

A MODEL STREPTOZOTOCIN MICE FOR STUDYING TYPE 2 DIABETES

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ABSTRACT

Animal models are always necessary in pathological research, especially for type 2 diabetes (T2D). It is a chronic metabolic disorder with a high rate of increasing and involves many physiological systems. This study aims to provide a chemically induced type 2 diabetes mouse model by administering streptozotocin (STZ). Adult Swiss Albino mice were divided into three main groups to inject different dosages of STZ, specifically low dose, high dose 1, and high dose 2. As a result, mice in the high-dose group with a total of 160 mg/kg STZ for two injections were considered to obtain type 2 diabetes. After 4 months of experiment, the mice did not lose weight and acted normally. Characteristic metabolic alterations in type 2 diabetes contained an average fasting blood sugar level of over 150 mg/dL, fatty liver, damaged kidneys, and HbA1c levels of over 6.5%. It can be said that this research is the initial basic step to developing an effective type 2 diabetes mouse model in the future.

Key words: Type 2 diabetes, STZ, chemically induced diabetes mouse model.

1. INTRODUCTION

Diabetes mellitus, also called diabetes, is characterized by a relative lack of insulin. The reasons are that the body cannot produce enough of the hormone insulin or cannot effectively use the insulin it produces [1]. Notably, insulin is a hormone that regulates blood glucose, so insulin deficiency causes the body to increase blood sugar levels, called hyperglycemia. Importantly, this chronic disease can lead to a variety of complications, such as neuropathy, nephropathy, and retinopathy, as well as an increased risk of cardiovascular disease [1-4]. According to the report of the IDF Diabetes Atlas (2021), 10.5% of the adult population (20-79 years) has diabetes, which will increase by 12.2% in 2045 (approximately 783 million, of which Southeast Asia accounts for 152 million). Total health expenditures due to diabetes are estimated at 966 billion in 2021 and 1054 billion in 2045. Besides, diabetes has also been related to an increased risk of cancers, Alzheimer's disease, or harm to bacterial, fungal, and viral infections [4]. It can be said that diabetes is the disease with a high mortality rate worldwide in the 21st century.

There are two main types of diabetes mellitus: type 1 (T1DM) and type 2 (T2DM), with differences in characteristics and prevalence. T1DM has a proportion of about 9.5% of all diabetes and is caused by an autoimmune disorder. It is characterized by deficient insulin production in which the insulin-producing pancreatic cells are destroyed. So, the body requires daily administration of insulin. Meanwhile, type 2 diabetes is the most common type of diabetes, accounting for over 90% of all diabetes worldwide, and is recognized by insulin resistance (IR) and β pancreatic-cell failure. The basis of type 2 diabetes management is initially encouraging lifestyle therapy, including a healthy diet, regular exercise, and maintenance of healthy body weight. After that, drug treatment will be prescribed if blood sugar levels remain high [2, 3].

However, the prevalence of type 2 diabetes is high and rising, confirming diabetes as a significant global challenge. Therefore, continuously researching T2DM is crucial to give effective treatments. Research with animal models for preclinical studies is fundamental, especially rodents, whose physiology is close to that of humans [3]. The main T2DM animal models include chemical, genetic/ spontaneous, surgical, and diet/nutrition models [2]. Among them, the chemical-induced

model of T2DM has advantages such as being inexpensive, simple, and easy to handle. Streptozotocin (STZ) is the most commonly used chemical substance, a highly selective pancreatic islet β -cell-cytotoxic agent [2, 5]. According to documents [2-6], the use of STZ to create a T2DM diabetic mouse model can also be combined with others, such as nicotinamide or high-fat diets (HFD) that accurately reflect T2DM, characterized by insulin resistance and progressive β cell dysfunctions. However, the dosage of high-fat feed, the duration of treatment, and the amount of additives affecting the effective control of the model are still controversial.

This paper aims to design a T2DM model for Swiss Albino mice by adjusting appropriately the STZ injection dosage. If the STZ injection dose is so high (≥ 200 mg/kg), it can destroy the pancreas, causing type 1 diabetes [3]. Therefore, the study focuses on injecting doses of STZ repeatedly, just enough to damage the pancreas, causing type 2 diabetic mice. The weight, blood sugar, HbA1c measurement, and H&E examination of the livers and kidneys of the mice were used to evaluate the model efficiency level.

