The Rat Model of Type 2 Diabetic Mellitus and Its Glycometabolism Characters

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Abstract: To develop a rat model of type 2 diabetic mellitus that simulated the common manifestation of the metabolic abnormalities and resembled the natural history of a certain type 2 diabetes in human population, male Sprague-Dawley rats (4 months old) were injected with low-dose (15 mg/kg) STZ after high fat diet (30% of calories as fat) for two months (L-STZ/2HF). The functional and histochemical changes in the pancreatic islets were examined. Insulin-glucose tolerance test, islet immunohistochemistry and other corresponding tests were performed and the data in L-STZ/2HF group were compared with that of other groups, such as the model of type 1 diabetes (given 50 mg/kg STZ) and the model of obesity (high fat diet). The body weight of rats in the group of rats given 15 mg/kg STZ after high fat diet for two months increased significantly more than that of rats in the group of rats given 50 mg/kg STZ (the model of type 1 diabetes) (595 ± 33 g vs. 352 ± 32 g, p<0.05). Fast blood glucose levels for L-STZ/2HF group were 16.92 ± 1.68 mmol/l, versus 5.17 ± 0.55 mmol/l in normal control and 5.59 ± 0.61 mmol/l in rats given high fat diet only. Corresponding values for fast serum insulin were 0.66 ± 0.15 ng/ml, 0.52 ± 0.13 ng/ml, 0.29 ± 0.11 ng/ml, respectively. Rats of type 2 diabetes (L-STZ/2HF) had elevated levels of triglyceride (TG, 3.82 ± 0.88 mmol/l), and cholesterol (Ch, 2.38 ± 0.55 mmol/l) compared with control (0.95 ± 0.15 mmol/l and 1.31 ± 0.3 mmol/l, respectively) (p<0.05). The islet morphology as examined by immunocytochemistry using insulin antibodies in the L-STZ/2HF group was affected and quantitative analysis showed the islet insulin content was higher than that of rats with type 1 diabetes (P<0.05). We concluded that the new rat model of type 2 diabetes established with conjunctive treatment of low dose of STZ and high fat diet was characterized by hyperglycemia and light impaired insulin secretion function accompanied by insulin resistance, which resembles the clinical manifestation of type 2 diabetes. Such a model, easily attainable and inexpensive, would help further elucidation of the underlying mechanisms of diabetes and its complications.

Key words: glycometabolism, mechanism, model, rat, type 2 diabetes

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**Introduction**

Type 2 diabetes is a kind of heterogeneous disease with complicated pathogenesis which is related to the genetic susceptibility and life style, especially the dietetic style. The establishment of its experimental model will be helpful in understanding its pathogenesis and the development of new treatments. Spontaneously or inducedly, several animal models are widely used for type 2 diabetes mellitus. Spontaneous, models such as Zucker diabetic fatty (ZDF) rat [1] and Goto-Kakizaki (GK) rat [2] are subject to the resources of the animal with the major factor being genetic predisposition. Induced, models use relatively high dosages of streptozotocin (STZ) (more than 50 mg/kg) [3] to reduce the synthesis and secretion of insulin in β cells and tend to manifest type 1 diabetes, even in combinations of 50 mg/kg STZ and fat fed diets [4]. These models differ from the common type 2 diabetes in many respects including pathogenesis and clinical symptoms. In most cases of type 2 diabetes, insulin resistance often precedes the onset of hyperglycemia, and a detect in insulin activity can be detected even when the plasma glucose level is normal [5]. Researches on a rat model of type 2 diabetes are still being undertaken. We aimed to establish a rat model of type 2 diabetes, similar to the metabolic abnormalities seen in humans, which is comparatively easy to produce.

**Materials and Animals**

*Animals and Treatments*

Ninety male SD (Sprague-Dawley) rats were obtained from BK Co. (Britain) weighing 250 g ± 20 g at an age of 2 months. The rats were exposed to a 12-h light/dark cycle beginning with light at 0800 and had free access to food and water. The animal experiments were conducted according to the “Guide for the Care and Use of Laboratory Animals” of the Shanghai Second Medical University.

