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Characterisation of Streptozotocin Induced Diabetes Mellitus in Swiss Albino Mice

[1]Sachin Arora, [1]Shreesh Kumar Ojha and [2]Divya Vohora

[2]Department of Pharmacology, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India

Abstract: The present study was designed to investigate the characteristics of diabetes mellitus induced by different doses of Streptozotocin in mice as assessed by blood glucose concentration. Swiss albino mice of either sex were intraperitoneally administered single dose of Streptozotocin 180 mg/kg, or 100 mg/kg and multiple low dose of Streptozotocin 40 mg/kg/day. A single injection of Streptozotocin 180 mg/kg was found to produce Type 1 (insulin-dependent diabetes mellitus) and multiple low dose Streptozotocin 40 mg/kg Type 2 (non-insulin-dependent diabetes mellitus). However, Streptozotocin 100 mg/kg failed to produce diabetes mellitus except a sustained hyperglycemia. Blood glucose concentrations were measured at every week after Streptozotocin injection. Multiple low doses of STZ 40 mg/kg, i.p. to mice for five consecutive days have been found suitable model to study long term complications of diabetes.

Key words: Multiple low dose · Mice · Streptozotocin · Diabetes mellitus

INTRODUCTION

Diabetes mellitus is the most common metabolic disorder. It is characterized by hyperglycemia that results from an absolute or relative insulin deficiency and is associated with long-term complications affecting the eyes, kidneys, heart and nerves [1]. Diabetes mellitus is classified in to 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 β cells [2,3]. Type II diabetes is characterized by peripheral insulin resistance and impaired insulin secretion.

To develop animal models of diabetes mellitus streptozotocin (STZ; N-nitroso derivative of glucosamine) has been commonly used to induce not only animal models of IDDM [4-7] but also NIDDM with hypoinsulinemia by STZ administration to neonates (1 or 2 day old mice [8,9,10]). It has been reported that STZ is capable of producing mild to severe types of diabetes according to the dosages used when it is given to animals by either single i.v. or i.p. injection [6]. Reports indicate that the nature of diabetes development varies with the doses, routes of drug and species of the animal [4-7,11-14].

In order to clarify the development and progression of hyperglycemia and diabetes mellitus of different type in mice, the present study has planned to investigate the characteristics of diabetes mellitus induced by administration of different doses of STZ. Therefore, to study the characteristic of diabetes mellitus three dose schedules were employed in the present study.

MATERIALS AND METHODS

Experimental Animals: Laboratory bred Swiss albino mice of either sex, 6-8 weeks of age, weighing 25-30 g were obtained from Central Animal House, Jamia Hamdard, New Delhi. All animal procedures were in accordance with the standards set forth in guidelines for the care and use of experimental animals by Committee for Purpose of Supervision of Experiments on Animals (CPCSEA). The study protocol was approved by Animal Ethics Committee of Jamia Hamdard, New Delhi. All mice were fasted for 20 hour before diabetes was induced with STZ. The animals were allowed to acclimatize for 2 weeks before the experiment. The animals were housed in polypropylene cages inside a well-ventilated room. Each cage consists of not more than 3 mice. They were maintained under standard laboratory conditions of temperature 24-28°C, relative humidity 60-70% and 12 hour light/dark cycle. They were fed a standard commercial pellet diet and water *ad libitum*. The diet consists of 71% carbohydrate, 18% protein, 7% fat, 4% salt mixture and adequate minerals and vitamins.

Corresponding Author: Sachin Arora, Department of Pharmacology, All India Institute of Medical Sciences, New Delhi-110029

Induction of Diabetes Mellitus: Streptozotocin was obtained from Sigma Chemicals Co., St. Louis, MO, USA. STZ was dissolved in cold 0.01 M citrate buffer, pH 4.5 and always prepared freshly for immediate use within 5 min. STZ injections were given intraperitoneally and the doses were determined according to the body weight of animals. The blood glucose concentration was measured every week from the day of STZ injection. The blood samples were collected from the tail vein once a week and the blood was deproteinized. The obtained supernatant was used immediately for the determination of blood glucose by glucose Oxidase/peroxidase method spectrophotometrically [15].

Experimental Groups and Protocol: The animals were distributed in to four experimental groups. Each group consisted of 8 mice in the beginning of the study. Animals in group I were intraperitoneally administered single injection of 180 mg/kg of STZ. Animals of group II were intraperitoneally administered a single injection of 100 mg/kg of STZ. Animals of group III were intraperitoneally administered multiple low doses of 40 mg/kg/day of STZ for five consecutive days. Animals of group IV served as control group were injected with equivalent amount of cold citrate buffer (pH 4.5). All the doses of STZ were administered at a volume not exceeding 1ml/100 g body weight of mice.

Statistical Analysis: The data were expressed as mean±SEM (standard error of mean). Statistical differences between groups were analyzed using one-way Analysis of Variance (ANOVA) followed by student-t test. The difference was considered statistically significant at p value <0.05. To obtain comparable results, data of six mice from each group was used for statistical analysis.

RESULTS

The values of blood glucose concentrations are presented in Table 1. All the animals were weighed weekly and their general conditions were also monitored through out the experimental duration.

