ORIGINAL ARTICLE

EFFECTIVE DOSE OF STREPTOZOTOCIN FOR INDUCTION OF DIABETES MELLITUS AND ASSOCIATED MORTALITY RATE IN WISTAR ALBINO RATS

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ABSTRACT

Background: To understand severity and complications of diabetes mellitus and to analyze effects of drugs, it is necessary to create diabetic animal model. There are different doses of streptozotocin to induce diabetes mellitus in rats that may be associated with mortality or may be insufficient for induction of DM. The objective of our study was to optimize the dose of streptozotocin to create a diabetic animal model with sustained hyperglycemia and to document the toxic dose at which there may be high mortality rate.

Methods: This experimental animal study was conducted at animal house, faculty of pharmacy Ziauddin University Karachi in April 2019. The sample size included 30 albino wistar rats divided into five Groups A, B, C, D and E "with 6 rats each group". Group A was the control, while streptozotocin at different concentrations was administered intraperitoneally in Group B, C, D and E respectively. Blood sample was drawn from lateral tail vein of animals and hyperglycemic profile was checked on 2nd, 4th, 6th, 8th and 10th day.

Results: When compared to control Group A, hyperglycemic profile (blood glucose level >180) was achieved in Group B, C, D and E after 48 hours. High mortality rate was observed in Group E followed by Group D. Group C had persistent hyperglycemia while Group B had reversible hyperglycemic profile.

Conclusion: Intraperitoneal dose of streptozotocin 60 mg/kg created diabetic animal model with persistent hyperglycemia. However, dose above increased the mortality rate and below failed to create diabetic animal model.

Keywords: Dose Optimization; Streptozotocin; Diabetic Animal Model; Persistent Hyperglycemia.

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INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disease, which is characterized by chronic elevation of blood glucose level[1,2]. The prevalence of diabetes mellitus is increasing yearly and different drugs are being used to treat the disorder[3]. To understand severity and complications of disease and to analyze effects of drugs in diabetes mellitus it is necessary to create diabetic animal models. From decades diabetic animal models have been used to understand the disease process and to understand the pharmacokinetics and pharmacodynamics of existing and newly developed drugs[4]. Various methods are being used in laboratories to create diabetic animal models under professional supervision, including surgical method such as pancreatectomy and pharmacological methods such as by alloxan monohydrate and streptozotocin. Use of streptozotocin particularly has shown predominance in selectivity as a diabetogenic agent [5, 6].

Streptozotocin is an antitumor antibiotic used for cancer chemotherapies[7]. It is a synthetic nitrosoureido glucopyranose derivative that inhibits DNA

synthesis in bacterial and mammalian cells[8]. Although the mechanism of action is not clear but it is thought that streptozotocin causes death of pancreatic β cells by DNA alkylation and produces diabetes mellitus in experimental animals by entering in β cells through cell membrane of GLUT2 glucose transporter[9,10]. Since the identification of streptozotocin as a diabetogenic drug, studies have documented different doses to induce diabetes mellitus in rats, few studies recommend a large dose (i.e., 100mg/kg) of streptozotocin[11], few studies support a moderate dose (i.e., 60mg/kg) and other researchers has documented that a small dose (i.e., 40mg/kg) of the drug will be sufficient to induce diabetes in rats[12,13].

Despite being an expensive drug for creating a diabetic animal model, streptozotocin is the preferred method for inducing diabetes mellitus in animal models over alloxan[14]. In the concern subject if researcher wants to create a diabetic animal model he should have a thorough knowledge of dose of streptozotocin to follow at which he may achieve a sustained diabetic profile in animal models with less mortality rate. The objective of our study was to optimize the dose of streptozotocin to create a diabetic animal model with sustained hyperglycemia and to document the toxic dose at which there may be high mortality rate.

