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A Comparative Study of Chemically Induced Animal Models of Diabetes Mellitus

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ABSTRACT

In diabetes research, animal models have been employed extensively. Although some studies are still carried out on larger animals, the majority of research is carried out on rodents. Chemically induced diabetic models are widely used, particularly in fundamental diabetes research. Streptozotocin and alloxan, among some other diabetogenics, cause hyperglycemia in rats and mice. These chemicals specifically harm ß cells, causing temporary suppression of insulin synthesis and/or secretion as well as lowering insulin's metabolic efficacy in target tissue. These compounds could be poisonous to the pancreas and other organs of the body, resulting in death of the animals. In this study, we compared three widely used chemically induced diabetic models with a new Alloxan +Nicotinamide (NAD) induced diabetes model in rats. Streptozocin (STZ) + Nicotinamide model outperformed all other models evaluated, in terms of successful induction, maximum stability, and lowest mortality. Our findings could aid in the selection of a suitable hyperglycemic model for diabetic research.

INTRODUCTION

Diabetes affects about 10% of the population, with Type-2 diabetes accounting for 90% of the cases. Hyperglycemia, hypercholesterolemia and hypertriglyceridemia are illnesses defined by insulin secretion abnormalities, impaired tissue sensitivity to insulin (insulin resistance), or a combination of both [1].

Selective inbreeding has resulted in various animal strains that can be used as models for Type 1 and Type 2 diabetes, as well as related traits including obesity and insulin resistance. Apart from their role in researching the disease's origin and consequences, all new diabetic treatments, such as islet cell transplantation and prevention methods, are first tested in rodents. Molecular biology approaches have yielded a slew of new animal models for diabetes research in recent years, including knock-in, generalised knock-out, and tissue-specific knockout mice [2]. In some of these models, insulin resistance predominates in association with obesity, dyslipidemia and hypertension, which provides valuable insights to study some events that are observed in human type 2 diabetes mellitus [3].

MATERIALS AND METHODS

Chemicals

Streptozocin (Sigma-Aldrich), Citric acid monohydrate, Trisodium citrate dehydrate (Fine Chemicals), Alloxan (Sisco Research Laboratories' Pvt, Ltd), Nicotinamide (Biophix), Glucose (Fine Chemicals).

Animals

Male albino Wistar rats of 3-4 weeks of age weighing between 180-250 g used. They were housed in groups six under standard laboratory conditions (temperature-25±1ºC, relative humidity-55±5% and 12.00:12.00h dark: light cycle) with standard pellet diet and water ad libitum. The overnight fasted (water ad libitum) animals were transferred to the laboratory at least one hour before the beginning of the experiment. The experiments were performed during day (9:00-12:00h) and as per the guidelines of the Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA), Government of India. The Institutional Animal Ethics Committee approved the study protocol.

Experimental Design

Animals were divided into five groups having 6 animals each. Diabetes was induced using various chemicals as per following protocol. During the experimental period Body weights, Water and food intake, Blood glucose levels are assessed on daily/periodic basis for duration of one month. Mortality during Experimental period was also recorded.

Group I: Control (No drug)

Group II: Streptazocin Induced Diabetic rats (STZ 45mg/kg)

Group III: Streptazocin + Nicotinamide induced Dibetes (NAD: 100mg/kg& STZ 50mg/kg)

Group IV: Alloxan induced diabetic rats (Alloxan 120mg/kg)

Group V: Alloxan + NAD induced Diabetic Rats (NAD: 100mg/kg & Alloxan 120mg/kg)

Induction of diabetes

Diabetes was induced in Group II to Group V. Diabetes was induced in Group II by Streptozotocin in citrate buffer (50 mg/kg i.p). In Group II animals diabetes was induced by Streptozotocin in citrate buffer (50 mg/kg i.p), 15 min before STZ injection Nicotinamide at a dose of 100mg/kg was administered intraperitoneally. In III Group, diabetes was induced by Alloxan monohydrate in Saline (120 mg/kg i.p) and Group IV animals received Niconamide (100mg/kg) followed by Alloxan monohydrate in Saline (120 mg/kg i.p) after 15 min. After induction all the animals received 20% glucose is provided to the induced animals for 24 hrs to prevent hypoglycemic shock in the animals. After 72 hrs of induction, rats showing fasting blood glucose levels > 200mg/dl were considered diabetic and included in the study.

