

ORIGINAL ARTICLE**Regeneration of islet β -cells in tree shrews and rats**

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Abstract

Background: Current understanding of injury and regeneration of islet β -cells in diabetes is mainly based on rodent studies. The tree shrew is now generally accepted as being among the closest living relatives of primates, and has been widely used in animal experimentation. However, there are few reports on islet cell composition and regeneration of β -cells in tree shrews.

Methods: In this study, we examined the changes in islet cell composition and regeneration of β -cells after streptozotocin (STZ) treatment in tree shrews compared with Sprague-Dawley rats. Injury and regeneration of islet β -cells were observed using hematoxylin and eosin (HE) staining and immunohistochemical staining for insulin, glucagon, somatostatin and PDX-1.

Results: Our data showed that in rats islet injury was most obvious on day 3 after injection, and islet morphologies were significantly restored by day 21. Regeneration of islet β -cells was very pronounced in rats, and mainly involved regeneration of centro-acinar cells and transformation of extra-islet ductal cells. In tree shrews, the regeneration of islet β -cells was not as significant. On days 3 and 7, only scattered regenerated cells were observed in the remaining islets. Further, no regeneration of centro-acinar cells was observed.

Conclusion: The results suggest that the repair mechanism of islet β -cells in tree shrews is similar to that of humans.

KEYWORDS

β -cell, rat, regeneration, tree shrew

1 | INTRODUCTION

Patients with type 1 diabetes (5%-10% of all patients with diabetes) suffer from an absolute deficiency of islet β -cells, while patients with type 2 diabetes (90%-95% of patients with diabetes) suffer from a relative

deficiency of β -cells or inadequate insulin secretion. However, normal blood glucose level can be restored in patients via β -cell transplantation (type 1 diabetes) or islet transplantation (type 2 diabetes).¹⁻³ Since it is difficult to find β -cell donors for transplantation, functional recovery or regeneration of endogenous islet β -cells is an attractive option.

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Current understanding of injury and regeneration of islet β -cells in diabetes is mainly based on rodent studies. Regeneration of islet β -cells in rodents has been confirmed using partial pancreatectomy,⁴ ligation of the pancreatic duct⁵ and other methods. Regeneration is dominated by replication of islet β -cells. However, regeneration of β -cells also occurs from precursor cells in the pancreatic duct. The regenerative function declines with age.^{6,7} Recently, Tomer et al⁸ constructed a mouse model of diphtheria toxin tissue-specific transgene, in which diphtheria toxin was expressed in islet β -cells after application of doxycycline, resulting in apoptosis of 70%-80% of β -cells. Shamsi et al⁹ have also developed a mouse model based on β -cell-specific genetic ablation of the transcription initiation factor 1A (TIF-1A) that induces a slow apoptotic response, eventually leading to protracted β -cell death. These models have provided a highly efficient method for studying replication and regeneration of islet β -cells.

In humans, growth and replication of β -cells mainly occurs in childhood. In adulthood, a few studies have found almost no evidence of β -cell replication.¹⁰⁻¹³ However, these results were mainly obtained from studies using cadavers or surgical specimens. Currently, it is impossible to directly detect the number of islet β -cells in a living human body, and assessment of β -cell function via measurement of insulin secretion is unlikely to accurately reflect the number of β -cells. Therefore, transplantation from human cadavers or biopsies into immunodeficient animals has been used to study the regeneration of islet β -cells in humans.¹⁴⁻¹⁸

Interestingly, Suarez-Pinzon et al¹⁸ found that human pancreatic duct cells in grafts introduced into diabetic mice had a large number of cells that were double stained for cytokeratin 19 (CK19) and insulin, and this number increased markedly to over 50% of the CK19 population after stimulation with gastrin and glucagon-like peptide 1 (GLP-1). These findings with double-stained cells provide evidence that neogenesis was active in these grafts and could be stimulated. Transplantation of human tissues into immunodeficient mice is unlikely to exclude the role of the mouse's microenvironment, suggesting that specific factors in mice initiate changes in stationary cells, or ischemia and temperature variation during specimen transplantation affect the expression of Ki67 and other genes.

