Study on optimization and stability of a single dose streptozotocin-induced diabetic modeling in rats

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Abstract

Background

Optimization of experimental conditions in streptozotocin induced diabetic model in Sprague Dawley (SD) rats to evaluate the stability of the model.

Methods

Male and female SD rats were randomly divided into control group, STZ 45 group (STZ: 45 mg / kg), STZ 65 group (STZ: 65 mg / kg), STZ 85 group (STZ: 85 mg / kg), high fat diet with STZ 45 group (STZ: 45 mg / kg), high fat diet with STZ 65 group (STZ: 65 mg / kg), high fat diet with STZ 85 group (STZ: 85 mg / kg). N = 6 in each group. The changes of body weight and blood glucose were observed dynamically.

Results

There was no significant difference in blood glucose or body weight between the STZ 45 group and the control group in both male and female rats, whether or not they were on a high-fat diet. However, there were significant differences in blood glucose between the high-dose STZ group and the control group in both male and female rats, regardless of whether the rats were on a high-fat diet or not ($P < 0.05$ or $P < 0.01$). Compared with the control group, there were significant differences in blood glucose levels ($P < 0.05$ or $P < 0.01$) and higher blood glucose levels in the male rats fed with the normal diet than that in those fed with the high-fat diet.

Conclusions

In this study, male rats fed with ordinary feed and injected STZ dose of 65 mg / kg were the most stable and ideal diabetic rat.

1. Introduction

Diabetes is a chronic metabolic disease characterized by a relative or absolute lack of insulin, leading to hyperglycemia. Chronic hyperglycemia, which causes multiple complications such as neuropathy, kidney disease and retinopathy, as well as an increased risk of cardiovascular disease, is currently one of the most important non-communicable diseases threatening global human health[1].

Diabetes is rising globally. According to the ninth edition of the Global Diabetes Map released by the International Diabetes Federation (IDF) in 2019, 9.3 percent of adults currently live with diabetes, and by 2045, nearly 700 million will be living with the disease. The prevalence of diabetes, diabetes-related deaths and medical costs place a huge burden on society, finance and the health care system[2]. To study the pathogenesis of diabetes and the selection of therapeutic drugs, it is necessary to establish an ideal animal model of diabetes.

Currently, there are many methods to establish animal models of diabetes, which are mainly divided into animal models of spontaneous diabetes such as Zucker Diabetic Fatty (ZDF) Rats, Biobreeding (BB) rats and Goto-Kakizaki (GK) rat, and animal models of diabetes through chemical induction such as alloxan and streptozotocin (STZ). ZDF rats were found in 1961 after crossbreeding between Merck (M-strain) and Sherman rats. They are characterized by a mutation in the leptin receptor that causes enormous appetite and the rats become obese by 4 weeks of age. These rats also had hyperinsulinemia, hyperlipidemia and hypertension, and showed impaired glucose tolerance[3]. BB rats is derived from a distant relative, Wistar rat. After puberty, BB rats developed diabetes, with rates similar between males and females, and about 90% of the rats developed diabetes between 8 and 16 weeks of age. The diabetes phenotype is quite severe and is characterized by the development of hyperglycemia, weight loss from hypoinsulinemia, and ketouria, which requires insulin therapy to survive[4]. GK rats developed mild hyperglycemia early in life, considered a non-obese model, and all adult animals of both sexes showed type 2 diabetes[5]. Alloxan and STZ are considered the most effective diabetes-promoting drugs used in diabetes research, and both are cytotoxic glucose analogues[6]. Although their cytotoxicity is achieved by different pathways, their selective action on β cells is the same, both causing insulin deficiency, where STZ is targeted β -cell apoptosis with chemical that induces DNA alkylation and alloxan is targeted β -cell destruction with chemical that induces oxidative stress[7–9]. Compared with alloxan, STZ is more stable and is the best choice for repeatedly inducing metabolic state of diabetes in experimental animals[10]. However, the physicochemical properties and related toxicity of STZ remain major obstacles for researchers who use STZ to treat diabetes in animals.

