Dose optimization of Alloxan for diabetes in albino mice

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ABSTRACT

Alloxan monohydrate induces diabetes in animals, although streptozotocin. The other diabetogenic chemical is less toxic but comparatively expensive. This study was conducted to provide an optimization of alloxan for diabetes induction in mice with single intraperitoneal injection. Twenty-four healthy Albino mice of a local strain weighing 25 to 30 gms were selected and injected with varying doses of alloxan monohydrate (from 100mg - 400mg)/ kg body weight in order to induce diabetes. The blood sugar random (BSR) and blood sugar fasting (BSF) were checked after 24, 72 hours and 7 days after injecting the alloxan. The mice did not sustain the 400 mg/kg of dose and showed 100% mortality. The optimized dose (200mg/kg) was found to lower the mice mortality by 50%. Diabetic mice attained the blood sugar levels ranging from 220mg/dl to 350mg/dl. There was statistically significant (p< 0.05) increase in feed intake in diabetic group as compared to control group. Whereas, decrease in body weight in diabetic group occurred during the same observational period. It was therefore concluded that our proposed regime may be beneficial for future researchers aiming to develop a similar animal model for the study of pathology of diabetes.

Key words: Alloxan monohydrate, streptozotocin, BSR (blood sugar random), BSF (blood sugar fasting).

INTRODUCTION

Diabetes mellitus is a hereditary disorder with multiple alleles located on different chromosomes. Defects either in secretion or action of insulin lead to characteristic elevated sugar level (Degirmenci et al., 2005; Kota et al., 2012). Type 2 diabetes (T2D) is one of the disease types in which deficiency of insulin occurs. Persons with diabetes produce insulin endogenously but gradually their body cells stop to respond and eventually cells do not take glucose from blood. In this way a sort of insulin résistance is developed (Wild et al., 2004). It is more common among the obese and occurs mostly above the age of 40years. Inadequacy of glucose control leads to vascular complications accounting for morbidities and mortalities associated with the disease. Glycemic control is helpful to reduce the risk of developing complications associated with T2D (ADA, 2012). T2D is found to be multifactorial and its relationship with obesity, hypertension and glucose intolerance is well documented (Steinberger & Daniels, 2003; Tan et al., 2006). Family history and urban residency are also linked with increased risks of diabetes. Certain factors like higher body mass index (BMI), sluggish lifestyle and hypertension aggravate the diabetes (Jayawardena et al., 2012).

Earlier studies have also shown those BMI and waist hip ratios (WHR) are risk factors for T2D independently (Wei et al., 2012).

Various diabetic animal models have been established either surgically (Black et al., 1980) or chemically (Szkudelski, T., 2001) using variety of modes, doses and routes of administration (Gosh & Suryawanshi, 2001; Sarasa et al., 2012; Akuodor et al., 2014). Alloxan in its monohydrate form is one of the chemical compounds used for the induction of diabetes since long (Etuk, 2010; Iranloye et al., 2011). The diabetogenic dose of alloxan varies considerably amongst species, age and metabolic state of the animal. To the best of our knowledge intraperitoneal (i.p) dose of alloxan monohydrate has not been optimized yet in laboratory mice. Therefore the present study was undertaken for this purpose.

MATERIALS AND METHODS

Animals

Healthy albino mice of 25 to 30 g were used and kept under observation for one week in the animal house under controlled conditions. The animals were fed pelleted diet having composition of carbohydrates 70%, proteins 18%, fats 4.9%, fiber 3.2%. The feed and water were available ad libitum.
All the mice were kept in the standard cages (8″ X 18″ X 10″) in groups of three to prevent from cannibalism and reared at 26±4˚C, relative humidity 55-60% and light dark cycle 12h: 12h.

Chemicals

All the chemicals were of analytical grade and obtained from commercial source as indicated: Alloxan monohydrate from Alfa Aesar Johnson Company Great Britain and Blood Glucose determination Glucosure strips from Apex Bio, Taiwan.

Experimental design

Vanitha et al. (2013) method was used to induce diabetes into the mice. Briefly overnight-starved mice were made diabetic with a freshly prepared dose of alloxan monohydrate in pyrogen free water. Single intraperitoneal dose of 100, 200, 300 and 400 mg/kg b.w. was injected into group I, II, III & IV, respectively (n=9). Blood was taken from tail; BSR (Blood Sugar Random) and BSF (Blood Sugar Fasting) were checked after 24, 72 hours and at day 07. According to Luka et al., (2013) mice with BSR and BSF more than 150mg/dl were considered as diabetic and were used for further study. Morphological and behavioral changes were observed throughout the experiment.