In vivo experiments in animals with pancreas tissue similar to humans in anatomy and function may alleviate the influence the microenvironment on tissue transplantation. The tree shrew is a small mammal that has been well-characterised in animal experimentation literature and is therefore highly appropriate for animal experiments. It is widely distributed in Southeast Asia and southwest China. Historically, it has been classified as a primate, but is now generally accepted as being among the closest living relatives of primates.¹⁹ Fan et al²⁰ conducted germ-line genome analysis which found that the tree shrew is very closely related to primates. Because of ethical concerns about primate research, the importance of the tree shrew as a laboratory animal has increased over the years. To date, the tree shrew has been extensively used in studies of hepatitis B and C infection, tumors, myopia, stress and depression, metabolic syndrome, as well as bacterial infection.²¹ These include studies investigating the role of STZ-induced diabetes models,²²⁻²⁴

which found that STZ destroyed islet β -cells and induced persistent diabetes in the tree shrew. Our study explored changes in islet structure, as well as regeneration of β -cells, after STZ treatment of tree shrews compared with rats, in an effort to provide basic data for application in studies on diabetes.

2 | METHODS

2.1 | Ethical approval

The experimental protocols were approved by the Institutional Animal Care and Use Committee of Chinese PLA General Hospital, and were implemented under its supervision (Approval document No. 2015-X10-45).

2.2 | Animals and treatment

Twenty five Sprague-Dawley (SD) male rats, aged 8 weeks and weighing 180-220 g each, were obtained from the Beijing Vital River Laboratory Animal Technology Co. Ltd. Animals were randomized into 5 groups: control and days 3, 7, 14, and 21 post-STZ injection, each containing 5 animals. After rats were fasted overnight, they were injected intraperitoneally with 60 mg/kg STZ, while rats in the control group were injected with the same dose of normal saline. Experiments were performed at the Animal Center of PLA General Hospital, and the experimental conditions were in accordance with National Standards for Barrier Environment (SYXK-(Military)-2012-0064).

Twenty-five male tree shrews, aged 8 weeks and weighing 120-160 g, were purchased from The Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences (CAMS). Experiments were also conducted at the center, under conditions in accordance with the National Standards for General Environment (SYXK-(Dian)-K2013-0001). Animals were grouped and treated in the same way as the rats, with an STZ dose of 150 mg/kg.

During the experiment, random blood glucose values were tested using a Contour TS Blood Glucose Meter (Bayer, Germany) at 8:00 AM daily. Since the tree shrews appeared to be in poor condition after treatment with STZ, animals with a blood glucose level higher than 20 mmol/L were injected with insulin Detemir (1 U/animal) from day 5 after treatment, while animals with sharply declined blood glucose were injected intraperitoneally with 500 μ L of 20% glucose. In addition, on days 3, 7, 14, and 21 after STZ treatment, fasting blood glucose values and glucose tolerance were tested in each group. Subsequently, blood was sampled for determination of insulin. At the end of the experiment, animals were euthanized, and pancreas was harvested and fixed with neutral formalin. Paraffin-embedded sections were obtained, followed by hematoxylin and eosin (HE) and immunohistochemical staining.

2.3 | Intraperitoneal glucose tolerance test

After overnight fasting, animals were injected intraperitoneally with 50% glucose (1.8 mL/kg). Fasting blood glucose and blood glucose

values at 10, 30, 60, 90 and 120 minutes after administration were measured.

2.4 | Analysis of fasting blood glucose and serum insulin

Blood was maintained at room temperature for 30 minutes and centrifuged at 4000 g for 10 minutes. The supernatant was collected and preserved at -80°C until further use. Blood glucose analysis was performed using an automated biochemical analyzer (Toshiba120, Japan), and serum insulin analysis was performed using radioimmunoassay (xh6080, China).

2.5 | Immunohistochemical staining

Immunohistochemical staining was conducted using conventional methods. The following antibodies were purchased from Abcam (Cambridge, UK): rabbit anti-insulin polyclonal antibody (1:20), mouse anti-glucagon monoclonal antibody (1:200), rabbit anti-somatostatin polyclonal antibody (1:800), and rabbit anti-PDX1 polyclonal antibody (1:500).

Five visual fields were selected from each section for photography (20 \times). All the islets in each visual field were depicted using the Image-Pro Plus pathological image analysis system, to calculate the islet number and islet area. Finally, the mean islet area and the mean islet number in each visual field were calculated for statistical analyses.

From each section, five sites containing islets were photographed under a high-power field (40 \times), and the integrated optical density (IOD) of the islets, or positive expression, and islet area (Area) in each visual field were measured. The mean optical density (MOD) was obtained by dividing IOD by Area for statistical analysis.

2.6 | Statistical analyses

All the results were expressed as mean \pm SD. Differences between means were tested for statistical significance using a one-way analysis of variance (ANOVA) and post hoc least significance tests. SPSS 13 program (SPSS[®], Chicago, USA) was used for all statistical analyses.