Another major challenge for STZ-induced diabetes models is how to maintain the suitability, repeatability, and inductivity of diabetes with minimal animal mortality. Lack of appropriate use of STZ was found to be associated with increased mortality and animal suffering[11]. Therefore, when using STZ in animals, attention should be paid to several factors such as preparation method, stability, appropriate dose, diet plan, animal species (related to age, weight and gender) and target blood glucose level representing hyperglycemia.

In this study, the effects of SD rats' gender, STZ injection dose and dietary conditions on body weight, blood glucose, modeling rate and mortality were compared.

2. Materials And Methods

2.1 Materials

Chow diet and high-fat diet (45% fat supply ratio) were both purchased from Guangzhou University of Traditional Chinese Medicine (University Town) Animal Center. Accu-Chek Active glucometer was purchased from Roche Diagnostic Corporation, Mannheim (German); streptozotocin (STZ, Sigma)

2.2 Animals

Adult male and female Sprague Dawley (body weight: 200 ± 20 g) rats were purchased from the Experimental Animal Center of Guangzhou University of Chinese Medicine (Certificate: SCXK20180002). The animals were housed in a specific pathogen-free (SPF) animal laboratory of the Experimental Animal Center of Guangzhou University of Chinese Medicine. All experimental procedures were performed according to the ethical principle guidelines approved by the National Research Council. The animals were maintained at 12 h of light and dark cycles and at a room temperature maintained within the range of 24–26 °C, and relative humidity from 50–70%. Food and water were given ad libitum.

2.2 HFD + STZ protocols

After acclimation period, male and female rats were fed a standard chow ad-libitum diet ($n = 48$) or high-fat diet (HFD) ($n = 48$) for 4 weeks. Each group was randomly divided into four subgroups ($n = 6$ each). After 12 h fasting, rats were undergone intra-peritoneal injection of three single doses of STZ (45, 65, and 85 mg / kg), while the respective control rats received the vehicle citrate buffer. The nomination of groups in this study is as follows: 1, Control ♂ (male + ad-libitum food); 2, STZ 45 ♂ (male + ad-libitum food + STZ 45 mg / kg); 3, STZ 65 ♂ (male + ad-libitum food + STZ 65 mg / kg); 4, STZ 85 ♂ (male + ad-libitum food + STZ 85 mg / kg); 5, HFD + STZ 45 ♂ (male + high-fat diet + STZ 45 mg / kg); 6, HFD + STZ 65 ♂ (male + high-fat diet + STZ 65 mg / kg); 7, HFD + STZ 85 ♂ (male + high-fat diet + STZ 85 mg / kg); 8, Control ♀ (female + ad-libitum food); 9, STZ 45 ♀ (female + ad-libitum food + STZ 45 mg / kg); 10, STZ 65 ♀ (female + ad-libitum food + STZ 65 mg / kg); 11, STZ 85 ♀ (female + ad-libitum food + STZ 85 mg / kg); 12, HFD + STZ 45 ♀ (female + high-fat diet + STZ 45 mg / kg); 13, HFD + STZ 65 ♀ (female + high-fat diet + STZ 65 mg / kg); 14, HFD + STZ 85 ♀ (female + high-fat diet + STZ 85 mg / kg).

2.3 Rat weights and fasting blood glucose (FBG)

Rat weights were measured on the same day and time of each week. At the end of acclimation week, rats fasted for 12 h, and a tip of the tail was snipped with sharp scissors and gently squeezed for a drop of blood. Then plasma glucose concentrations were assessed by glucometer (Accu-Chek Active; Roche Diagnostic Corporation, Mannheim, German). FBG was determined every week on the same day and time during the experiment. Glucometer was calibrated with the help of calibrators provided by the manufacturer.

2.4 Statistical analysis

Statistical software SPSS 20.0 and GraphPad 5.0 were used to conduct statistical processing on all experimental data, and the mean values $\pm$ standard error of the mean ($\bar{x} \pm \text{SEM}$) was used to represent the experimental data. ANOVA statistical method was used to test the significant difference between the two groups, and $P < 0.05$ was used to indicate the significant difference between the two groups.