Food and water intake

Each group received premeasured food and water on a daily basis, with the remaining food and water being measured every 24 hours. For a month, food/water was monitored on a daily basis in this way. The following calculation represents the average food consumption per animal. After that, the average monthly food and water consumption was computed.

Average Food/water consumption of each Group/day = Water or Food provided-Water/Food left animals/day

Measuring of Blood glucose levels

After an overnight fast, blood glucose level was measured on weekly basis. The blood was collected from the tail vein of the animal and blood glucose levels of animals in each group was measured using automatic glucose analyzer (Accu-check, Blood glucose monitoring kit) on weekly basis. These procedures were repeated for every seven days.

RESULTS AND DISCUSSION

Effect of chemically induced Diabetes on body weights

There is increase in body weights in control animals by 8% during one month experimental period. But in all diabetic groups, drastic decrease in body weights was observed. 28.6%, 24.8%, 21.1% and 8.8% decrease in body weights of Group 2, Group 3, Group 4 and Group 5. Maximum % reduction was found in Group 2 i.e. STZ induced diabetic model. These models include the characteristics of Type I Diabetes. Thus, more loss in body weights was observed.

Table No. 1. Changes in body weights in normal and diabetic animals

Group	0 week	1 week	2 Week	3 Week	4 Week
Group-1	191.42±9.2	198.3±5.5	201.1±14.6	200±1.31	206.7±4.7
Group-2	192.8±0.53	184.17±0.53	186±0.86	150.17±8.45	137.67±4.64
Group-3	190.56±2.42	158.2±4.5	151.5±6.8	153±2.0	143.3±3.13
Group-4	198.4±10.12	201±5.19	190±2.12	142±12.12	156.5±13.12
Group-5	208.3±16.24	184±24.27	183.8±31.6	193.8±31	190±2.94

Values are Mean ± SEM. (n=6)

Blood glucose levels in various chemically induced models of Diabetes

After 3 days of induction, the blood glucose was high in 90% of the animals. All the animals showing blood glucose levels > 250 mg/dl were included in study. Blood glucose levels were comparatively high in only STZ induced and only Alloxan induced diabetic rats. The

nicotinamide and Alloxan model showed decreased blood glucose levels after one month indicating pancreatic regeneration. 39% reduction was observed after one month diabetes. So optimizing this model with either increased Alloxan or decreased Nicotinamide administration is required to have further utility.

