Induction of Diabetes Mellitus in Rats Using Intraperitoneal Streptozotocin: A Comparison between 2 Strains of Rats

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Abstract

The purpose of this study was to determine species related differences in the diabetogenic response to streptozotocin (STZ) after single intraperitoneal injection. Twenty adult, male, nude rats (NR, strain Crl:NIH-Fox1Rnu) and 8 adult, male Sprague-Dawley (SD) rats were used to induce diabetes using streptozotocin. A single, 150mg/kg STZ was injected intraperitoneally in both strains of rats. Four rats (2 from each strain) were injected buffer and served as control. Severity of the induced diabetic state was assessed by daily monitoring of body weights, clinical signs, and blood glucose levels. Four rats in the NR group died in an average of 5 days following STZ injection and 4 rats of the SD group died in an average of 2 days. In the NR group, non fasting serum glucose levels (267 ± 80 mg/dl) rose significantly (P ≤ 0.05) only in 7 (35%) rats while all SD group experienced glucose levels above 300 mg/dl (324 ± 20 mg/dl) and required insulin treatment all over the
observation period (21 days). It appears that SD rats are far more susceptible to a single intraperitoneal injection of 150mg/kg STZ than NR.

**Keywords:** Streptozotocin • nude rats • Sprague-Dawley rats • Diabetes

**Introduction**

Diabetes mellitus is a chronic, widely spread human disease. Experimental induction of diabetes mellitus in animal models is essential for the advancement of our knowledge and understanding of the various aspects of its pathogenesis and ultimately finding new therapies and cure. Several methods have been used to induce diabetes mellitus in laboratory animals with variable success and many difficulties. Surgical removal of the pancreas is effective method; however, to induce diabetes, at least 90-95% of the pancreas has to be removed [1]. Injection of anterior hypophysis extract has been used to induce diabetes with less reliable results [14]. Another method which is more uniformly effective and widely used is the injection of streptozotocin.

Streptozotocin (STZ; N-nitro derivative of glucosamine) is a naturally occurring, broad-spectrum antibiotic and cyto-toxic chemical that is particularly toxic to the pancreatic, insulin-producing beta cells in mammals [6,17,18,19]. Induction of experimental diabetes in the rat using streptozotocin is very convenient and simple to use [2,10,19]. Streptozotocin injection leads to the degeneration of the Langerhans islets beta cells [9,15,19]. Clinically, symptoms of diabetes are clearly seen in rats within 2-4 days following single intravenous or intraperitoneal injection of 60mg/kg STZ. Understanding strain differences in the diabetogenic activity of STZ is essential for the use of this diabetic agent in different animal models. The objective of this study was to evaluate the diabetogenic activity of single intraperitoneal injection of STZ in 2 common strains of laboratory rats; namely nude rats (NR) and Sprague-Dawley (SD) rats following single intraperitoneal injection.

**Materials and Methods**

**Animals**

Twenty adult, male, nude rats (average body weight 275 ± 25g; strain Crl:NIH-Fox1^RNU, Charles River, Wilmington, MA) and 8 adult, male Sprague-Dawley (Locally produced) rats (average body weight 350 ± 40g) were used to induce diabetes. Animals were housed individually in a special clear-sided cages at controlled temperature (22º C) with a 12:12-h light:dark cycle and had free access to water and chow diet over a 2-wk adaptation period. Body weights were measured twice weekly. All experimental procedures were approved and supervised by the Jordan University of Science and Technology Animal Care and Use Committee (JUST-ACUC) which conforms to the principles laid by the National Research Council Guide for the Care and Use of Laboratory Animals.

**Induction of diabetes**

Rats were fasted for 12-h before diabetes was induced using STZ. NR and SDR received a single intraperitoneal injection of 150mg/kg of STZ (Sigma, St. Louis, MO, USA). STZ was freshly dissolved in 0.05 M citrate buffer, pH 4.5. For the i.p. injection of STZ, the rat was held in one hand in dorsal position, the injection site was swabbed using povidone-iodine solution and the designated amount of STZ was injected in the caudal abdominal cavity using sterile 25g needle. Four rats (2 from each strain) were injected citrate buffer and served as control.

**Serum Glucose Levels**

Severity of the induced diabetic state was assessed by daily monitoring of blood glucose levels. For the determination of blood glucose using Glucocheck (Biotest Medical Corp., Tortola, VI, USA), whole
blood was from the tail vein from all rats immediately before STZ injection (Time 0; T0) and daily until euthanatized. STZ was dissolved in citrate buffer (pH 4.5) and injected intraperitoneally within 10 min after preparation. Animals whose blood glucose level exceeded 200 mg/dl at 24 h after treatment were considered diabetic. Rats with blood glucose levels exceeding 400 mg/dl were administered 10 units insulin (Mixtrad 30, Novo Nordisk, Denmark) subcutaneously. In addition whole blood was collected in plain blood tubes at T0 and on days 1, 3, 7 and 14 (non-fasting) following STZ injections. Serum was separated using a centrifuge (ALC Centrifuge 4206, Milano, Italy) at 1500 g for 5 minutes. Serum was then placed in labeled plastic tubes and stored at –20ºC until testing. Glucose serum levels were determined using Trinder method (Glucose GOP-PAP).

