Can the Mortality Rate be Reduced in the Diabetes Induction Model in Rats? A Protocol Study

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Abstract- The use of animal models of diseases is essential for the study of the effects of various drugs and the discovery of new drugs. One of the most common problems in researches on diabetes in animal models is the high rate of mortality after diabetes induction. This leads to disrupt the grouping of diabetic animals for interventional evaluations. We introduce a protocol to reduce the rate of mortality in diabetic rats. To do this, we used alloxan at dose of 185 mg/kg to induce diabetes in rats. In this study in addition to provide 5% glucose solution in drinking water from 2 to 12 hrs post alloxan injection, 2 ml of 50% dextrose solution orally gavaged with 2 hrs intervals up to 12 hrs post diabetes induction, and also from 12 to 24 hrs following alloxan injection 2 ml of 5% glucose solution was given by gavage every 2 hrs. Furthermore, from 6 to 48 hrs post alloxan injection the rats were orally received 1 ml of Ringer's serum with 2 hrs intervals via gavage route. Moreover, one unit of Novolin 70/30 (70% NPH and 30% insulin regular) was subcutaneously injected in the back of the neck area for 5 consistence days from the second day post alloxan injection. In the sixth day following alloxan injection the level of glucose was at level of 280 mg/dL which considered as diabetic level. Our findings showed that by abovementioned interventions the rate of mortality significantly reduced (2 percent) and diabetes well established.

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Keywords: Diabetes; Protocol; Reduce mortality; Diabetes establishment

Introduction

The use of animal models in researches is necessary to get access to new investigations and treatments for various diseases (1,2). Alloxan monohydrate and streptozotocin is commonly used to induce diabetes in rodents (3,4). Based on the experiences and results from previous studies in particular conducted by this research team on using alloxan monohydrate for induction of diabetes in rats the following problems have arisen: Failure in diabetes induction in a significant number of rats with the prescribed dose based on the previous studies (5), severe lethargy in rats at the onset of the study, and most importantly, the presence of mortality rate of 50-70% during the first week of induction which disrupts the grouping of animals to be included in the study.

Based on the pilot studies of this research team, the possibilities that caused high mortality as well as the possible protective interventions were considered in this study. These possibilities are including the low blood glucose level due to keeping them in a fasted state for 12 hrs prior to the diabetes induction (6), hypoglycemic shock during the first hours post alloxan injection due to severe damage to the pancreas leading to increase in blood insulin (7), hypovolemic shock caused by fatigue, not drinking water, and diuresis caused by high blood glucose (8,9), water-electrolyte imbalance (10), diabetic coma (11), and finally, possibility of occurrence of multiple stresses in the early hours post alloxan injection. Symptoms of fatigue in rats are including: Skewering hairs, drooping of mustache, increasing rate of respiration and heart rate. In this conditions, rats gathering in the corner of the cage and take refuge in each other and not eating. According to the experiences gained from previous studies, mortality begin

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approximately 4 hrs post alloxan injection and the number of casualties increases over time such that more than 50% 48 hrs and more than 70% at the end of first week following injection. Therefore, the grouping of animals in order to receive therapeutic interventions in various studies faces problems (12,13).

Thus, by focusing on the above-mentioned problems in experimental diabetes studies, this study was conducted to introduce a protocol for reducing the rate of mortality in alloxan-induced diabetes in rats in order to make a condition to stabilize diabetes in rat.

Materials and Methods

Animals and conditions

Twelve male wistar rats weighting 200-250 g were bought from Tehran Pasteur Institute, Iran. For environmental adaptation, all rats were then transferred to animals' house of Shahrekord University of Medical Sciences and kept there in standard conditions at an ambient temperature of 31° C under a 12:12-hrs light: dark cycle for one week without doing any intervention. Animals were kept on straw bedding in 20×45×30 cm special cages in groups of four. All rats were allowed free access to water and food y. All experimental procedures in this study were approved by the Ethics Committee of Shahrekord University of Medical Sciences, Shahrekord, Iran (ethical number: IR.SKUMS.REC1394.233), and NIH Guidelines for the Care and Use of Laboratory Animals, revised 2011.

Diabetes induction method

To induce diabetes, alloxan was injected at dose of 185 mg/kg of body weight (BW) in two divided doses on two consecutive days (6,14,15). Alloxan was dissolved in cold saline and injected intraperitoneally at dose of 120 mg/kg BW on the first day and at a dose of 65 mg/kg BW on the second day. The animals were fasted 12 hrs (overnight) before induction of diabetes (18-16).

Blood glucose measurement

The blood glucose levels in animals were measured using K meter Match Glucometer, made in Taiwan, by pricking the tail with a lancet. The blood glucose level was measured 12 hrs post alloxan injection. The blood glucose levels in animals were also measured on the second day with 12 hrs intervals. Also we measured glucose levels daily from days 3 to 6.