Effect of Single STZ Injection (180 Mg/kg, I.p.) On Blood Glucose: All the animals develop diabetes mellitus within a week after administration of 180 mg/kg STZ. A significant rise in blood glucose concentration was observed till 3rd week in comparison with control. By the completion of 5th week >20% mortality was observed (2 out of 8 animals died) because of which we have included only the data of six mice survived till the end of the study

Table 1: Blood glucose levels with different doses of Streptozotocin in mice

Time period	STZ 180 (n=6)	STZ 100 (n=6)	STZ 40 (n=6)
Blood Glucose (mg/dl)			
0 day	131.8±3.7	139.7±4.5	133.9±3.9
Week 1	508.3±46.2	141.4±5.6	199.1±6.9
Week 2	539.1±45.7*	161.4±4.9	291.4±8.4*
Week 3	617.8±97.7	179.8±3.0	334.16±17.5*
Week 4	612.3±84.3	164.0±4.2	325.7±30.8
Week 5	602.2±91.5	141.4±3.8	295.8±23.2

The data are expressed as mean ± SEM (standard error of mean) of six experiments.*P<0.05, when compared to control

Effect of Single STZ Injection (100 Mg/kg, I.p.) On Blood Glucose: None of the animal develops diabetes with STZ 100 mg/kg. Though a insignificant increase in blood glucose concentration was observed at the 3rd and 4th week after STZ injection. But the blood glucose concentrations were far below the threshold value for the animals to be considered as diabetic. No mortality was observed in this group.

Effect of Multiple STZ Injection (40 Mg/kg × 5 Day, I.p.) On Blood Glucose: Administration of multiple low doses of STZ produces significant hyperglycemia from 1st week. The animals were observed to be diabetic from 2nd week and remained in diabetic state till 5th week. One mice died by the end of the study in this group.

DISCUSSION

Streptozotocin induced diabetes is a well-documented model of experimental diabetes. Previous reported literature indicates that the type of diabetes and characteristics differ with the employed dose of STZ and animal and species used [4-7]. Streptozotocin induced diabetes provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia [16]. STZ is a pancreatic β cell toxin that induces rapid and irreversible necrosis of β cells [6]. Whereas a single diabetogenic dose of STZ (70-250 mg/kg, body weight) has been demonstrated to induce complete destruction of β cells in most species within 24 hour, multiple sub-diabetogenic doses of STZ partially damage islets, thereby triggering an inflammatory process leading to macrophage and subsequent lymphocyte infiltration, which is followed by the onset of insulin deficiency[17,11].

In the present study, we studied the diabetogenic response in mice using three models, one specific for type I by administering a single i.p. injection of STZ 180 mg/kg, another specific for type II by administering a single i.p. injection of STZ 100 mg/kg and the third following administration of multiple low dose i.p. injection of STZ 40 mg/kg 5 days for the induction of diabetes mellitus of Type 1 and 2.

Present study results demonstrate that a single i.p. injection of STZ 180 mg/kg produced diabetes mellitus in the very first week and the animals remained in that state till the 3rd week similar to a previous study report in which mice administered 200 mg/kg STZ induced a sharp rise with an accompanying marked fall in serum insulin levels from the first day after STZ administration in mice and produced type I or insulin dependent diabetes mellitus [12]. Furthermore, this study also reported that mice administered 100 mg/kg STZ, non-fasting serum glucose level continued to increase gradually after STZ administration without affecting the non-fasting serum insulin levels and shown to produce Type 2 or non-insulin-dependent diabetes mellitus. However, STZ 100 mg/kg administered mice failed to produce diabetes mellitus in our study. STZ 100 mg/kg only produced hyperglycemia in the 3rd and 4th week and the blood glucose begin to decline after 5th week and the values were far below the threshold value (250 mg/dl) for the animals to be considered as diabetic. Although, the findings are not in agreement with previous other reports[13,14] where STZ 100 mg/kg has been reported to produce type II or non-insulin-dependent diabetes mellitus and blood glucose concentrations remained high till 9th week. Our study assessed the blood glucose level till 5th week only because it was observed that blood glucose concentration was abating in 5th week. The previous study report that animals administered STZ 100 mg/kg have the tendency to secrete insulin from β cells and the remaining β cell escaping from the attack of STZ may cause an over secretion of insulin to maintain normoglycemia which could be a probable explanation of observed normoglycemia in the present study results.

In multiple low dose STZ administered mice a significant hyperglycemia was observed from the first week to 3rd week. A gradual and progressive increase in blood glucose concentration was observed and animals produce type I or insulin dependent diabetes. The present study findings are in consonance with a previous study which reports that mice intraperitoneally injected multiple low dose of STZ 40 mg/kg developed diabetes (fed blood glucose concentration < 250 mg/dl) 2-4 weeks after STZ injection. Multiple low dose STZ diabetes in mice believed to resemble human type I diabetes in many aspects [17]. However, there is conflicting reports which demonstrate that administration of multiple low dose injection of STZ in the first few days induce Type 2 diabetes and in progression produce Type 1 diabetes.

In summary, the present study results indicate that single i.p. injection of STZ 180 mg/kg produces type I or insulin dependent diabetes and STZ 100 mg/kg i.p. failed to produce diabetes mellitus. However, multiple low doses STZ produce type I or insulin dependent diabetes mellitus similar to STZ 180 mg/kg. The severity and mortality of diabetes with STZ 180 mg/kg is more in comparison to multiple low doses STZ 40 mg/kg. The long-term complications of diabetes mellitus and the characteristics of progressive diabetes mellitus could be studied employing multiple low doses STZ injections to mice. Using multiple low doses of STZ a cost effective and comprehensive animal model mimicking human type I diabetes could be utilized for future studies.

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