3 | RESULTS

3.1 | Changes in fasting blood glucose and serum insulin levels

The fasting blood glucose value is controlled by multiple factors, and is the key parameter for diagnosis of diabetes. STZ treatment decreased insulin levels following injury to a large number of islet β -cells, resulting in persistently elevated fasting blood glucose levels from day 3. Since there are few reports of STZ treatment in tree shrews, we determined the STZ dose mainly based on studies by Liang et al²⁵ and short-term pre-experimental trials. We later found that a single application of 150 mg/kg STZ was excessive, resulting in animals in a poor condition. In order to increase the survival rate,

we treated the animals with insulin (when blood glucose was higher than 20 mmol/L) and glucose (when blood glucose was less than 1 mmol/L). The fasting blood glucose level was persistently elevated starting from day 3 in tree shrews (Figure 1A).

Insulin is the key factor regulating blood glucose absorption, which mainly affects liver, muscles and adipose tissues, promoting glucose usage and inhibition of glucose synthesis. By day 3 after application of STZ in rats, serum insulin was significantly increased. However, the values at subsequent time points did not show a significant difference compared with the control group.

STZ treatment of tree shrews significantly increased the mean serum insulin levels at each time point compared with the control group (Figure 1B). These results suggest that insulin secretion persisted after STZ administration in rats and tree shrews. The insulin values were never less than in the control group, which was probably because the blood was sampled immediately after the glucose tolerance test.

3.2 | Changes in blood glucose levels following IPGTT

The glucose tolerance test measured changes in blood glucose after administration of glucose, to evaluate the effect of β -cell function and regulation of blood glucose levels. Similar variations in glucose levels were found in rats at each time point after administration of STZ (days 3, 7, 14, and 21) (Figure 1C). Blood glucose rose after administration of glucose, peaking at 60-90 minutes, and decreased to pre-treatment level after 2 hours. Compared with the control group, the baseline blood glucose value was significant raised. However, the blood glucose value peaked at a later stage after administration of glucose, indicating a slow recovery. These results suggest that STZ administration significantly decreased blood glucose regulation, and insulin secretion was deficient until day 21 post STZ.

Blood glucose variation in tree shrews was comparable to that of the control group on days 7, 14 and 21, following STZ treatment. Blood glucose peaked at 10 minutes after administration, and was restored to pre-treatment levels at 60 minutes, or even below the pre-treatment level on days 7 and 21. On day 3 after administration of STZ, blood glucose varied from that of the control group, with levels persistently elevated after administration, and only slightly restored at 90 minutes (Figure 1D). These results suggest that after administration of STZ blood glucose regulation was maintained except on day 3. We speculate that the tree shrew is a highly active species, in which glucose utilization in peripheral tissues, especially skeletal muscle, is highly sensitive to insulin.

3.3 | STZ-mediated changes in islet morphology

HE staining of pancreatic sections showed that islets were scattered between pancreatic acini in controls. Pancreatic islets are elliptical or circular structures with a lucid structure, staining a light colour, and a clear boundary with the exocrine gland. In control tissue, the size of islets varied greatly, and giant islets were occasionally observed