Interventions in the first 24 hrs post alloxan injection

Considering the abovementioned possibilities, previous studies have provided solutions to prevent fatal hypoglycemia and reduce mortality like use of 5% glucose solution in drinking water (19-21) or use 15-20 ml intraperitoneal injection of 20% glucose solution (20). Despite using these methods, mortality rate is still high during the first 24-48 hrs post alloxan injection. As rats are nocturnal animals and mostly eat food during the night, providing 5% glucose solution cannot alone sufficiently increase the blood glucose level and consequently reduce mortality post alloxan injection. It's could be due to long time from alloxan injection till night (about 12 hr). On the other hand, intraperitoneal injection of high volume of 20% glucose solution (15-20 ml) in addition to impose high stress to rats could not significantly reduced mortality following diabetes induction. Therefore, in this study in addition to provide 5% glucose solution in drinking water from 2 to 12 hrs post alloxan injection, 2 ml of 50% dextrose solution orally gavaged with 2 hrs intervals up to 12 hrs post diabetes induction, and also from 12 to 24 hrs following alloxan injection 2 ml of 5% glucose solution was given by gavage every 2 hrs.

Measurement of volume of urine during 24 hrs period

The rats usually consume 10-12 ml water per 100 grs BW per day and produce a urine volume of 13-33 ml/24 hr (22). Following injection of alloxan, significant polyuria occurs (23). In previous studies, the volume of 24-hour urine in alloxan induced diabetic rats was measured using metabolic cages. The obtained results showed that diabetic rats excreted 45-83 ml urine in 24 hrs (24). With this volume of urine, a significant amount of fluids and electrolytes excreted from the body which undoubtedly led to high rate of mortality. Considering these conditions, excreted water and electrolytes should be replaced to avoid mortality. In the other words, we should provide balance between intake and output of electrolytes (25-28). To resolve this problems, in the present study, from 6 to 48 hrs post alloxan injection the rats were received orally 1 ml of Ringer's serum with 2 hrs intervals via gavage route.

Blood glucose level

In previous studies, the rats' blood glucose increased post alloxan injection up to 600 mg/dL and even more than 600 mg/d. Considering the increased diuresis and severe increase in blood glucose levels, the possibility of incidence of diabetic coma in rats in the first days post alloxan injection is raised. These conditions are similar

to excessive increase in blood glucose levels in humans, which eventually lead to shock, coma, and finally death (8). To overcome this problem, in the present study, one unit of Novolin 70/30 (70% NPH and 30% insulin regular) was subcutaneously injected in the back of the neck area for 5 consistence days from the second day post alloxan injection.

Diabetes stabilization

No intervention was done from the sixth day post alloxan injection (considering the above-mentioned intervention methods up to the sixth day) and rats had free access to water and food. In the six th day following alloxan injection the level of glucose was measured. The obtained results showed that all rats have high blood glucose at level of 280 mg/dL which according to the previous studies, this level considered as diabetic level (29,30). Thus, with low mortality rate (2 %), diabetes was established and animals were in access for interventional studies.

Discussion

Rats are nocturnal and do most of their activities, such as feeding, at night. They actually sleep during the day or eating so little during day (31). In order to induce diabetes rats kept in the fasted state at night before alloxan injection. The real duration of fast state seems to be 24 hr because they have eaten no food from the day before the diabetes induction. This condition lead to moderate to severe reduces in the blood glucose level which could be caused hypoglycemic shock. Consequence of alloxan injection the pancreas destroy and a large amount of insulin secreted into the blood; this alone causes severe hypoglycemia (7). This condition lead to fatigue and anorexia (do not ability for eating and drinking). Therefore, the incidence of hypoglycemic shock and mortality is expected (32). In order to overcome this problem in addition to provide 5% glucose solution in drinking water from 2 to 12 hrs post alloxan injection, 2 ml of 50% dextrose solution orally gavaged with 2 hrs intervals up to 12 hrs post diabetes induction, and also from 12 to 24 hrs following alloxan injection 2 ml of 5% glucose solution was given by gavage every 2 hrs. In some studies, 15-20 ml of 20% glucose solution was intraperitoneally injected to manage hypoglycemia post alloxan injection (20). We did not use this method because intraperitoneal injection of 20 ml solution is painful and create an overestimate stress to the animals which can worsen the condition.

Following injection of alloxan, significant polyuria occurs which led to high excretion of liquid and electrolytes which undoubtedly led to high rate of mortality (23). We should replace electrolytes to reduce mortality rate. To do this, from 6 to 48 hrs post alloxan injection the rats were received orally 1 ml of Ringer's serum with 2 hrs intervals via gavage route.

Following diabetes induction, the level of blood glucose increase hazardously which lead to incidence of diabetic coma and death in the first days post alloxan injection (8). To overcome this problem, in the present study, one unit of Novolin 70/30 (70% NPH and 30% insulin regular) was subcutaneously injected in the back of the neck area for 5 consistence days from the second day post alloxan injection.

Following our interventions, we successfully established diabetes. The blood glucose level was 280 mg/dL which considered as diabetic level. Our exciting finding is that we observed low rate of mortality by our interventions (2%).

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