METHODS

It was an animal based experimental study conducted at Animal House of Faculty of Pharmacy Ziauddin University Karachi. The sample size included n=30 albino wistar rats 9 weeks old, weighing 200-250g, animals were purchased from Agha Khan University. The study was approved by Animal Ethics Committee of Ziauddin University and Protocol No. 2018-003 was allotted to this animal-based experimental trial. All the animals were given twelve-hour light and dark cycle, and before start of treatment animals were acclimatized with the environment. Animals were dealt through all procedure according to CARE guidelines[15]. Induction of Diabetes Mellitus was performed at different concentrations of Streptozotocin i.e., 40mg/kg, 60mg/kg 80mg/kg and 100mg/kg. The drug was diluted in 1ml of normal saline and administered intraperitoneally as single dose to animal groups; rats were kept deprived of their feed and water for twelve hours before administration of streptozotocin. Blood glucose levels were obtained after 48 hours by using Abbott FreeStyle Optium Xceed Glucometer. Rats with blood glucose level >180mg/dl were considered as diabetic[4]. Blood were drawn from lateral tail vein[16] of all the rats to analyze the hyperglycemic profile on 2nd, 4th, 6th, 8th and 10th day. Animals were randomly selected for grouping, Group A: was considered as Control group (Normal Saline was administered intraperitoneally), in Group B, C, D, and E streptozotocin at concentration of 40mg/kg, 60mg/kg, 80mg/kg and 100mg/kg was administered intraperitoneally. Follow up data was recorded on a proforma and results were compared in the end of analyses.

RESULTS

When compared to the control group hyperglycemic profile was achieved in all the groups on 2nd day at various doses of streptozotocin (i.e., 40mg/kg, 60mg/kg, 80mg/kg and 100mg/kg). Mortality was observed in Group D (80mg/kg) where n=1 rat expired and Group E (100mg/kg), where n=3 rats perished. On 4th day, Hyperglycemic profile was present in all intervened groups. Mortality was again observed in Group D (80mg/kg) and Group E (100mg/kg) where a total of n=4 rats perished, two in each group respectively. On 6th day it was noticed that in Group B (40mg/kg) there was a gradual decrease in glucose levels and there was no change in glucose levels in Group C (60mg/kg) while n=1 rat from Group D (80mg/kg) and the last surviving rat from Group E (100mg/kg) had died. On 8th day, Glucose profile in Group B (40mg/kg) and C (60mg/kg) remained same and there was no mortality in any of the group. In the end of follow-up i.e., on 10th day Glucose levels in Group B (40mg/kg) was decreasing gradually and in n=2 rats the glucose levels were found to be below 120mg/dl. There was no change in glucose profile of Group C (60mg/kg) while n=1 rat from Group D (80mg/kg) died (Table 1 expresses the blood glucose level in all animals on different days (i.e., 4th, 6th, 8th and 10th).

Table 1: Glucose levels in animal groups and mortality after administration of streptozotocin on 2nd, 4th, 6th 8th and 10th day.

Glucose level in mg/dl					
2nd day after streptozotocin administration					
Experimental Animals	Group A	Group B	Group C	Group D	Group E
	Control	40mg/kg	60mg/kg	80mg/kg	100mg/kg
rat 1	90	189	289	300	280
rat 2	98	195	270	296	325

rat 3	89	225	344	290	350
rat 4	95	180	289	190	Died
rat 5	93	240	295	243	Died
rat 6	96	275	293	Died	Died
4th day after streptozotocin administration					
rat 1	98	190	240	289	300
rat 2	89	200	276	225	Died
rat 3	65	245	237	290	Died
rat 4	94	180	300	Died	-------
rat 5	95	225	290	Died	-------
rat 6	90	140	310	------	-------
6th day after streptozotocin administration					
rat 1	80	140	224	288	Died
rat 2	87	166	278	284	-------
rat 3	95	155	289	Died	-------
rat 4	78	147	245	-------	-------
rat 5	69	180	340	-------	-------
rat 6	88	192	310	-------	-------
8th day after streptozotocin administration					
rat 1	68	140	300	330	-------
rat 2	79	144	285	357	-------
rat 3	76	128	289	-------	-------
rat 4	81	134	300	-------	-------
rat 5	89	145	258	-------	-------
rat 6	102	150	310	-------	-------
10th day after streptozotocin administration					
rat 1	90	117	289	310	-------
rat 2	85	180	224	Died	-------
rat 3	89	138	310	-------	-------
rat 4	90	129	278	-------	-------
rat 5	110	200	245	-------	-------
rat 6	70	110	190	-------	-------

Difference in mean value on 2nd and 10th day of Group A, B and C were compared (Figure 1) and Group D and E were excluded from this analysis.