Selected dose of Alloxan (group II) was further used to check its effect on feed intake and body weight of mice compared with non-diabetic (negative control). Body weight of each mouse was measured and recorded for each mouse throughout the experiment. Measured quantity of pelleted diet was put into cage and at the end of 24 hours remaining quantity of feed was weighed.

Calculation of LD 50.

LD 50 was calculated according to Reed and Munch formulae.

Statistical analysis

The data was presented as Mean ± S.E.M. One Way Analysis of variance (ANOVA) was performed on means to determine the significant (p < 0.05) difference among the groups.

RESULTS

During the experiment certain behavioral (sluggish body movements, shivering and timmed eyes) and morphological changes (thinning of body hairs and khyphosis) were observed in diabetic group. The dose of alloxan monohydrate for each mouse was selected carefully based not only on the body weight but also on the general conditions of the animal were also noticed. Most of the mice became diabetic on first administration. The animal did not sustain the 400 mg/kg of dose and showed more than 70% mortality.

Table I: Calculation of LD 50.

<table>
<thead>
<tr>
<th>Doses Selected (mg/kg)</th>
<th>Number of mice uninfected</th>
<th>Number of mice infected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Uninfected</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
</tr>
<tr>
<td>100</td>
<td>09</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>200</td>
<td>03</td>
<td>06</td>
<td>09</td>
</tr>
<tr>
<td>300</td>
<td>0</td>
<td>09</td>
<td>09</td>
</tr>
<tr>
<td>400</td>
<td>0</td>
<td>09</td>
<td>09</td>
</tr>
</tbody>
</table>

a The sum of all of the uninfected mice at that dose and higher.

b The sum of all of the infected mice at that dose and lower.

c The total infected divided by the sum of the total uninfected and total infected multiplied by 100.

Fig. 1: Flow sheet showing calculation of LD 50.
Using the numbers in the table above following calculations were performed:

\[
\begin{align*}
50 \text{-} 40 \text{ (the percent infected below 50\%)} \\
72 \text{ (the percent infected above 50\%)} \text{-} 40 \text{ (the percent infected below 50\%)} \\
= 0.31 \\
\log_{10} 2 \text{ (dose at which less than 50\% of the mice become infected)} = 2 \\
0.31 + 2 = 2.31 \\
\text{Inverse log 2.31} \\
\text{So LD50 = 204 or } 2.04 \times 10^2 \text{ mg/kg}
\end{align*}
\]

Those mice which were not diabetic after 24h were given booster of 100 mg/kg of alloxan monohydrate. All the surviving diabetic mice had blood sugar levels ranging from 220 mg/dl to 350 mg/dl. As they survived without insulin injections, the mice had developed TD2 which was the requirement of our animal diabetic model. Blood sugar levels of 24 mice were recorded before and after alloxan administration given in Table II (a).

**Table II (a): BSR & BSF of mice before and after 24 hours of Alloxan Administration**

<table>
<thead>
<tr>
<th>Group</th>
<th>% Mortality</th>
<th>Dose selected (mg/kg)</th>
<th>BSR (mg/dl)</th>
<th>BSF Levels after Alloxan induction (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before Alloxan induction</td>
<td>After 24 hour of Alloxan induction</td>
</tr>
<tr>
<td>I</td>
<td>Nil</td>
<td>100</td>
<td>80±3.46</td>
<td>100±3.45</td>
</tr>
<tr>
<td>II</td>
<td>50</td>
<td>200</td>
<td>80±3.0</td>
<td>250±3.06</td>
</tr>
<tr>
<td>III</td>
<td>75</td>
<td>300</td>
<td>80±2.96</td>
<td>350±4.0</td>
</tr>
<tr>
<td>IV</td>
<td>100</td>
<td>400</td>
<td>80±3.16</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table II (b): BSR & BSF of mice before and after 72 hours and 7 days of Alloxan Administration**

<table>
<thead>
<tr>
<th>Group</th>
<th>BSR (mg/dl)</th>
<th>BSF (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 72 hours</td>
<td>After 07 days</td>
</tr>
<tr>
<td>I</td>
<td>95±2.65</td>
<td>95±3.65</td>
</tr>
<tr>
<td>II</td>
<td>250±3.4</td>
<td>278±4.10</td>
</tr>
<tr>
<td>III</td>
<td>345±3.0</td>
<td>356±5.90</td>
</tr>
<tr>
<td>IV</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

Values represent Means of triplicates with Mean±S.E.M. (*) = died.