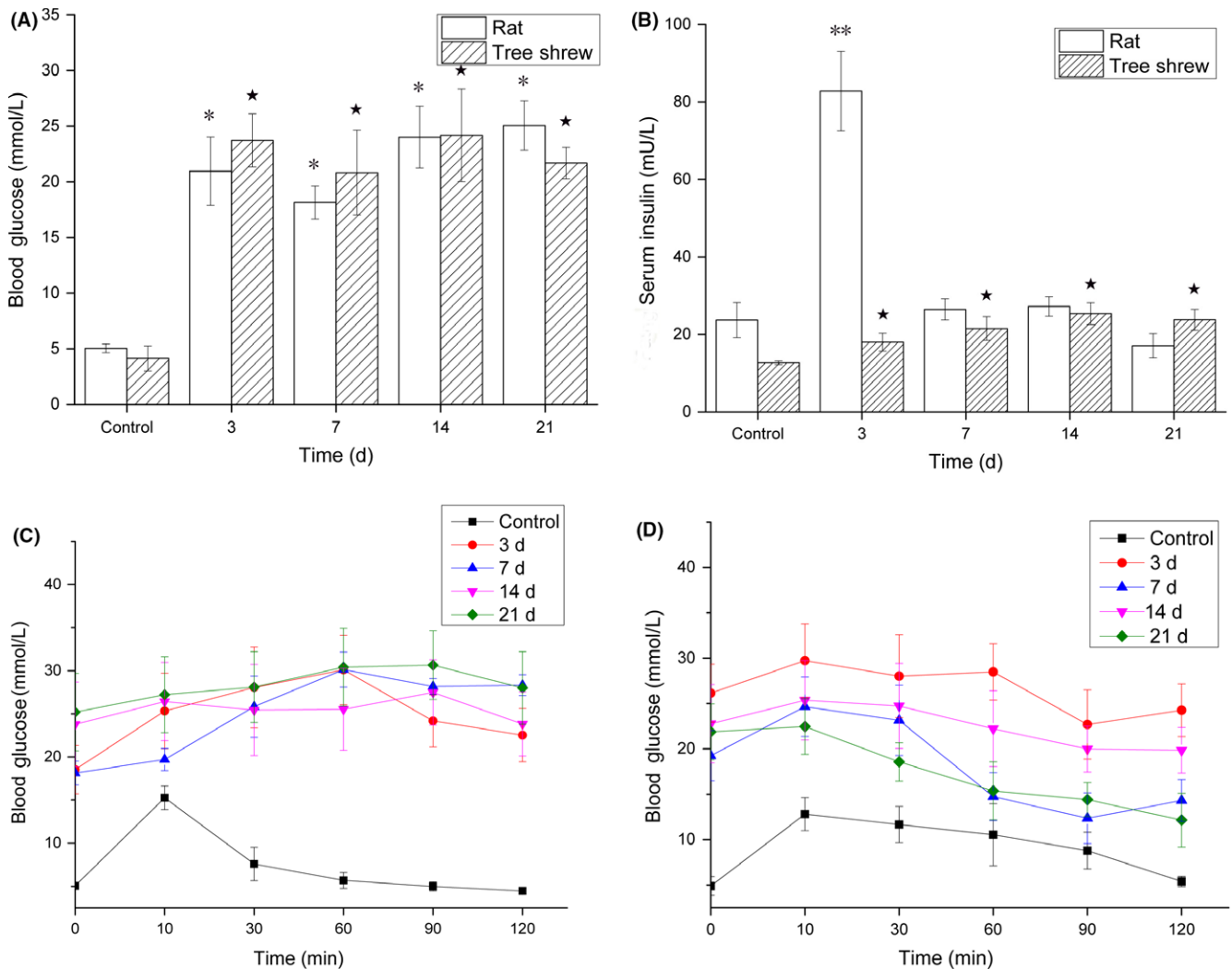


FIGURE 1 A, Changes in fasting blood glucose levels after STZ treatment. *STZ treatment group vs control group of rats, $P < .05$; ★STZ treatment group vs. control group of tree shrews, $P < .05$; B, Changes in serum insulin level after STZ treatment. **STZ treatment group vs. control group of rats, $P < .01$; C, Changes in blood glucose levels following IPGTT at different time points after rats were treated with STZ; D, Changes in blood glucose levels following IPGTT at different time points after tree shrews were treated with STZ

(Figure 2A). The islets were generally small in normal tree shrew tissue; ultra-small islets (cell masses consisting of a few cells) were commonly found, while giant islets were not found (Figure 2F). The number of islets in each visual field was significantly larger in tree shrews than in rats ($P < .05$), and the mean islet area in tree shrews was significantly smaller than in rats ($P < .05$) (Table 1).

Three days after STZ treatment in rats, the number of islets was reduced. Islets were squeezed by exocrine pancreatic acini and had an irregular periphery, suggesting necrosis in a large number of β -cells and diminished islet area. Cells inside the islets were irregular and densely arranged in a few areas, with frequent signs of cell degeneration and necrosis (Figure 2B). Seven days after application of STZ, islet peripheries were still irregular in shape. Cell degeneration and necrosis were still visible in a few islets, and cell regeneration was clearly visible in a few islets (Figure 2C). At 14 days post STZ, islet peripheries and intra-islet cells were still irregular, with occasional cell degeneration, necrosis and regeneration (Figure 2D). On day 21 post STZ, islet morphologies were significantly restored.

However, cells were still densely arranged locally, with evident cell degeneration and necrosis (Figure 2E).

STZ treatment led to similar changes in islet morphology in tree shrews. However, 3 days after application of STZ, the number of islets was significantly reduced from normal, compared to rats. The degeneration and necrosis of intra-islet cells was more obvious, which was probably associated with the larger dose of STZ. By 7 days after application of STZ, intra-islet cell regeneration was still only scattered (Figure 2G-J).

