

INDUCTION OF DIABETES BY STREPTOZOTOCIN IN RATS

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ABSTRACT

The objective of this study is to induce experimental diabetes mellitus by Streptozotocin in normal adult Wistar rats via comparison of changes in body weight, consumption of food and water, volume of urine and levels of glucose, insulin and C-peptide in serum, between normal and diabetic rats. Intra-venous injection of 60mg/kg dose of Streptozotocin in adult wistar rats, makes pancreas swell and at last causes degeneration in Langerhans islet beta cells and induces experimental diabetes mellitus in the 2-4 days. Induction of experimental diabetes mellitus is indeed the first step in the plan of purification of pancreatic Langerhans islet cells of normal rats for transplanting under the testis subcutaneous of experimentally induced diabetic rats. Streptozotocin induces one type of diabetes which is similar to diabetes mellitus with non-ketosis hyperglycemia in some animal species. For induction of experimental diabetes in male adult rats weighted 250-300 grams (75-90 days), 60mg/kg of Streptozotocin was injected intravenously. Three days after degeneration of beta cells, diabetes was induced in all animals. The diabetic and normal animals were kept in the metabolic cages separately and their body weight, consumption of food and water, urine volume, the levels of serum glucose, insulin and C-peptide quantities in all animals were measured and then these quantities were compared. For a microscopic study of degeneration of Langerhans islet beta cells of diabetic rats, sampling from pancreas tissue of diabetic and normal rats, staining and comparison between them, were done. Induction of diabetes with Streptozotocin decreases Nicotinamide-adenine dinucleotide (NAD) in pancreas islet beta cells and causes histopathological effects in beta cells which probably intermediates induction of diabetes. In this study, we used Streptozotocin for our experiments in induction of experimental diabetes mellitus. After Induction of diabetes, consumption of food and water, volume of urine and glucose increased in the diabetic animals in comparison with normal animals, but the weight of body and the volume of insulin and C-peptide decreased in the diabetic animals. Sampling and staining of pancreas tissue of diabetic and normal rats showed that the Langerhans islet beta cells of diabetic rats have been clearly degenerated. In three days, Streptozotocin makes pancreas swell and at last causes degeneration in Langerhans islet beta cells and induces experimental diabetes. It also changes normal metabolism in diabetic rats in comparison with normal rats. Consumption of water and food, volume of urine, serum glucose increases in diabetic animals in comparison with normal rats but the levels of serum insulin, C-peptide and body weight decreases.

KEY WORDS

Diabetes Induction, Streptozotocin, Islet cells.

INTRODUCTION

Diabetes is a chronic disease that is relatively common throughout the world. In recent decades, various

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epidemiological studies have been carried out on prevalence of diabetes mellitus in Iran, according to which the population of diabetics was estimated to exceed 1.5 million. In the year 2004, according to the World Health Organization reports, more than 150 million people throughout the world suffered from diabetes (1) while the mankind has been unable to solve this problem. The only simple, inexpensive, easy and available way is to refine the Langerhans islets and to graft them under the testis subcutaneous. Inducing experimental diabetes mellitus is indeed the first step in the plan for transplanting

the pancreatic Langerhans islets under the testis subcutaneous. Experimental diabetes mellitus has been induced in laboratory animals by several methods. The generally effective method is to take the pancreas out of the body. However, to induce a notable form of diabetes, at least 90-95% of the pancreas has to be removed. Otherwise, the Langerhans islets in the remaining pancreas may undergo hypertrophy and secrete a sufficient amount of insulin for fulfilling the natural metabolic needs. The second method for creating diabetes in animals is injecting drugs such as alloxan or Streptozotocin. These materials inflate and ultimately degenerate the Langerhans islets beta cells (2). A less reliable method for creating diabetes is injection of the anterior hypophysis extract (3). The final symptoms of insulin deficiency are clearly seen in rats afflicted with diabetes chemically by Streptozotocin (4). Using 60mg/kg Streptozotocin dose can begin an autoimmune process that results in the destruction of the Langerhans islets beta cells and the 60mg/kg Streptozotocine dose results in the toxicity of beta cells with emergence of clinical diabetes within 2-4 days (5). For the purpose of transplantation of Langerhans islets of healthy rats under the testis subcutaneous of diabetic rats, we had to induce experimental diabetes in order to study the effect of grafting the Langerhans islets in diabetic rats. Therefore, the study made us, first, to induce experimental diabetes mellitus in order to study the effect of transplantation of the Langerhans islets in diabetic rats with Streptozotocin so as to be able to study the clinical parameters before and after the pancreas islet cells transplantation.

