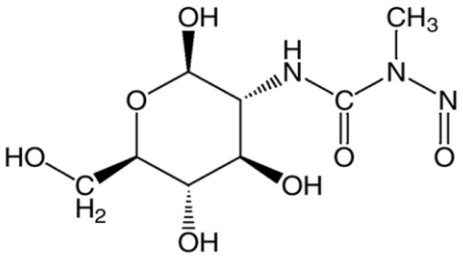

Figure 3. chemical structure of streptozotocin [19].

The severity of damage is dependent of different factors including the dose, number of STZ administration, animal species, strain and age [6]. Reports also indicate that the nature of diabetes development varies with administration route [13]. Our study indicated that severity of diabetes was affected by dose, sex, administration route, injection frequency and duration time of induction. Additionally, species or strain rodents maybe have contribution for development of diabetes. Hayashi et al (2006) has reported that only in ICR and ddY mice, and not BALB/c and C57BL/6 mice, was slowly progressive NIDDM induced by a single i.p. injection of a sub-diabetogenic low dose of STZ (100 and 125mg/kg), respectively [8]. Abeeleh et al. (2009) has also reported a similar finding that average of blood glucose in SD rats is higher than in Nude rat [15]. In comparison, Kunming mice induced by STZ at dose of 150 mg/kg, i.p, single injection for 3 days showed average of fasting blood glucose up to 14 mmol/L, more high than another strain or species such as BALB/c mice, Swiss mice and Nude rat. Genetic factors are a main reason considered as cause of difference in diabetogenic activity response.

In summary, induction of STZ on Kunming male can be administrated either intravenously or intraperitoneally. In addition to administration route, another variable such as STZ doses, sex, duration time of induction and injection frequency must be adapted in order to obtain mild diabetes referring to type 2 diabetic models (See Table 6).

Table 6. Optimized-Induction Matrix for Type 2 Diabetic Kunming Mouse Model

Administration route	STZ doses	Animal sex	Duration of induction	Injection frequency
Intravenously	100 mg/kg	male	3 days	Single
Intraperitoneally	180 mg/kg	male	7 days	Single

V. Conclusion

STZ is the common diabetogenic agent that can induce type 2 diabetic experimental model on male Kunming (KM) mice when administrated either intravenously at dose of 100 mg/kg or intraperitoneally at dose of 180 mg/kg, in single injection for duration of 3 and 7 days, respectively. Additionally, mild diabetes refers to type 2 diabetes when fasting blood glucose level is between 11.1-24.9 mmol/L.

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