**Effect of diabetes on feed intake and body weight**

There was an increase in the feed intake from 2.80±0.12 gms to 3.40±0.12 gms /24 hrs from day 0 to post 21 days in non diabetic group, respectively. Whereas, in diabetic group the feed intake increased from 2.97±0.23 gms to 6.20±0.23 grams /24 hrs from day 0 to post 21 days. The result indicated that there was statistically significant (p< 0.05) increase in feed intake in diabetic group as compared to normal group (Table III). There was an increase in the weight of mice from 30.03±0.69 on day 0 to 34.60±0.64 gms post 21 days in normal group. Whereas, in diabetic group the decrease in body weight occurred from 30.47±0.55 to 25.27±0.78 gms during the same observational period. There was significant decrease (p< 0.05) in body weight in diabetic group as compared to normal group (Table II).
Table III: Changes in feed intake and body weight in normal and diabetic mice in grams

<table>
<thead>
<tr>
<th>No. of Days</th>
<th>Feed intake</th>
<th></th>
<th>Body weight</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Diabetic</td>
<td>Normal</td>
<td>Diabetic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.80±0.12</td>
<td>2.97±0.23</td>
<td>30.03±0.69</td>
<td>30.47±0.55</td>
</tr>
<tr>
<td>7</td>
<td>2.90±0.06a</td>
<td>3.60±0.26</td>
<td>32.40±0.40a</td>
<td>27.57±0.38b,c,d</td>
</tr>
<tr>
<td>14</td>
<td>3.23±0.09ab</td>
<td>4.80±0.21a,b,c,d</td>
<td>33.57±0.48a</td>
<td>26.10±0.78a,b,c,d</td>
</tr>
<tr>
<td>21</td>
<td>3.40±0.12a</td>
<td>6.20±0.23a,b,c,d</td>
<td>34.60±0.64a</td>
<td>25.27±0.7878ab,c,d</td>
</tr>
</tbody>
</table>

Means with the same superscript differ significantly at p < 0.05. Similar alphabets represent statistically significant values.

DISCUSSION

Various chemical or surgical diabetic animal models have been established to study the disease. Alloxan monohydrate is one of the routinely used chemical compound since long (Etuk, 2010; Iranloye et al., 2011). It varies considerably amongst species, age and metabolic state of the animal. In the present study single intraperitoneal injection of alloxan monohydrate (200 mg/kg) produced experimental diabetes after 07 days in albino mice used as animal model. However, others workers reported its different doses for induction of diabetes. Sarasa et al., 2012 reported 120mg/kg of alloxan monohydrate induced diabetes in mice, Gosh & Suryawanshi, 2001; Akuodor et al., 2014 reported 150 mg/kg of alloxan monohydrate induced diabetes in rats.

The most plausible explanation for induction of diabetes due to alloxan is that following its administration, it may have accumulated in the islets of Langerhans and in the liver where it is reduced to dialic acid. Lenzen, (2008) reported alloxan to be an effective pro-oxidant selectively cytotoxic to ß cells of the pancreatic islets of Langerhans. Alloxan induced diabetes is also suggested to result from initial islet cell inflammation followed by activation of macrophages and lymphocytes that might be the source of cytotoxic oxygen radicals (Trivedi et al., 2004). In the present study changes in feed intake were also noticed in grams/24 hrs. It was observed that in diabetic mice it was almost twice as compared to normal. The increase in feed intake might be associated with the increased body metabolism. Similar results of change in feed intake were reported by Vanitha et al., 2013. On contrary Mallick et al. (2007), reported decrease in the amount of feed intake in streptozotocin induced diabetic rats. Furthermore, body weight of diabetic mice was significantly reduced in diabetic group post 21 days exposure. Luka et al. (2012) have indicated that diabetes induction with 100 mg/kg of alloxan monohydrate caused reduction in the amount of weight which may be associated with the increased body metabolism on day 14.

Conclusion

It is concluded from the above findings that single intraperitoneal injection of alloxan monohydrate at the dose of 200 mg/kg b.w. produced symptoms of diabetes in mice along with rise of blood sugar random and blood sugar fasting levels more than 150 mg/dl from day 0 to 21 days post induction.

REFERENCES


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