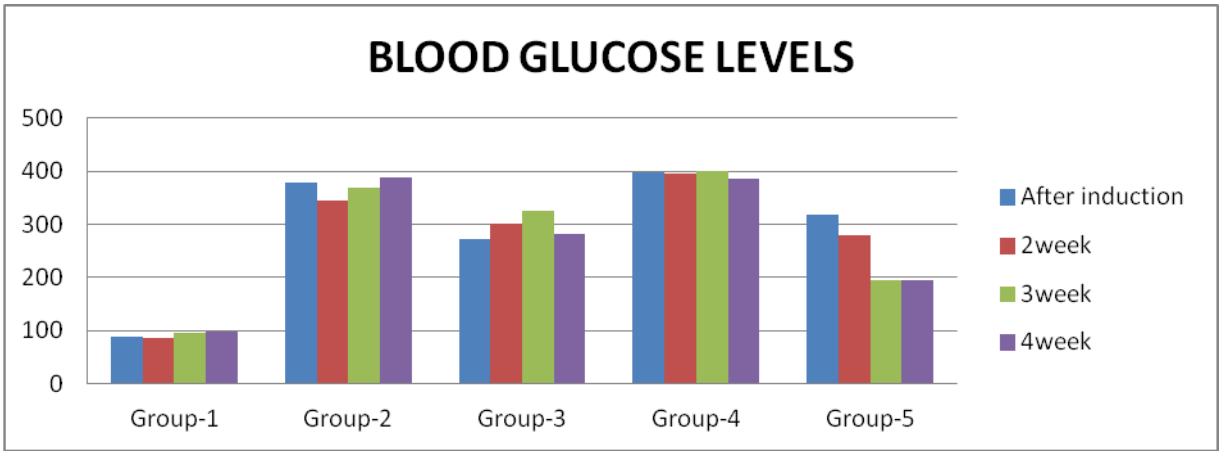

Figure No. 1:Weekly blood glucose leves in normal and diabetic animals

Values are Mean ± SEM. (n=6)

Effect of Chemically induced diabetes on Food and water intake

The water and food intake are relatively high in all diabetic groups especially Polydipsia and polyphagia are two important characteristics of diabetes. 59.4 %, 106.3%, 86.3% and 36.3% raise in food intake in group 2, 3, 4 and 5 respectively compared to the control group. 48.4%, 48.4%, 108.9 % and 9.7% rise in average water intake in group 2, 3, 4 and 5 respectively compared to the control group. Group 3 and 4 exhibited very much increased intake of both water and food.

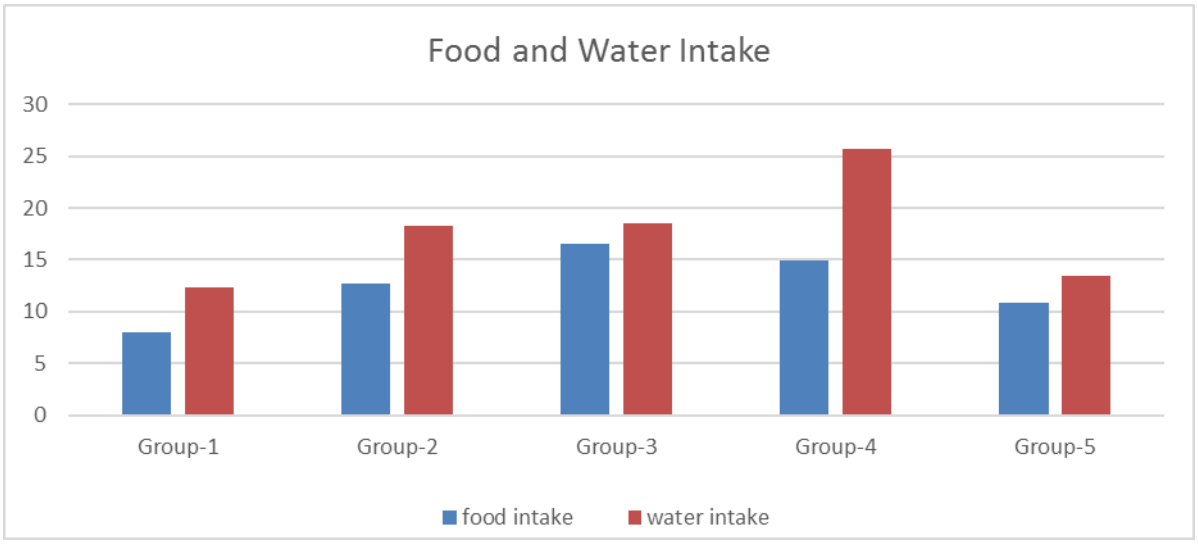

Figure No. 2: Weekly food and water consumption in normal and diabetic animals

Mortality observed in different chemically induced models of Diabetes

The number animal dead in each group during the one month of experimental was observed and percentage mortality was calculated by using the following formula

%Mortality = (No of animals dead ÷ Total no of animals) ×100

Table No. 2. Percentage mortality in various models of Diabetes

Model	**% Mortality rate**
Streptozocin induced model	20%
Streptozocin + Nicotinamide induced model	11%
Alloxan induced model	50%
Alloxan + Nicotinamide induced model	40%

Least mortality was found in case of streptozocin and nicotinamide induced model and highest mortality was found with alloxan only model.