Diet composition: conventional chow included 60% carbohydrate, 22% protein, 10% fat (mostly bean oil), 8% fiber and other ingredients; high fat diet included 50% carbohydrate, 13% protein, 30% fat (mostly zoosterol), 7% fiber and other ingredients.

The rats were divided into 6 groups. (1) Group A (normal control, NC) were conventional chow fed rats injected with vehicle of 0.1 mol/l citric acid buffer (pH4.5) via the caudal vein once. (2) Group B (control, low dosage of 15 mg/kg STZ, STZ was dissolved in 0.1 M citrate buffer (pH 4.5), L-STZ) (STZ, Sigma Chemical Co.) were conventional chow fed rats injected with 15 mg/kg STZ once. (3) Group C (the model of type 1 diabetes, high dosage of 50 mg/kg STZ, H-STZ) were conventional chow fed rats injected with 50 mg/kg STZ once. (4) Group D (the model of obesity, high fat diet, HF) were high fat diet rats injected with vehicle once. (5) Group E (the model of type 2 diabetes, 15 mg/kg STZ injected after high fat diet for 2 months, L-STZ/2HF) were rats injected with 15 mg/kg STZ once after high fat diet for 2 months. (6) Group F (control, 15 mg/kg STZ injected after high fat diet for 1 month, L-STZ/1HF) were rats injected with 15 mg/kg STZ once after high fat diet for 1 month. All of groups were fed with the same diet at other times.

Specimens Preparation and Sample Analysis

Rat body weights and food and water intakes were recorded at 2 days and 2 months after STZ or vehicle injection respectively.

Their blood was collected respectively at 2 days and 2 months after STZ or vehicle injection (i.e. 4 and 6 months old respectively) from the caudal vein in a fasting state. After separation of the plasma, an aliquot was taken for the glucose test and measurement of triglyceride and cholesterol concentrations. The remainder was stored at 4°C for insulin measurement by radioimmunoassay in which a specific rat insulin antibody (Linco Research, Inc) was employed.

Insulin-glucose tolerance test: at 2 months after STZ injection, the rats were intraperitoneally (i.p.) anesthetized with pentobarbital sodium 50 mg/kg in the nonfasting state. The two femoral blood vessels were exposed by incision through the skin in the inguinal area and an i.v. insulin-glucose tolerance test [6] was performed. The rats were injected with glucose 0.7 g/kg into the femoral vein, immediately followed by insulin with a dose of 0.175 u/kg body weight. Blood specimens, 0.2 ml, were obtained by venipuncture from the femoral artery. At approximately 0, 2, 4, 6, 8, 10, 20 and 30 min after the insulin injection, blood was collected for glucose determination. Insulin sensitivity was measured by the glucose disappearance rate within 10 min, illustrated with the average slope K in the fit-
Immunohistochemistry: at 2 months after STZ or vehicle injection, rats in each group were sacrificed after intraperitoneally (i.p.) anesthetizing with pentobarbital sodium 50 mg/kg, and pancreata were removed. Pancreata were fixed in 4% paraformaldehyde overnight and embedded in paraffin by a routine procedure. Consecutive 4 µm paraffin sections were made. The primary antibody used for immunohistochemistry was the mouse monoclonal antibody against insulin. Immunostain was completed by the UltraSensitive™ S-P Kit (Maxim Co., Streptavidin Peroxidase method). The insulin-immunoreactive area in the section was measured by a colour image analyzer (KS400) under the same conditions of resolution, contrast, brightness and subjected to a quantitative analysis.