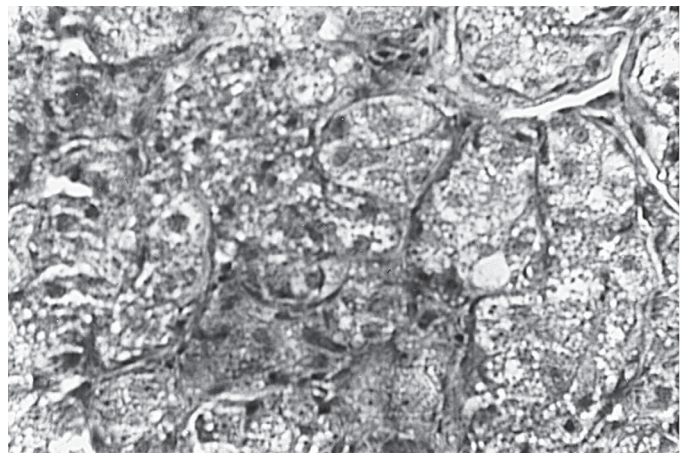
MATERIALS AND METHODS

Material : Streptozotocin or Streptozocin or Izoctazin or Zanosar (STZ) is a synthetic antineoplastic agent that is classically an anti-tumor antibiotic and chemically is related to other nitrosureas used in cancer chemotherapy. Streptozotocin sterile powders are provided and prepared as a chemotherapy agent. Each vial of sterilized Streptozotocin powder contains 1 gr. of Streptozotocin active ingredient with the chemical name, 2-Deoxy-2-[[[(methylnitrosoamino)-carbonyl] amino]-D-glucopyranose and 200 mg. citric acid. Streptozotocin was supplied by Pharmacia Company. Streptozotocin is available for intravenous use as a dry-frozen, pale yellow, sterilized product. Pure Streptozotocin has alkaline pH. When it is dissolved inside the vial in distilled water as instructed, the pH in the solution inside the vial will be 3.5-4.5 because of the presence of citric acid. This material is prepared in 1-gr vials and kept in cold store and refrigerator temperature (2-8 °C) away from light.

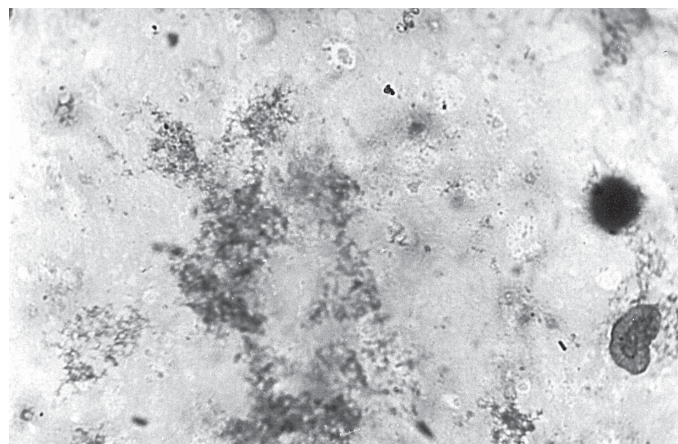
Induction of diabetes in rats : Six adult Wistar rats weighting 250-300 grams (75-90 days old) were used for inducing diabetes. The animals were injected by streptozotocin at the dose of 60 mg/kg of the body weight intravenously. Streptozotocin induces diabetes within 3 days by destroying the beta cells (6). Diabetic animals and non-diabetic control group were kept in metabolic cages individually and separately and under feeding and metabolism control. Glucose in the blood of diabetic rats exceeded that of the non-diabetic control ones. Food consumption was measured in terms of (gr.), water consumption was measured in terms of (ml) and urine volume was measured in terms of (ml) on a daily basis while every 2-4 weeks in 80 days the levels of C-peptide, insulin and glucose in blood serum were also measured, so that chemical diabetes was verified in rats injected with Streptozotocin (7).