2. MATERIALS, ANIMALS AND METHODS

2.1. Materials and Animals

2.1.1. Materials

Streptozotocin (STZ) was bought from Aladdin (China). Citrate buffer (pH 4.5) and distilled water were provided by the lab of Ho Chi Minh City University of Industry and Trade (Viet Nam).

2.1.2. Animals

The male Swiss Albino mice (20–25 g) aged 4–5 weeks were obtained from the Pasteur Institute in Ho Chi Minh City.

2.2. Methods

2.2.1. Experimental Model

After 2 weeks of stabilizing the living environment and diet, the mice were injected with the first STZ dose and the second dosage 1 month later. Due to the limited conditions, the experiment was carried out in 2 phases as follows:

Phase 1:

Eight male mice were divided into two groups, containing the control group (NC) and the low-dose group (LD).

Phase 2:

Twelve male mice were classified into three groups with the same numbers, including the control group (NC), the high-dose group 1 (HD1), and the high-dose group 2 (HD2).

The NC mice were only injected with the citrate buffer solution (pH 4.5) to mimic the STZ injections. The LC mice were designed with the first STZ dose of 40 mg/kg and the next 60 mg/kg. Two groups of high-dose mice had the same amount of 160 mg/kg STZ drug, but the doses of the two injections were reversed. Specifically, the animals of the HD1 were initially injected with a dose of 100 mg/kg and then 60 mg/kg, while the HD2 mice were administered with the opposite dose, respectively.

2.2.2. Evaluation Parameters

The weight of the mice was checked weekly. The mice blood samples of all experimental groups were collected from the tail vein for glucose measurement by an On-Call Ez II glucometer of ACON. HbA1c levels were determined by high-performance liquid chromatography (HPLC). The hematoxylin-eosin (HE) staining test for histopathological examination of liver and kidney organs of mice was performed at the University of Medicine and Pharmacy, Ho Chi Minh City.

2.2.3. Data and statistics

This article includes all data generated or analyzed during the study. Statistics and graphical presentation of data were carried out using Excel software, and results were expressed as the mean $\pm$ SD.

3. RESULTS AND DISCUSSION

3.1. Weight change of mice after STZ injection

The common point is the mice behaved normally during the experiment and gained weight quickly in the first month, followed by small, steady increases. The weight gain rate of STZ injection mice is the same as that of the control mice in Figs. 1 and 2. The little difference is that the weight gain rate of the high-dose group was 20% in the first month, higher than the average of 14% of the low-dose group. It did not confirm the effect of STZ on the body weight of mice yet. The reason may be the initial weight of the mice in the high-dose group was lighter than that of the low-dose group. Note that the sign of type 1 diabetic mice is often weight loss [7].

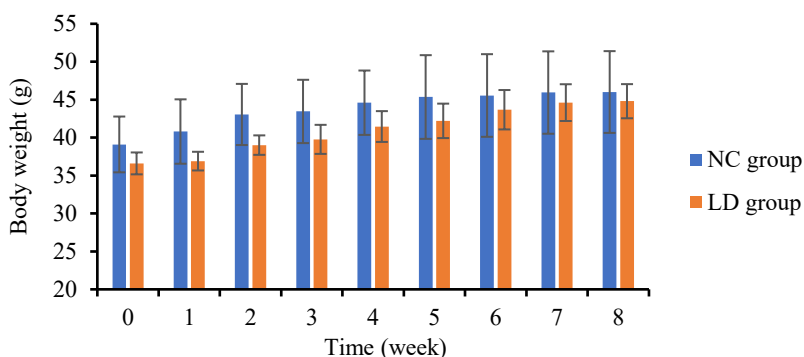


Fig. 1. Body weight of mice in time after injecting the STZ low dose [8]

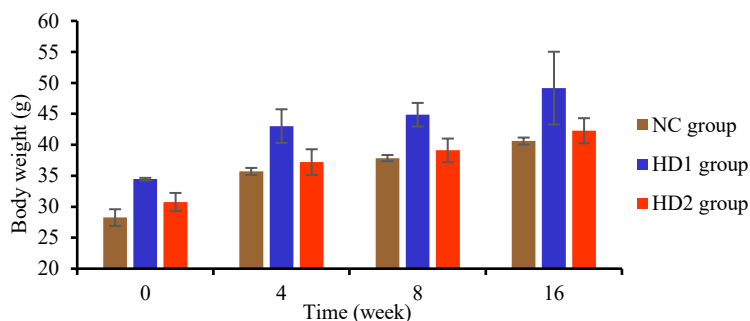


Fig. 2. Body weight of mice in time after injecting the STZ high doses [8]