**Table 1.** Comparison of glycometabolism related indices (4 months old, n=6)

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
<th>Group F</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mmol/l)</td>
<td>5.65 ± 0.77</td>
<td>5.10 ± 0.32</td>
<td>28.7 ± 5.36*</td>
<td>5.34 ± 0.25</td>
<td>23.4 ± 2.76**</td>
<td>5.63 ± 0.77</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.11 ± 0.20</td>
<td>0.96 ± 0.12</td>
<td>1.23 ± 0.34*</td>
<td>1.97 ± 0.63*</td>
<td>1.85 ± 0.64*</td>
<td>1.92 ± 1.00*</td>
</tr>
<tr>
<td>Ch (mmol/l)</td>
<td>1.40 ± 0.47</td>
<td>1.38 ± 0.21</td>
<td>1.37 ± 0.52</td>
<td>1.42 ± 0.23</td>
<td>1.51 ± 0.31</td>
<td>1.39 ± 0.68</td>
</tr>
</tbody>
</table>

*the others vs. Group A, *Group C, E vs. Group D, P<0.05. A is the group of normal control, B is the group given 15 mg/kg STZ, C is the group given 50 mg/kg STZ (H-STZ group), D is the group given high fat diet (HF group), E is the group given 15 mg/kg STZ after high fat diet for 2 months (L-STZ/2HF group), F is the group given 15 mg/kg STZ after high fat diet for 1 month. FBG, fasting blood glucose; TG, triglyceride; Ch, Cholesterol.

**Results**

**Glycometabolic parameters**

Fast blood glucose levels for the L-STZ/2HF group at 6 months old were 16.92 ± 1.68 mmol/l, versus 5.17 ± 0.55 mmol/l in normal control, 5.59 ± 0.61 mmol/l in rats given high fat diet only. The corresponding values for fast serum insulin were 0.66 ± 0.15 ng/ml, 0.52 ± 0.13 ng/ml, 0.29 ± 0.11 ng/ml, respectively. Symptoms of polydysia, polyuria and polyphagia appeared in the groups of model type 1 and 2 diabetes. The body weight of rats in the group of rats given 15 mg/kg STZ after high fat diet (L-STZ/2HF group) for two months increased significantly more than that of rats in the group of rats given 50 mg/kg STZ (H-STZ group) (595 ± 33 g vs. 352 ± 32 g, p<0.05). Besides, rats of model type 2 diabetes had elevated levels of TG (3.82 ± 0.88 mmol/l) and Ch (2.38 ± 0.55 mmol/l) compared with control (0.95 ± 0.15 mmol/l and 1.31 ± 0.3 mmol/l, respectively) (p<0.05). The detailed information is showed in Tables 1 and 2.

**Insulin glucose tolerance tests**

The plasma glucose curves indicating the glucose disappearance rate after insulin challenge in the insulin-glucose tolerance test are summarized in Fig. 1 and Table 1. The K value, representing the level of insulin resistance, was reduced in the model groups of type 1 diabetes, type 2 diabetes and obesity when compared with that of the control group. Among these groups, insulin sensitivity in the L-STZ/2HF group was the most seriously impaired.

**Morphometry**

In the pattern of immunostaining, islets in the control rats (Groups A and B) were in good order with phanero-edge. There were many atrophy islets in the type 1 diabetic model rats (Group C). While hyperplasia islets in disorder could be found in the obese model rats and type 2 diabetic model rats (Groups D and E).

By image and quantitative analysis, the content of insulin deduced from the optical density of immunostaining per unit in β cells increased in type 2 diabetic model rats compared with that of the control, but decreased in type 1 diabetic model rats. Figure 2 shows the images of the expression of insulin stained...
by immunohistochemistry. The index (Ins-p) indicating the insulin content is shown in Table 1.

### Discussion

For a long time, people have been looking for an ideal model that closely simulates the natural history and metabolic characteristics of patients with type 2 diabetes. According to the new classification and diagnostic criteria for diabetes proposed by the American Diabetes Association (ADA) [7], type 1 diabetes is characterized with absolute insulin deficiency due to the destruction of β cells. The development of type 2 diabetes is linked to impaired insulin sensitivity (insulin resistance) coupled with a failure of pancreatic β cells to compensate by adequate insulin secretion [8]. Reduced insulin sensitivity and impaired pancreas function are two major factors in the pathogenesis of type 2 diabetes. By combining high fat diet and STZ administration, we initiated our efforts to establish a rat model which became insulin resistant after a given period of high fat diet, and then added a relative low dose (15 mg/kg) of STZ administration. When hyperglycemia occurred, the insulin secretion function presented only light impairment and relatively insulin deficiency.