3.4 | Expression of insulin, glucagon, somatostatin, and PDX-1

3.4.1 | Semi-quantitative analysis of insulin expression

β -Cells secrete insulin, so changes in islet β -cells can be determined by observing insulin-positive cells. Insulin staining in rats revealed

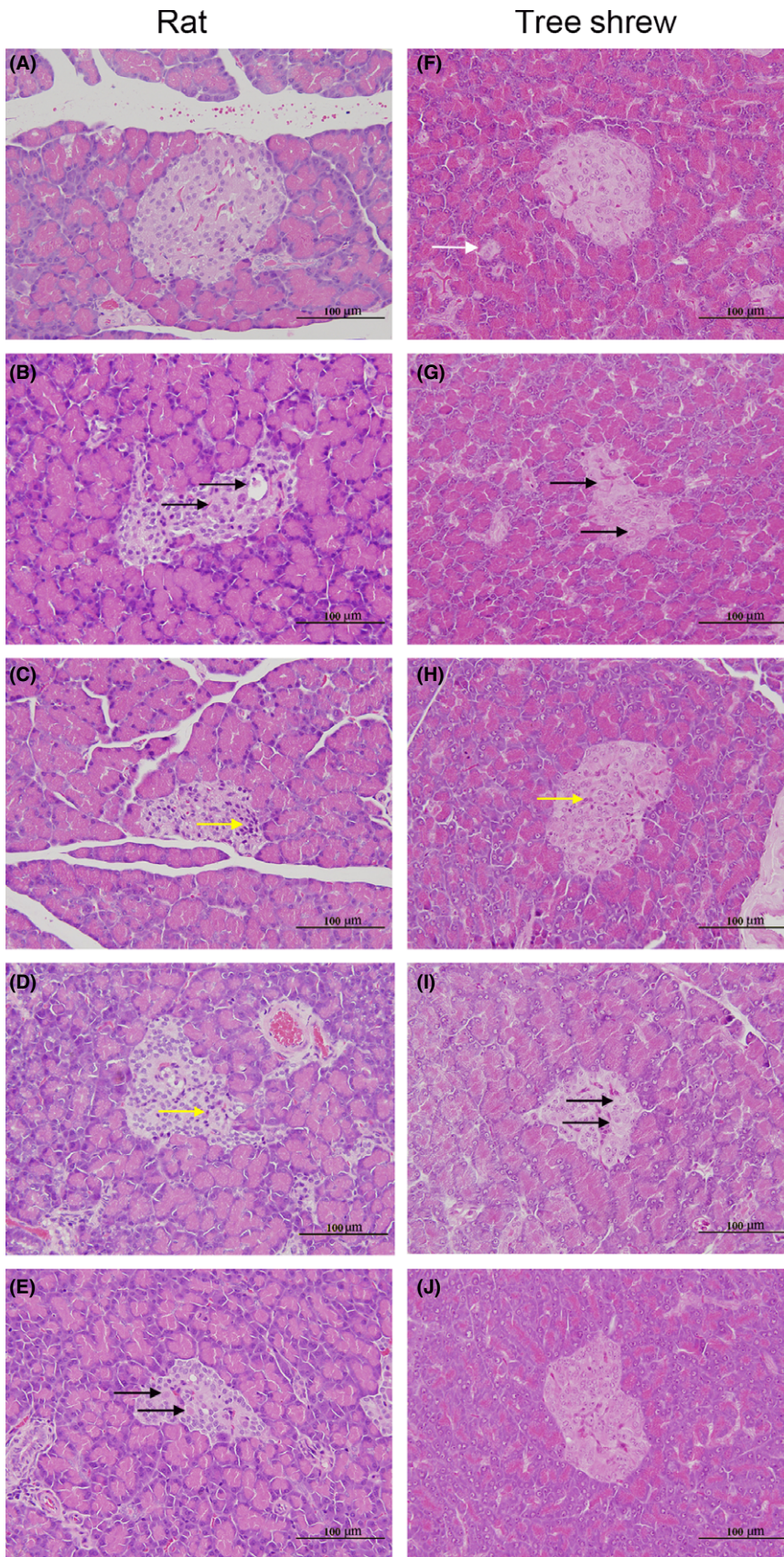


FIGURE 2 HE staining of islet morphology after STZ treatment. A, Islets of rat control group. B, 3 d after STZ treatment of rats, islets were smaller and deformed, with visible cell degeneration and necrosis (black arrow). C, 7 d after STZ treatment of rats, apparent cell regeneration was occasionally visible in islets (yellow arrow). D, 14 d after STZ treatment of rats, scattered cell regeneration was occasionally visible in islets (yellow arrow). E, 21 d after exposure to STZ, scattered cell degeneration and necrosis were still visible in islets (black arrow). F, Islets of tree shrew control group, with ultra-small islets (white arrow). G, 3 days after STZ treatment of tree shrews, islets were smaller and deformed, with visible cell degeneration and necrosis (black arrow). H, 7 d after STZ treatment of tree shrews, scattered cell regeneration was occasionally visible in islets (yellow arrow). I, 14 d post STZ, scattered cell degeneration and necrosis were still visible in islets (black arrow). J, 21 d after exposure to STZ, intra-islet cells were still arranged irregularly