DISCUSSION

Many diabetic animal models are used to show and research common traits of diabetes types, such as hyperglycemia effects or diabetic complications, and cannot necessarily be classified as Type 1 or Type 2 Diabetes Mellitus.

STZ is a nitrosourea analogue in which the N-methyl-N-nitrosourea (MNU) molecule is linked to the carbon-2 of hexose. The DNA alkylating activity of STZ's methyl-nitrosourea moiety determines the mode of action of its toxicity. The transfer of the methyl group from STZ to the DNA molecule produces DNA fragmentation by causing damage along a predetermined sequence of events [4]. Alloxan is a toxic chemical that damages ß cells, resulting in a state of primary insulin insufficiency. Alloxan inhibits glukokinase, which has a signal-recognition function in linking insulin secretion to glucose levels. Indeed, the enzyme's sulphydryl groups may be the major intracellular target for alloxan and ultimately accountable for ß cell toxicity [5]. By separate mechanisms, both Alloxan and STZ cause hyperglycemia through pancreatic damage. Nicotinamide is an antioxidant that protects pancreatic ß cell mass from the cytotoxic effects of STZ by scavenging free radicals and produces relatively moderate damage to type-2 diabetes patients. In adult rats, partial protection from the -cytotoxic effect of streptozotocin (STZ) provided by appropriate doses of nicotinamide resulted in a new experimental diabetic syndrome that appears to be closer to NIDDM than other available animal models in terms of insulin responsiveness to glucose and sulfonylureas. As a result, this model is viewed as a useful tool for studying insulin sensitivity [6]. In rats, co-administration of alloxan and nicotinamide causes biochemical and clinical abnormalities comparable to those seen in type 2 diabetic mellitus [7].

Our findings show that, though each individual's sensitivity to the different models differs, the hyperglycaemia state was created in all models after 48 hours. A rapid biphasic increase in insulin production is normally elicited by a rise in normal glucose levels. As a result of ß cell loss, chronic hyperglycemia causes this reaction to almost completely diminish.

STZ, STZ+NAD, and Alloxan induced diabetic rats maintained continuous hyperglycemia for a month, according to our findings. However, due to the peak hyperglycemic effect, there was a relatively significant mortality rate in the Alloxan and STZ groups, which may necessitate insulin administration for animal survival. Our findings are consistent with earlier research [8]. Body weights of STZ injected animals were decreased drastically because of severe hyperglycemia. Much increased food and water intake was found in alloxan group and it might be due to maximum hyperglycemia. But maximum polyphagia was observed in STZ+ NAD group. By the end of 30 days, the Alloxan+ NAD group had reversed hyperglycemia, which could be attributable to pancreatic regeneration [9]. In all aspects, STZ+ NAD caused diabetic rats demonstrated good consistency. Streptozotocin is chosen

over alloxan because it has various advantages over alloxan, including a longer half-life, a longer duration of hyperglycemia, and better established diabetic complications.

Though, most of the time, these models are chosen for their utility in researching the processes of diabetic complications, physiological responses, or current regeneration or transplantation technologies, rather than for their capacity to simulate one form of diabetes or the other [10].

CONCLUSION

Three existing models and one novel model were investigated. STZ and nicotinamide models outperformed all other models evaluated in terms of successful induction, maximum stability, and lowest mortality. The Alloxan + Nicotinamide model is also a potentially useful model, although it requires good standardization. This strategy has the potential to become more cost-effective and consistent. However, all researchers should be aware of the ethical implications of using animal models in their research.

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