The results seem contradictory compared with the study in the document [9], although the Swiss mice and the injection dose of STZ 40 or 60 mg/kg were the same. That means the mice lost weight after the injection. This is due to different research methods. According to [9], the mice were fed lipid-rich food before injection and were continuously injected 4 times after the first dose. The result of the mice losing weight was as much as the phenomenon of type 1 diabetic mice. This study showed that two injection doses of STZ to mice, with a distance of 1 month, resulted in mice losing almost no weight.

Briefly, the STZ injection protocol in this study was appropriate for the T2D mice model. The blood sugar level results of the mice in the next section also demonstrate this protocol.

3.2. Blood glucose levels

Fasting plasma glucose was assessed in all animals monthly. For the LD group, the glucose concentration increased gradually to 101.4 mg/dL, 112.8 mg/dL, and 129.5 mg/dL after the 1st, 2nd, and 4th months respectively, in Fig. 3. These values were approximately 45 mg/dL higher than in the control mice. Although the average blood glucose level after 4 months was up to 129.5 mg/dL, it was still not convincing enough to confirm that the mice were diabetic because this value just exceeded the diagnostic threshold slightly (>126 mg/dL). This blood glucose threshold was securely considered impaired fasting glucose (110-125 mg/dL), an approach to signs of type 2 diabetes [1].

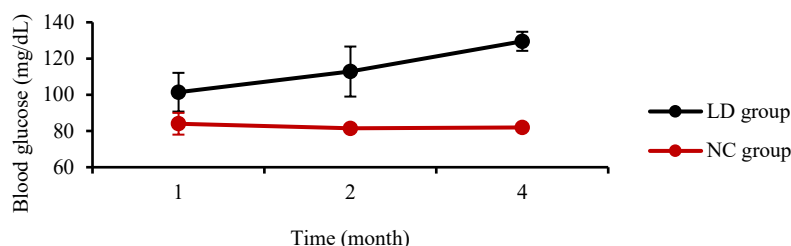


Fig. 3. Time course changes in the blood glucose level of NC and LD group [8]

Fig. 4 shows the blood sugar levels of the group of mice injected with high doses of STZ drug. Generally, the curves had the same shape, increasing rapidly from 1st to 2nd month and stabilizing, lightly decreasing in the following months. Specifically, the glucose concentration of the HD1 group had an average of 140 mg/dL after the first month, increased to 270 mg/dL in the second month, and decreased slightly to 262.5 mg/dL at 4 months. Meanwhile, the HD2 group had plasma glucose of 93.3 mg/dL, 164.3 mg/dL, and 156 mg/dL in order 1st, 2nd, and 4th months respectively. With this blood sugar threshold higher than 87 mg/dL in control mice, it was predicted that the mice would have diabetes.

Regarding the study result, the highest-value glucose of HD1 can be affected by the first high dose of 100 mg/kg injection. It could damage the number of β cells in the pancreas, leading to an enhancement of the effectiveness of the second injection (60 mg/kg) that strongly impacts the health of the mice. Besides, at the fourth month, the glucose content had a trend of decreasing as the reason of long-term studies confounded by the ability of the remaining beta cells to compensate/regenerate [6].

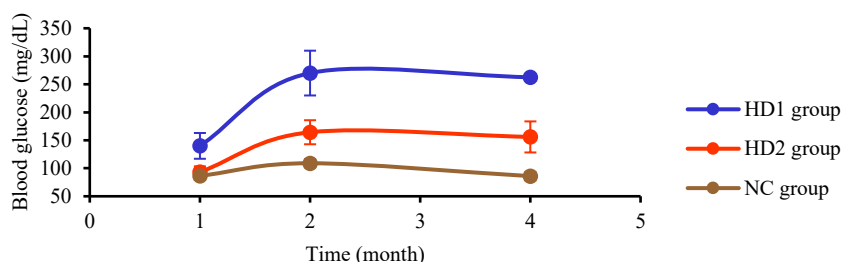


Fig. 4. Time course changes in the blood glucose level of NC and HD group [8]

3.3. Histological analysis and HbA1c level

The HE results in Fig. 5a and 5b show that the liver and kidney tissues of the LD group exhibited no signs of damage. They were completely normal.