**Oral Glucose Tolerance Test**

To determine animals with apparently low blood glucose (Blood glucose less than 200 mg/dl) 5 days after STZ injection are actually diabetic, oral glucose tolerance test was performed on 13 NR. Rats were fasted for 12 h before the test and 2 g/kg glucose solution was administered orally. Blood samples were taken by severing the tip of the tail 1 h before and at 0.5 and 1 and 2 h after glucose administration.

**Statistical Analysis**

Results are expressed in mean ± S.E.M. Data were analyzed using one-way analysis of variance and Duncan's multiple range test or nonparametric statistics. Statistical analyses were performed using Graphpad Prism for windows (Graphpad, San Diego, CA). P value ≤ 0.05 was considered significant.

**Results**

Twenty, nude adult NR and 8 SDR were used in this study. Fasting blood glucose levels before STZ injection were 133 ± 15 mg/dl and 142 ± 22 mg/dl in NR and SDR respectively (Table 1). In the NR group, non fasting serum glucose levels rose significantly (P ≤ 0.05) only in 7 (35%) rats and required insulin treatment while all SDR group experienced glucose levels above 300 mg/dl (324 ± 20 mg/dl) and required insulin treatment all over the observation period (21 days). The mean ± SD of blood glucose level in 7 NR rats was 414 ± 76 mg/dl while in the rest of the rats it was 221 ± 54 mg/dl. Collectively NR had 267 ± 80 mg/dl blood glucose levels after the first STZ injection.

**Table 1:** Mean ± standard deviation and ranges of blood glucose levels in nude rats and Sprague Dowely rats following STZ injection (mg/dl).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Control</th>
<th>First STZ n= 20</th>
<th>Second STZ n=13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 3</td>
<td>Day 7</td>
</tr>
<tr>
<td>Nude rats</td>
<td>133±14</td>
<td>171±8*</td>
<td>140±12</td>
</tr>
<tr>
<td></td>
<td>111-161</td>
<td>110-225</td>
<td>123-256</td>
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<tr>
<td></td>
<td>111-161</td>
<td>324±33*</td>
<td>400±24*</td>
</tr>
<tr>
<td>Sprague- Dawley</td>
<td>142 ± 22</td>
<td>235-567</td>
<td>267-524</td>
</tr>
<tr>
<td></td>
<td>122-154</td>
<td>324±33*</td>
<td>400±24*</td>
</tr>
</tbody>
</table>

P ≤ 0.05
NA= not performed

Four rats in the NR group died in an average of 5 days following STZ injection and 4 rats of the SDR group died in an average of 2 days. The rest of the rats were euthanatized at the end of the study. Rats in the SD group lost weight significantly (350 ± 40g to 280 ± 35g) and appeared dull with rough hear coats compared to the body weight and condition of the ND (275 ± 25g to 267 ± 20g) and control groups.
Oral glucose tolerance test was carried out in 13 NR with blood glucose levels less than 200 mg/dl, 5 days after STZ injection. In 6 rats, blood glucose level (280 ± 20 mg/dl) was significantly elevated and remained high during the test indicating their diabetic condition. The remaining 7 NR and none of the SDR required a second i.p. injection of STZ. The second STZ injection was performed 1 week after the first injection. The mean non fasting glucose level following the second STZ injection was 220 ± 17 mg/dl) in all NR group.

Discussion
Streptozotocin has been widely used to induce type 1 diabetes in animal models especially rats and mice [6]. It has been reported that STZ induce dose-dependant diabetes administered either intravenously or intraperitoneally [6]. Intraperitoneal injection of STZ led to physiologic alterations consistent with reports of spontaneous and chemically induced diabetes in other animals [8,12,13]. Alterations in serum glucose revealed a dose–response relationship between STZ and the severity of diabetes in this and other studies [4,11,16]. In rats, it has been reported that a dose ranging from 25 to 100 mg/kg STZ injected intravenously was successful in inducing a dose dependent hyperglycemia [6]. In nude mice, it was reported that 150mg/kg STZ dose sufficient to induce pathological blood glucose [18].

Different strains of mice have been reported to respond differently to STZ injection using doses raging from 75 to 200 mg/kg intraperitoneally where only 100 mg/kg STZ was found to induce slowly-progressive diabetes mellitus [4,10,11]. In this study, we used 125 mg/dl of STZ intraperitoneally in a single dose to compare its diabetogenic activity in 2 common strains of experimental rats; nude rats and Sprague-Dawley rats. Such comparison between these 2 strains of rats has never been addressed in the recent literature.

It is clearly demonstrated in this study that SD rats are far more susceptible to intraperitoneal injection of STZ than their NR counterparts. SD rats had significantly higher blood glucose levels post STZ injection, suffered more mortality with a significantly shorter period of time, required more frequent insulin treatment throughout the observation period and did not require a second STZ injection. Similarly, it has been found that different strains of rats react differently to 125 mg/kg STZ intraperitoneal injection [6].

Results of the OGTT performed in the NR group showed that blood glucose levels were not significantly impaired following the first STZ injection indicating that ND are probably resistant to low dose STZ and may require higher doses to induce diabetes or frequent doses. Following the second STZ injection, blood glucose levels were significantly elevated through out the observation period. In conclusion, it appears that SD rats are far more susceptible to a single intraperitoneal dose of 150mg/kg STZ than NR. Future studies are required to determine the optimal dose to induce diabetes in nude rats.

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