Measurement of Glucose, Insulin and C-Peptide in Rats' Serum : Normal and diabetic rats were anesthetized with ether (two min. contact with ether does not affect blood glucose, insulin or C-peptide concentrations). One ml. of blood was taken from rats in order to measure glucose, insulin and C-peptide (8). Blood was taken from the heart. The samples were collected in sterilized tubes and kept at 4 °C and, after separating the clot, the serum was separated by centrifuging. Blood glucose was measured by the glucose-oxidase method, insulin and C-peptide by radio-immunoassay method. This phase of the work was carried out once every 2-4 weeks for 80 days in diabetic and control counterparts (9).

Pancreatic biopsy of normal and diabetic rats : For the study and comparison of pancreas Langerhans islet beta cells in diabetic rats induced by Streptozotocine, and normal rats, pancreatic biopsy of normal and diabetic rats was done and samples were fixed in 10% formalin, stained by Hematoxylin& Eosin and photographed by Leitz microscope with 4000 times



(Picture1) Pancreatic biopsy of normal rats

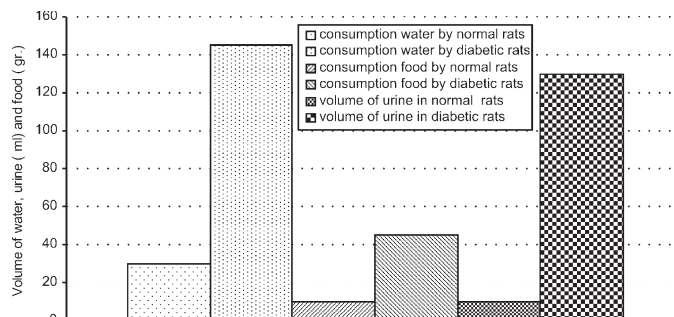


(Picture 2) Pancreatic biopsy of diabetic rats that confirms the destruction of islets and cells due to the effect of Streptozotocin

enlargement (pictures 1, 2). The comparison of these pictures shows that the tissue of Pancreatic Langerhans and the beta cells of diabetic rats have been degenerated irreversibly (10).