that β -cells are distributed throughout the islets and form the majority of islet cells; no extra-islet insulin-positive cells were found (Figure 3A[a]). Three days post STZ, deeply stained insulin-positive cells

were visible in the remaining islets (Figure 3A[b]). On day 7, insulin-positive cells were occasionally visible in exocrine centro-acinar cells, as well as in the remaining islets (Figure 3B[e]). At the same

time, insulin-positive staining was also visible in the epithelial cells and lumen of pancreatic tubules (Figure 3B[f]). On days 7 and 14, the distribution of insulin-positive staining was similar. However, positive staining was more commonly found in pancreatic tubular lumen (Figure 3B[g]). On day 21, cell masses consisting of insulin-positive cells were found in exocrine centro-acinar cells (Figure 3B[h]). In addition, image analyses showed that a much lower proportion of insulin-positive cells was found in the remaining islets after STZ application (Figure 3C).

In tree shrew controls, islet β -cells were distributed throughout the islets, forming the majority of cells (Figure 3A[c]). After 3 and 7 days post STZ, insulin-positive cells were found only in the remaining islets (Figure 3A[d]), while on days 14 and 21 post STZ, positive cells were also visible in exocrine centro-acinar cells, and positive staining was visible in pancreatic tubules. Image analyses showed that after STZ application, the proportion and expression of insulin-positive cells in the remaining islets were significantly decreased (Figure 3C).

3.4.2 | Semi-quantitative analysis of glucagon expression

Islet α -cells secrete glucagon, so changes in islet α -cells were determined by observing glucagon-positive cells. In the control group of rats, islet α -cells accounted for about 30% of the tissue, and were

TABLE 1 Comparison of islet area and numbers in rats and tree shrews

	Rat	Tree shrew
Area of islets analyzed	22443.91 \pm 8836.59	9371.70 \pm 2230.98 ^a
Number of islets analyzed	1.83 \pm 0.35	3.88 \pm 0.52 ^a

^a $P < .05$.

mainly distributed in the peripheral regions of the islets (Figure 4A[a]). During the entire observation period after application of STZ, glucagon secretion did not vary significantly. However, the distribution and proportion of islets varied. Starting at day 7 after STZ treatment, the proportion of α -cells in the islets increased, and the cells were distributed throughout the islets (Figure 4A[b]). Expression of α -cells after STZ treatment increased compared with that of the control group, but the difference was not statistically significant ($P > .05$) (Figure 4B). These results indicate that the replication and proliferation of islet β -cells were insignificant after injury. After absorption of necrotic cells, they were replaced by α -cells, resulting in diminished islet area.

In the tree shrew control group, after STZ treatment the proportion and distribution of islet α -cells, as well as changes in the islet α -cells, were similar to those in rats (Figure 4A[c] and 4A[d]). However, the proportion of α -cells in the islets was higher than in rats, but was not significantly different compared with the control group ($P > .05$) (Figure 4B). These findings suggest that replication and proliferation of the injured islet β -cells in tree shrew were not significantly affected. However, the number of necrotic islet β -cells was higher than in rats, possibly due to the excessively large dose of STZ.

3.4.3 | Semi-quantitative analysis of somatostatin expression

Islet δ -cells secrete somatostatin. Therefore, changes in δ -cells were determined by observing somatostatin-positive cells. In the control group of rats, islet δ -cells accounted for about 5%-10% of the tissue, mainly distributed in the peripheral areas of the islets. During the entire observation period after treatment with STZ, somatostatin expression in animals after STZ treatment showed no significant difference compared with the control group (Figure 5A), but the proportion of δ -cells was slightly increased, and the cells were