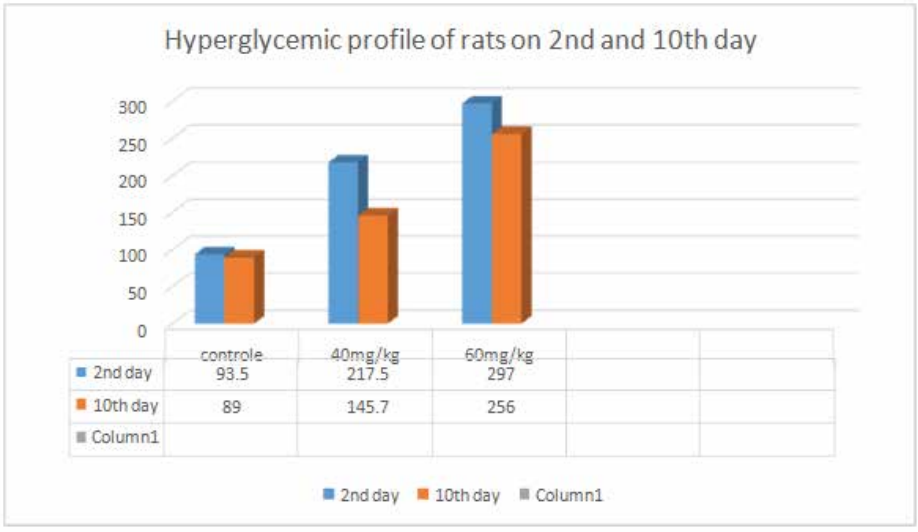

Figure 1: Mean blood glucose level in Group A, B and C.

DISCUSSION

Route of drug administration, adequate dose and proper technique are key points to produce a diabetic animal model as stated by Jain and Arya, who compared the different doses and routes of administration for alloxan to achieve a perfect diabetic animal model[16]. After administration of streptozotocin hyperglycemia is achieved in 72 hours[17], while in our study, we checked the blood glucose levels after 48 hours and found hyperglycemia in all intervened groups. In multiple studies it is suggested that the streptozotocin should be diluted in buffer solutions with pH range 4.0-5.5[17,18], but Deeds et al.[19] recommended normal saline should be used for dilution of drug. In our study streptozotocin was diluted in normal saline and hyperglycemic profile was achieved that is parallel to findings of Tay et al[20]. It was observed in our study that animals who received an intraperitoneal injection of streptozotocin at 60mg/kg dose (Table 1) developed a sustained hyperglycemic profile as reported by Huber et al.[21] and Ali et al[22]. Similar to our study, Akberzadeh et al., in 2007 have also highlighted that the same dose of streptozotocin i.e., 60mg/kg produced hyperglycemia (≥350 mg/dl) without causing any harm to animals.

However, the drug was administered intravenously[12]. As depicted in Table 1, we found high mortality rates in two Groups D and E who received streptozotocin 80 mg/kg and 100mg/kg respectively. Contrary to our results few studies have documented that single intraperitoneal injection of streptozotocin at a dose of 100 mg/kg will produce immediate hyperglycemia in animals without harming them[13,23,24] while in our study we found high mortality rate in two groups who received streptozotocin 80 mg/kg and 100mg/kg. Mostafavinia et al. concluded their study by stating that 40 mg/kg dose of streptozotocin will give a persistent diabetic profile[25]. Whereas in our study we observed that few rats from Group B which were intervened by 40 mg/kg dose of streptozotocin become euglycemic by 10th day of intervention that was a similar finding as reported by Ar` Rajab and Ahre'n[26]. This study will help in deciding the accurate dose of streptozotocin for induction of diabetes in wistar albino rats in our scenario.

This is the first study conducted as follow-up after streptozotocin administration. After administration of streptozotocin we just observed the hyperglycemic profile of animals for 10 days only. Due to financial issues histopathologic examination of pancreas in the animals was not performed. Future studies with longer duration of follow up that is more than 10 days should be conducted to find out the exact time of insulin reversal after administration of streptozotocin at 40mg/kg dose. Furthermore, histopathologic examinations should be performed immediately after streptozotocin administration and it should be repeated after the insulin reversal to analyze the recovery of β islets from streptozotocin toxicity.

CONCLUSION

Administration of 60mg/kg intraperitoneal dose of streptozotocin created diabetic animal model with persistent hyperglycemia. Dose of 100mg/kg was found to be lethal to animals indicated by high mortality rate. Streptozotocin at 40mg/kg dose was insufficient to create persistent diabetic profile in animal model.

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CONFLICT OF INTEREST

There is no conflict of interest.

ETHICS APPROVAL

Animal Ethics Committee of Ziauddin University approved the study.

AUTHORS CONTRIBUTION

AA generated the concept of study, did the drafting, data analysis and finalized the results. ZM designed the project and SS critically reviewed the manuscript. FA performed the statistical analysis. KM helped in the implementation of the experimental protocol and SA did final drafting of the manuscript.

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