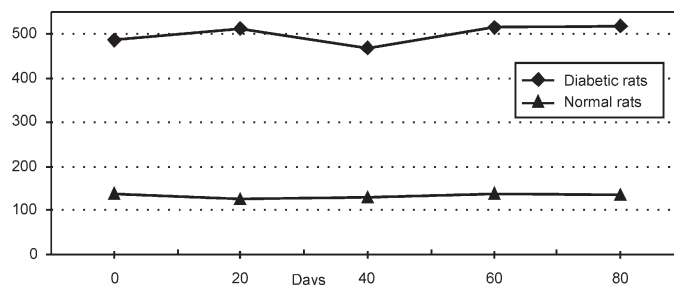
RESULTS AND DISCUSSION

Normal levels of glucose, insulin and C-peptide in healthy adult rats were measured as 135±5 mg/dl, 2±0.2 mIU/L and 0.056±0.003 ng/ml, respectively. Daily consumption of water and food in healthy adult rats were measured as 30±5 ml. and 10±2 gr., respectively. Daily urine in healthy adult rats was measured as 10±1ml (Table 1 and Fig 1). But in diabetic rats the levels of glucose, insulin and C-peptide were measured as 500±20 mg/dl, 1.5±0.2 mIU/L and 0.052±0.002 ng/ml, respectively and daily consumption of water and food in them were measured as 145±5 ml. and 45±4 gr. Daily urine volume in diabetic rats was measured as 130±5ml (Table 1 and Fig 1). Changes of body weight in adult and non-adult diabetic rats varied. Since the non-adult diabetic rats are in the growing age, diabetic loss of weight is not seen in them and they even show a slight weight gain (Fig 5). In adult rats, however, diabetes is accompanied by loss of weight (Fig 6). In each group, there were individual changing trends in respect of the amount of glucose, insulin, C-peptide and adult rats' weight. Using replicated measurements, the data including a diabetic and a normal group underwent analysis of variance by SPSS.



(Fig 1) Shows the mean of water, food consumption and volume of urine in 6 head normal and diabetic rats

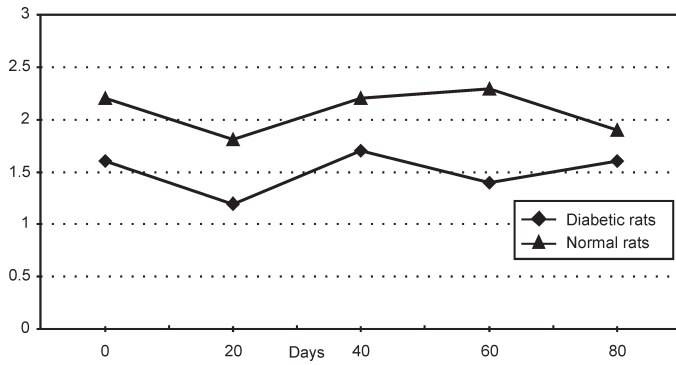
12 Program up to 80th day. According to the One-way ANOVA, a significant effect on diabetic and normal rats occurred, with rat blood glucose of SEM=61.08, F=1304.4, d.f=1,8 and P<0.001 (Fig 2), rat blood insulin of SEM=0.11, F=19.3, d.f=1,8 and P<0.002 (Fig 3), rat blood C-peptide of SEM=0.002, F=34.3, d.f=1,8 and P<0.001 (Fig 4) and adult rats weight of SEM=12.09, F=56.3, d.f=1,38 and P<0.001 (Fig 6). Which shows the success of induction of diabetes by streptozotocin in rats. In addition, the changes in healthy and diabetic rats are apparently distinctive because, in addition to thinness of diabetic rats, the tails of the healthy rats are pink and they have a white velvet coat. Due to induction of diabetes, the tail becomes dark in color and stained and their coat turns from white velvet into pink or gray behind the head and in the lower part of the body. If the environment of the rats is kept clean, there will be a change of color from white to pink. Otherwise, the change will be from white to gray (10). Pancreatic biopsy of normal and diabetic rats confirmed that the islet cells were



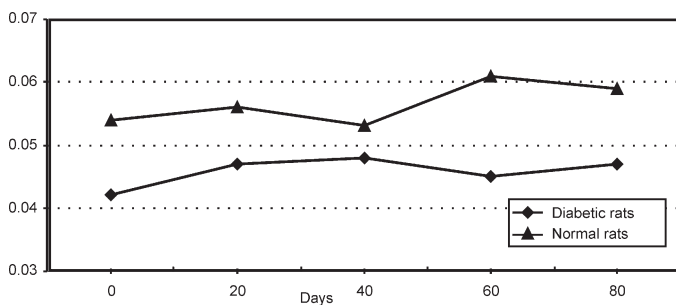
(Fig 2) shows the changes of average level of glucose in serum of 6 head diabetic and normal rats during 80 days.