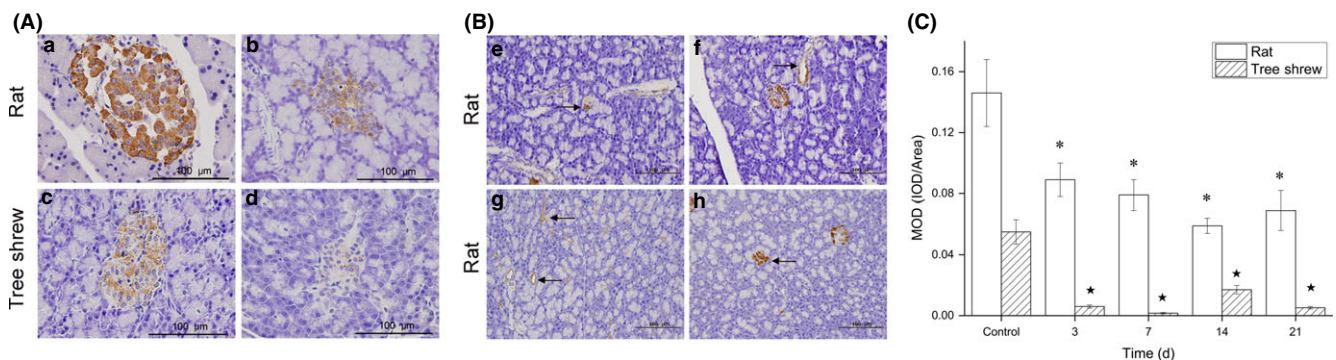


FIGURE 3 A, Immunohistochemical staining of insulin after STZ treatment. (a) Rat control group; (b) 3 d after administration of STZ in rats; (c) tree shrew control group; (d) 3 d after STZ treatment of tree shrew; B, Immunohistochemical staining of exocrine pancreas after application of STZ in rats. (e) 7 d after STZ treatment, insulin-positive cells were visible in centro-acinar cells (arrow); (f) 7 d post STZ, insulin-positive reaction in pancreatic ductal epithelial cells (arrow); (g) 14 d post STZ, a large number of insulin-positive secretions were visible in pancreatic acinar lumen and pancreatic ductal lumen (arrow); (h) 21 d, insulin-positive centro-acinar cell masses are visible (arrow). C, Semi-quantitative analysis of insulin expression in animals after STZ treatment. *STZ treatment group vs control group of rats, $P < .05$, ★ STZ treatment group vs control group of tree shrews, $P < .05$

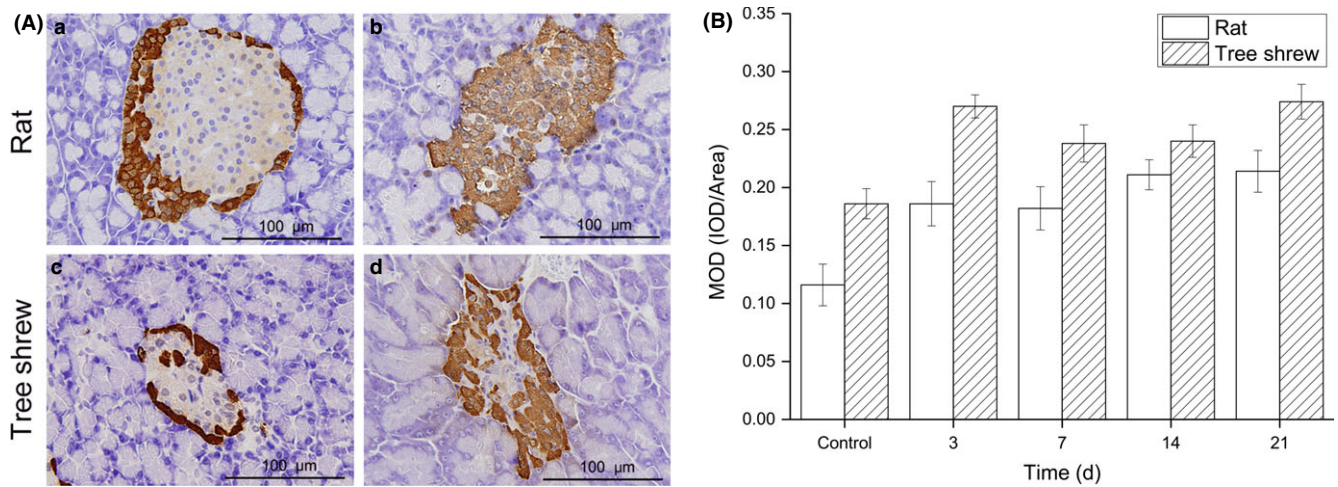


FIGURE 4 A, Immunohistochemical staining of glucagon in rats and tree shrews after STZ treatment. (a) Rat control group; (b) 14 d after application of STZ in rats; (c) Tree shrew control group; (d) 14 d after STZ treatment of tree shrew. B, Semi-quantitative analysis of glucagon expression in rats and tree shrew after STZ treatment, showing no significant difference compared with the control group ($P > .05$)