3. Results

3.1 Effect of STZ on weight and serum glucose in male rats

Typical feature of diabetes is loss weight and a persistent increase in blood sugar. Fasting blood glucose and body weight of rats were measured on day 0, day 7, day 14, day 21, and day 28 after animal modeling. Compared with the control group, the male rats injected with STZ dose of 65 mg/kg intraperitoneally lost weight on the 7th, 14th and 28th day, which the modeling rate were 1 / 6 (dead rats / group) and no rats died. While lost weight significantly on the 7th, 14th, 21th, 28th day with STZ dose of 85 mg/kg of male rats, which the death rate and incidence of diabetes in rats were 1 / 6 and 5 / 6 (dead rats / group) respectively. The blood glucose of male rats treated with a dose of 65 mg / kg or 85 mg / kg were all greater than 8.3 mmol / L at five time points. In addition, blood glucose and body weight of male rats treated with a dose of 45 mg / kg was no significant difference compared with control group, which the mortality were 2/6 and all the rats were modeled (Fig. 1, Table 1, Table 2).

3.2 Effect of STZ on weight and serum glucose in female rats

Compared with the control group, the female rats injected with STZ dose of 65 mg / kg and 85 mg / kg intraperitoneally lost weight on the 7th, 14th, 21th and 28th day. The serum glucose of female rats in STZ 85 group were higher greatly than that in control group at five time points, and they had a mortality rate of 3 / 6 and 6 / 6 modelling rate. Compared with the control group, the female rats injected with STZ dose of 65 mg / kg have an increased serum glucose on the 3th, 21th and 28th day, which have a incidence of diabetes of 5 / 6 and no mortality. Besides, blood glucose and body weight of male rats treated with a dose of 45 mg / kg was no significant difference compared with control group, and none of them are modeled or dead (Fig. 2, Table 1, Table 2).

3.3 Effect of STZ on weight and serum glucose in male rats using high fat diet

Compared with the control group, the weight of HFD + STZ 45 group, HFD + STZ 65 group and HFD + STZ 85 group showed no significant difference at 5 time points, and the weight change was relatively stable. In terms of blood sugar, compared with the control group, the male rats injected with STZ dose of 65 mg / kg and 85 mg / kg have an increased serum glucose on the 3th, 7th, 14th, 21th and 28th day. What's more, the rats in HFD + STZ 45 group did not die and were not modeled, while the modeling rate was 5 / 6 and the mortality rate was 2 / 6 in HFD + STZ 65 group. And the mortality rate for HFD + STZ 85 group also was 2 / 6, which are all developed into diabetes (Fig. 3, Table 1, Table 2).

3.4 Effect of STZ on weight and serum glucose in female rats using high fat diet

Compared with the control group, on day 7, 14, 21 and 28, the HFD + STZ 45 group and HFD + STZ 65 group decreased their weight and showed significant differences. Furthermore, compared with the control group, on day 7, 14, 21, and 28, the HFD + STZ 45 group showed a slight increase in blood glucose, but no significant difference, which only one of the six died and one modeled. However, on day 3, 7, 14, 21, and 28, the HFD + STZ 65 group was greater than 8.3 mmol / L, and there was a significant difference compared with the control group, which the modeling rate was 5 / 6 and the mortality rate was 2 / 6. After an intraperitoneal injection of 85 mg / kg of STZ and given high-fat diet, all female SD rats were modeled but died within seven days (Fig. 4, Table 1, Table 2).