High fat diet influenced the blood level of triglyceride and cholesterol, as was indicated in rats of the HF group (Group D). Due to substance competition among fatty acids and glucose metabolic related production [9], hyperglycemia affected the lipid level in blood, and the triglyceride and glucose level increased in blood in rats of the H-STZ group (Group C). The nature of insulin resistance is that the reduced capability of glucose utilization is stimulated by insulin. Acquired metabolic dysfunction results in impairments in the intracellular biological response to

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>FBG (mmol/l)</th>
<th>TG (mmol/l)</th>
<th>Ch (mmol/l)</th>
<th>FSI (ng/ml)</th>
<th>Ins-p</th>
<th>K value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>550 ± 35</td>
<td>5.17 ± 0.55</td>
<td>0.95 ± 0.15</td>
<td>1.31 ± 0.3</td>
<td>0.52 ± 0.13</td>
<td>82.09 ± 1.71</td>
<td>55.72 ± 3.79</td>
</tr>
<tr>
<td>B</td>
<td>553 ± 37</td>
<td>5.35 ± 0.59</td>
<td>0.98 ± 0.22</td>
<td>1.3 ± 0.5</td>
<td>0.53 ± 0.11</td>
<td>81.09 ± 0.99</td>
<td>56.44 ± 4.36</td>
</tr>
<tr>
<td>C</td>
<td>352 ± 32</td>
<td>17.93 ± 2.40</td>
<td>2.58 ± 0.52</td>
<td>1.38 ± 0.43</td>
<td>0.29 ± 0.11</td>
<td>63.48 ± 2.04</td>
<td>49.68 ± 6.02</td>
</tr>
<tr>
<td>D</td>
<td>670 ± 105</td>
<td>5.59 ± 0.61</td>
<td>2.5 ± 0.42</td>
<td>1.92 ± 0.62</td>
<td>0.93 ± 0.13</td>
<td>89.12 ± 1.57</td>
<td>45.6 ± 2.97</td>
</tr>
<tr>
<td>E</td>
<td>595 ± 33</td>
<td>16.92 ± 1.68</td>
<td>3.82 ± 0.88</td>
<td>2.38 ± 0.55</td>
<td>0.66 ± 0.15</td>
<td>83.95 ± 1.15</td>
<td>38.72 ± 3.47</td>
</tr>
</tbody>
</table>

*the others vs. Group A, * Group C, E vs. Group D, P<0.05. A is the group of normal control, B is the group given 15 mg/kg STZ, C is the group given 50 mg/kg STZ (H-STZ group), D is the group given high fat diet (HF group), E is the group given 15 mg/kg STZ after high fat diet for 2 months (L-STZ/2HF group), F is the group given 15 mg/kg STZ after high fat diet for 1 month. BW, body weight; FBG, fasting blood glucose; TG, triglyceride; Ch, Cholesterol; FSI, fasting serum insulin; Ins-p, insulin content (optical density per unit) in islets. K value indicates the level of insulin sensitivity, which was measured in the insulin-glucose tolerance test.
insulin, making the high fat diet rats more susceptible to hyperglycemia. First, hyperlipidemia, especially increased plasma triglycerides and cholesterol is considered to be one of the major factors inducing insulin resistance [11]. Fatty acid oxidation inhibits both glucose oxidation and its ability to enter cells by regulating the activity of glucose transporters and the glycogen synthesis enzyme subunit [12]. Then, prolonged hyperglycemia induces the impairment of insulin action and results in the occurrence and severity of insulin resistance. Abnormal glycogen biosynthesis and impaired receptor kinase are considered to be involved in
hyperglycemia-induced insulin resistance [13, 14]. Thus the index of insulin resistance (K value) decreased in rats of H-STZ, HF, and L-STZ/2HF groups (Group C, D and E). Especially in rats of the L-STZ/2HF group (Group E), insulin resistance was more serious because of the actions of both hyperglycemia and hyperlipidemia.