Table.1 Shows data of number, weight, age, amount of Streptozotocine, glucose, insulin, C-peptide of blood, consumed water, food and volume of urine in normal and diabetic rats

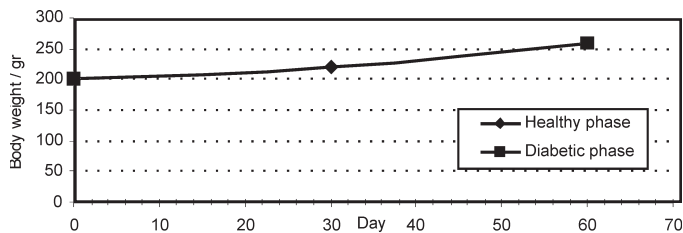
State of Rats	Blood glucose (mg/dl)	Blood insulin (m IU/ml)	Blood C-peptide (ng/ml)	consumed water (ml)	consumed food (gm)	volume of urine (ml)
Normal (n = 6)	135±5	2±0.2	0.056±0.003	30±5	10±2	10±1
Diabetic (n = 6)	500±20	1.5±0.2	0.52±0.002	145±5	45±5	130±5



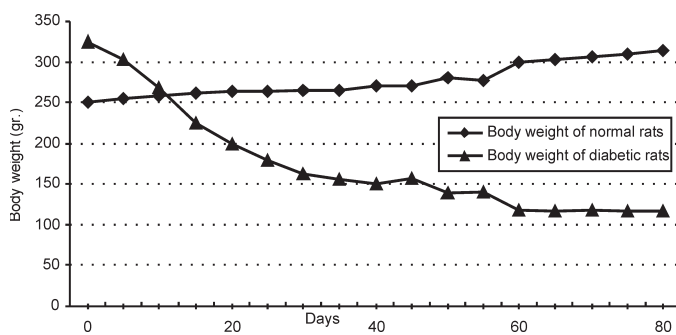
(Fig 3) shows the changes of average level of insulin in serum of 6 head diabetic and normal rats during 80 days.



(Fig 4) shows the changes of average level of C-peptid in serum of 6 head diabetic and normal rats during 80 days.



(Fig 5) shows continuous changes in average of body weight in 6 head non-adult rats in two healthy and diabetic phase



(Fig 6) Shows a comparison of the curves relating to the average of body weight in two groups of normal and diabetic rats. This graph reveals loss of weight and thinness in diabetic adult rats.

destroyed due to the effect of Streptozotocin in diabetic rats.

Streptozotocin prevents DNA synthesis in mammalian and bacterial cells. In bacterial cells, it renders special reaction with cytosine groups, resulting in degeneration and destruction of DNA. The biochemical mechanism results in mammalian cell death. Streptozotocin prevents cellular reproduction with a much smaller dose than the dose needed for inhibiting the substrate connection to the DNA or inhibiting many of the enzymes involved in DNA synthesis (10). Although Streptozotocin prevents entry of cells into mitosis but no special phase of the cellular cycle is especially sensitive to its mortal effects. Streptozotocin, which is used in intravenously form by rapid injection or constant short diffusion, stimulates the tissues. Metabolically, a slight deviation of the glucose-bearing pain from the normal limit has been seen in patients treated with a certain dose of Streptozotocin, which is generally reversible. However, the insulin shock, which is one of its other effects, is irreversible (11). In this study, the clinical manifestations and also the amount of glucose, insulin and C-peptide after using a 60 mg/kg dose of Streptozotocin, ensured induction of diabetes in rats. Hyperglycemia, hypoinsulinemia, polyphagia, polyuria and polydipsia accompanied by weight loss were seen in adult rats within three days of Streptozotocin treatment and, within one week to ten days, the amounts of the relevant factors were almost stable, which indicates irreversible destruction of Langerhans islets cells moreover, Researchers around the world have used streptozotocin to create experimental diabetes because it is a simple, inexpensive and available method (9, 10, 11). Our results are similar with those of Elias, 1994 (4), Ikebukuro, 2002 (2), we found their results similar to ours with no significant difference between them.

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