Table 1. Weight (g) of male and female SD rats

	male							female						
	Control (n = 6)	STZ 45 (n = 6)	STZ 65 (n = 5)	STZ 85 (n = 4)	HFD + STZ 45 (n = 6)	HFD + STZ 65 (n = 4)	HFD + STZ 85 (n = 3)	Control (n = 6)	STZ 45 (n = 6)	STZ 65 (n = 6)	STZ 85 (n = 3)	HFD + STZ 45 (n = 5)	HFD + STZ 65 (n = 4)	
0d	223.00 ± 3.85	206.58 ± 4.43	211.14 ± 5.25	205.50 ± 6.18	232.80 ± 3.48	232.68 ± 2.39	236.90 ± 4.57	197.07 ± 2.63	200.65 ± 6.27	207.5 ± 4.02	197.53 ± 8.86	205.30 ± 3.66	205.20 ± 6.03	
7d	278.93 ± 6.10	261.07 ± 12.13	223.10 ± 11.49*	210.53 ± 17.11**	306.07 ± 7.51	280.08 ± 15.55	251.03 ± 8.89	244.20 ± 5.04	230.73 ± 9.74	200.62 ± 9.35*	178.83 ± 13.69**	220.96 ± 4.54*	206.73 ± 4.31**	
14d	312.27 ± 6.77	264.93 ± 16.15	216.88 ± 24.53**	197.98 ± 25.74**	346.72 ± 10.82	306.35 ± 24.38	263.03 ± 12.78	254.28 ± 5.18	246.25 ± 11.49	210.18 ± 9.20*	167.80 ± 13.42**	234.06 ± 5.49	196.35 ± 6.70**	
21d	320.17 ± 6.54	299.00 ± 19.37	237.80 ± 26.66	212.75 ± 28.85*	357.32 ± 11.56	297.83 ± 35.36	256.80 ± 8.48	263.75 ± 5.14	240.87 ± 14.27	209.57 ± 11.01*	163.63 ± 14.94**	235.94 ± 6.12*	208.45 ± 8.39**	
28d	352.53 ± 8.12	309.95 ± 24.99	240.60 ± 33.27*	238.40 ± 34.37*	376.67 ± 15.37	301.00 ± 35.12	249.70 ± 6.37*	277.08 ± 6.38	251.37 ± 16.69	209.25 ± 12.78**	156.53 ± 18.29**	240.24 ± 4.53*	219.95 ± 12.76**	

Data representation ($\bar{x} \pm \text{SEM}$). Significance is denoted by* $P < 0.05$, ** $P < 0.01$ different from control group.

Abbreviation: STZ 45, the STZ dose was 45 mg / kg; STZ 65, the STZ dose was 65 mg / kg; STZ 85, the STZ dose was 85 mg / kg; HFD + STZ 45, the STZ dose was 45 mg / kg and followed a high-fat diet; HFD + STZ 65, the STZ dose was 65 mg / kg and followed a high-fat diet; HFD + STZ 85, the STZ dose was 85 mg / kg and followed a high-fat diet.

Table 2. Blood glucose levels (mmol / L) of male and female SD rats

	male							female						
	Control (n = 6)	STZ 45 (n = 6)	STZ 65 (n = 5)	STZ 85 (n = 4)	HFD + STZ 45 (n = 6)	HFD + STZ 65 (n = 4)	HFD + STZ 85 (n = 3)	Control (n = 6)	STZ 45 (n = 6)	STZ 65 (n = 6)	STZ 85 (n = 3)	HFD + STZ 45 (n = 5)	HFD + STZ 65 (n = 4)	
3d	4.25 ± 0.44	5.18 ± 0.30	13.46 ± 2.36	17.75 ± 5.52**	5.03 ± 0.24	7.70 ± 0.93*	17.60 ± 1.80**	4.95 ± 0.28	5.55 ± 0.87	10.00 ± 1.58**	26.13 ± 2.07**	4.50 ± 0.53	8.43 ± 0.96**	
7d	6.95 ± 0.23	8.72 ± 2.05	11.88 ± 2.60	20.03 ± 5.47*	5.22 ± 0.25	10.03 ± 2.48	15.77 ± 0.68**	4.70 ± 0.14	5.17 ± 0.92	7.08 ± 0.88	22.03 ± 1.03**	5.62 ± 0.35	10.63 ± 2.45*	
14d	4.23 ± 0.26	10.62 ± 2.86	17.46 ± 3.78*	16.40 ± 4.66	12.23 ± 2.30	15.28 ± 3.12*	20.90 ± 4.17**	4.38 ± 0.25	7.20 ± 1.36	7.20 ± 1.06	19.53 ± 1.84**	9.50 ± 2.55	12.65 ± 3.04*	
21d	4.05 ± 0.35	7.62 ± 2.69	22.02 ± 4.37**	21.05 ± 5.16*	5.53 ± 0.36	19.43 ± 4.87**	19.97 ± 1.20**	4.58 ± 0.13	7.82 ± 0.73	14.78 ± 2.69**	24.47 ± 1.96**	7.60 ± 0.90	12.55 ± 3.49*	
28d	3.53 ± 0.22	7.17 ± 2.50	16.80 ± 3.05**	20.93 ± 2.07**	4.70 ± 0.19	10.25 ± 2.15**	23.13 ± 1.71**	4.53 ± 0.27	4.65 ± 0.66	15.32 ± 2.73**	20.33 ± 3.32**	6.16 ± 0.72	16.00 ± 4.07**	