In most cases, reduced insulin sensitivity was compensated for by hyperinsulinemia (the compensatory ability of pancreas) which maintained normal glucose homeostasis, a condition similar to the prediabetic state in humans [15]. In rats of HF group (Group D), the hyperinsulinemia may have been caused by islet hyperplasia and insulin content enhancement. After the high fat diet for a given period, insulin resistance in the rats of L-STZ/2HF group (Group E) developed to some degree or the ability of insulin secretion required to overcome insulin resistance lessened. The L-STZ/2HF rats still didn’t develop diabetes until they were injected with a dose of STZ that would have little effect on the blood glucose level in conventional chow fed rats. In the case of L-STZ/2HF rats the administration of a small amount of STZ would have led to the loss of a certain number of insulin-secreting cells and the decrease of a certain amount of insulin secretion, but hyperinsulinemia still existed. Since hyperinsulinemia couldn’t compensate for insulin resistance any more, diabetes occurred. This resembled the metabolic characteristics of patients with high risk of type 2 diabetes due to insulin resistance. The L-STZ group was also a negative control. It should be pointed out that the effect of the low dose (15 mg/kg) of STZ is not enough to destroy β cells morphologically and functionally (Group B), so that its single administration would not have affected basal insulin secretion. The two combined conditions, high fat diet for a given period of time and the administration of a low dose (15 mg/kg) of STZ, were time and dosage dependent. Although given the same dosage of STZ as L-STZ/2HF rats (Group E), the rats in the L-STZ/1HF group (Group F) did not develop diabetes because they had been fed with the high fat diet for only 1 month before STZ administration. At that time their insulin resistance was underdeveloped.

The high fat diet would have led to the increase in compensatory insulin secretion in the rats of the HF and L-STZ/2HF groups (Groups D and E), but destruction of β cells with low-dose (15 mg/kg) of STZ and incessant hyperlipidemia would also have affected the insulin secretion. So the basal insulin level in L-STZ/2HF rats (Group E) decreased to the level of NC rats (Group A), which was lower than that in HF rats (Group D). Since the compensatory function of β cells was not enough to overcome insulin resistance, type 2 diabetes developed.

In conclusion, the newly established rat model of type 2 diabetes (i.e. the L-STZ/2HF of study this study) has the following character (1) It simulates the development of common type 2 diabetes, first insulin resistance and then lightly impaired the function of insulin-secreting cells, both of which play important roles in the development of diabetes. (2) The dosage of STZ is very low. The severity of β cells destruction induced by STZ depended on the dosage in the same strain of rats. There have been reports on apoptosis of β cell line INS-1 induced by low-dose STZ and necrosis induced by high-dose STZ [16]. So it’s important to find out an appropriate dosage that is low enough to guarantee the occurrence of diabetes. The low dose of STZ did not induce the classical type 1 diabetes, which often results from exhaustion of insulin synthesis and secretion (just as H-STZ rats showed). Moreover, since the damage of STZ to the pancreas is relatively selective, the lower the dosage of STZ administration, the less damage to the other tissues. Especially in the research of the gene expression profile of diabetic chronic complications such as kidney or cardiovascular disease, the use of a lower dose of STZ would reduce its effect allowing research to focus on molecular differential expression resulting from hyperglycemia, hyperinsulimia, or hyperlipidemia. In principle, the longer the time of the high fat diet, the lower the dosage of STZ required to induce type 2 DM. Given the age of the rats and the time spent on the observation of diabetic chronic complications, 2 months of high fat fed are suggested. (3) Type 2 DM induced rats might live for a long time (the follow-up observation indicated that their life-span was at least 1 year) without insulin or other oral treatment, so it could be used for remedy estimation or rapid and chronic complication related investigations. Besides, such a model could also be applied to the further study of the molecular mechanisms regulating insulin sensitivity and the relationship among blood levels of insulin, glucose, lipid and insulin resistance. This model, eas-
ily producable and inexpensive, is worthy of further investigation.

Acknowledgments

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