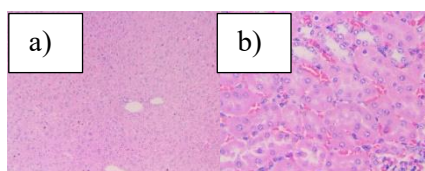


Fig. 5. Photomicrographs of HE-stained sections of the LD group: a) liver and b) kidney [8]

This is also consistent with the low HbA1c value of 3.4% in Table 1. Note that an HbA1c value of more than 6.5% is considered a diagnostic threshold for diabetes [1]. Although blood glucose levels increased near the diabetic threshold, other clinical signs clearly showed that the LD mice group did not have the potential to develop diabetes. In contrast with the LD group, analysis of the liver tissues of high-dose group mice in Fig. 6a and 6d showed fatty liver. Kidney tissues were damaged, including lymphocytic, plasma cell infiltration, and renal thrombosis in Fig. 6b and 6e. Only the heart part did not reflect any injury in Fig. 6c, 6f. All of those indicated a sign of diabetes, a type 2 diabetes, which can cause slight degeneration of various mice's organs [10, 11]. In addition, the HbA1c values in Table 1 for the high-dose group were 8.6% and 6.7% for HD1 and HD2, respectively.

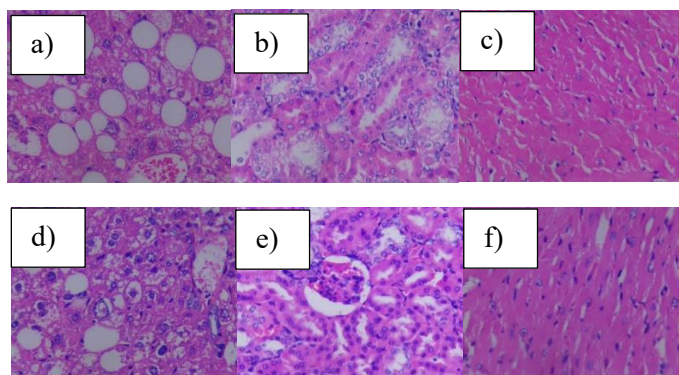


Fig. 6. Photomicrographs of HE-stained liver, kidney, and heart sections of the high-dose STZ group: a,b,c present of the HD1 group; d,e,f illustrate the HD2 group respectively [8]

Table 1. The HbA1c value of mice

Group	LD	HD1	HD2
HbA1c (%)	3.4	8.6	6.7

According to the literature [1], diagnosing “prediabetes” is recommended with HbA1c values between 5.7–6.4% and fasting plasma glucose within 100–125 mg/dL. Thus, it is clear that among the three research models, the HD1 group showed the most convincing results. Both the HbA1c and fasting blood sugar were much higher than the warning threshold. Meanwhile, the parameters of the HD2 group had just exceeded the threshold of prediabetes, and those of the LD group were below the threshold.

4. CONCLUSION

The model of repeated injection of mice with different doses of STZ created a model of type 2 diabetes mice. With a total dose of STZ of 160 mg/kg for two injections, the mice showed signs of type 2 diabetes, such as no weight loss, high blood sugar over 150 mg/dL, fatty liver and kidney damage, and high-value HbA1c (> 6.5%). However, the HD1 model firmly exhibited type 2 diabetes more than the HD2 model, whose measured values were all initial thresholds for diabetes. The outstanding feature of this study is not high-cost and easy to control, in contrast to genetically induced T2D animal models, which are greatly appreciated but technically difficult and costly. If this research is expanded, the model may become more feasible, creating conditions for effective diabetes research. In the future, the HD1 model is proposed for studying numerous mice to show statistical validity and check the influence of mouse age on the results.

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Additional information

Author contributions

Hoang Thanh Long, Le Thi Ngoc Tuyen: Assisted in conducting experiments and data analysis; Nguyen Thi Thanh Hien: Conceptualized the methodology, performed experiments, managed data, supervised the study, and contributed to drafting, revising, and finalizing the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest and no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI. No AI tools were used in the preparation of this manuscript.

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