Data representation ($\bar{x} \pm \text{SEM}$). Significance is denoted by * $P < 0.05$, ** $P < 0.01$ different from control group.

Abbreviation: STZ 45, the STZ dose was 45 mg / kg; STZ 65, the STZ dose was 65 mg / kg; STZ 85, the STZ dose was 85 mg / kg; HFD + STZ 45, the STZ dose was 45 mg / kg and followed a high-fat diet; HFD + STZ 65, the STZ dose was 65 mg / kg and followed a high-fat diet; HFD + STZ 85, the STZ dose was 85 mg / kg and followed a high-fat diet.

4. Discussion

In the present study, we investigated blood glucose and weight in 14 male and female rat models of diet-induced and drug-induced diabetes. An ideal rat model must typically and mimic the disease pattern in diabetes. It is known that the characteristics of diabetes are mainly included chronic high blood sugar. Additionally, the model must be inexpensive and easy access. The rat models described in the current study including rats injected with low or high dose STZ[12]; rats injected with nicotinamide and STZ[13]; and rats received high-fat diet and STZ injection[14]. We attempted to identify a suitable non-genetic rat model to mimics the pathogenesis and clinical features of diabetes for further research.

In the current study, we made several important observations. In the first place, we proved that the fasting blood glucose of male diabetic rats induced by STZ is more stable than that of female. In the study of animal models, the physiological systems involved in metabolic homeostasis showed sex differences, which sex hormones play an important role[15]. Researchers often use male rodents because they show better metabolic diseases than females[16]. Here we confirmed that stability of blood glucose in the diabetic male rats induced by STZ is better than that of female rats, and male rats showed more sensitive to STZ than female mice, which may be related to sex hormones. STZ can induce diabetes by causing damage to β cells of the pancreas, while female sex steroids could prevent it happening. Gonadal hormone 17-estradiol (E2) is associated with reproductive, skeletal, cardiovascular, and neuronal physiology[17]. E2 promoted the survival of mouse pancreatic cells through estrogen receptor (ER) [18]. E2 through the estrogen receptor α (ER- α) to promote the survival of pancreatic β cells in mice. E2 activates the nuclear estrogen receptor through the estrogen response element (ERE). E2 also activates non-genomic signals through extracellular forms of ER and G-protein-coupled estrogen receptor (GPER). In diabetic rodent model, use E2 treatment can protect pancreatic β cells from oxidative stress, toxicity of amyloid peptide, lipid toxicity and apoptosis[17]. Therefore, estrogen therapy can protect β cells from STZ-induced apoptosis, help maintain insulin and prevent diabetes[19, 20]. In this study, compared with male rats, regardless of whether they were given normal diet or high-fat diet, glucose the blood glucose after modeling in female rats, and glucose level was lower than that of male rats in the same period, which was speculated to be related to estrogen in female rats.

Second, we proved that blood glucose concentrations in fat-fed rats are not higher than values in chow-fed rats. This conclusion is consistent with previous reports that fasting glucose challenge insulin concentrations were significantly higher in high-fat rats, it appears likely that insulin-mediated glucose disposal was decreased in these animals, which may lead to the lower blood glucose[21]. In Carvalho's study, compared with STZ mice fed with low-fat diet, STZ mice fed with HFD had lower blood glucose and higher body weight[22]. And there was no significant difference between high fat and low fat. Considering the cost, the STZ-induced (65 mg / kg) model without HFD was better.

The dosage of STZ was not clearly consistent when using it to model diabetes in rats. For example, Wang use single dose intraperitoneal injection of 50 mg / kg STZ to induce diabetes[23]. In Luippold's study, male SD rats with an age of 8–9 weeks were pre-treated with a single intraperitoneal dose of 60 mg / kg STZ to induce experimental diabetes[24]. In order to screen a better injection dose, three STZ dose groups of high, medium and

low were selected. Cost, operability, modeling rate and mortality were taken into account. In our study, the rats treat with a single 65 mg / kg and 85 mg / kg dose of STZ have higher blood glucose than that in other groups (45 mg / kg). However, it showed a higher mortality in 85 mg/kg dose group compared with 65 mg / kg dose group. The single high-dose STZ-induced (65 mg / kg) diabetic rats without feeding high-fat diet is more suitable for diabetes research. Since the severity of diabetes in STZ rats will gradually increase with the dose of STZ, any further ongoing research is likely to add to the complexity of the explanation. In the future, we plan to study the STZ dose group of 65 to 85 rats separately for a longer period of time to determine whether the protective effect against diabetic complications persists.

Conclusions

Compared with blood glucose and body weight, male SD rat injected intraperitoneally with STZ dose of 65 mg/kg and fed with ordinary diet is the most stable and ideal diabetic rat model.

Abbreviations

STZ: Streptozotocin; SD: Sprague Dawley; HFD: High-fat diet

Declarations

Notes

Yao Zhang and Jiao Zhang contributed equally to this work.

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Authors' Contributions

HC conceived, designed and directed this study; YZ, JZ, MH, JH, RW performed the experiments; YZ, JZ, MH, JH, RW, BT substantial contributed to analysis, interpretation of data and drafted the manuscript. HC, YZ, JZ, MH, JH, RW, BT, PH all participated in revision of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The raw data for the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All experimental procedures were performed according to the ethical principle guidelines approved by the National Research Council. All SD rats were purchased from Guangdong Experimental Animal Center and housed in the Experimental Animal Center of Guangzhou University of Chinese Medicine (No. SYXK2018-0085, Guangzhou, China).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Weight (g) on A and blood glucose levels (mmol / L) on B of male SD rats. STZ45: The STZ dose was 45 mg / kg; STZ65: The STZ dose was 65 mg / kg; STZ85: The STZ dose was 85 mg / kg. Data representation ($\bar{x} \pm \text{SEM}$) (n = 6 for control group; n = 6 for STZ 45 group; n = 5 for STZ 65 group; n = 4 for STZ 85 group). Significance is denoted by *P < 0.05, **P < 0.01 different from control group.

Figure 2

Weight (g) on A and blood glucose levels (mmol / L) on B of female SD rats. STZ 45: The STZ dose was 45 mg / kg; STZ 65: The STZ dose was 65 mg / kg; STZ85: The STZ dose was 85 mg / kg. Data representation ($\bar{x} \pm \text{SEM}$) (n = 6 for control group; n = 6 for STZ 45 group; n = 5 for STZ 65 group; n = 3 for STZ 85 group). Significance is denoted by *P < 0.05, **P < 0.01 different from control group.

Figure 3

Weight (g) on A and blood glucose levels (mmol / L) on B of male SD rats. HFD + STZ 45: The STZ dose was 45 mg / kg and followed a high-fat diet; HFD + STZ 65: The STZ dose was 65 mg / kg and followed a high-fat diet; HFD + STZ 85: The STZ dose was 85 mg / kg and followed a high-fat diet. Data representation ($\bar{x} \pm \text{SEM}$) (n = 6 for control group; n = 6 for HFD + STZ 45 group; n = 4 for HFD + STZ 65 group; n = 3 for HFD + STZ 85 group). Significance is denoted by *P < 0.05, **P < 0.01 different from control group.