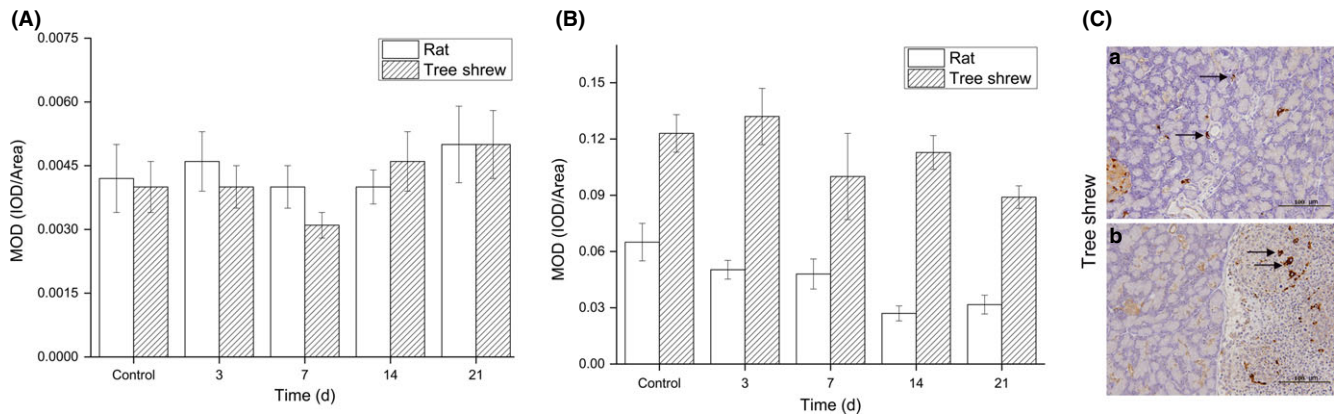


FIGURE 5 A, Semi-quantitative analysis of somatostatin expression in animals after STZ treatment, showing no significant difference compared with the control group ($P > .05$). B, Semi-quantitative analysis of PDX-1 expression in animals after STZ treatment, showing no significant differences compared with the control group ($P > .05$). C, Immunohistochemical staining of pancreatic PDX-1 in tree shrew control group. (a) Strongly positive PDX-1 expression in pancreatic interstitial cells (arrow); (b) strongly positive PDX-1 expression in dendritic cells of pancreatic lymph nodes (arrow)

distributed throughout the islets. We speculate that a large number of necrotic β -cells were absorbed and replaced by other cells. In the tree shrew, the proportion and distribution of islet δ -cells was similar to that of rats.

3.4.4 | Semi-quantitative analysis of PDX-1 expression

PDX-1 is the most important transcription factor in the development of pancreas. It controls the expression of transcription factors, and induces stem cell differentiation into β -cells. It is also an important factor in maintaining the functionality of differentiated β -cells. In adult rats, PDX-1 is mainly expressed in β -cells secreting insulin and δ -cells secreting somatostatin. It regulates important functions of β -cells, such as insulin transcription. Its high expression in ductal and

islet cells during the regeneration of islets in rodents has been confirmed. In this experiment, changes in islet PDX-1 expression were similar to the results obtained with insulin staining, which further indicated limited replication and regeneration of β -cells in islets locally (Figure 5B). In addition, positive PDX-1 expression was found in pancreatic interstitial cells (Figure 5C[a]), and strong positive PDX-1 expression was detected in the dendritic cells of pancreatic lymph nodes in the tree shrew (Figure 5C[b]).

4 | DISCUSSION

Generally, STZ is used for treatment of metastatic islet cell adenoma. Due to its selective toxicity for β -cells secreting insulin, it is often used to experimentally induce type 1 diabetes in animals, including

mice, rats, rabbits, dogs, pigs, monkeys, baboons, tree shrews, and other species, using a dose ranging from 40 to 150 mg/kg. In this experiment, the application of 60 mg/kg STZ in rats induced significant hyperglycemia until day 21 after administration, and animals survived without any treatment. Administration of 150 mg/kg of STZ to tree shrews induced significant hyperglycemia, which continued until day 21 after treatment. However, from day 5 dead animals were found and these were excluded from analysis. Therefore, the one-time intraperitoneal injection of 150 mg/kg STZ used to induce a type 1 diabetes in tree shrews was excessively high.

Wu X-Y et al²³ and Wu X et al²⁴ developed a tree shrew model of type 1 diabetes using 80 mg/kg STZ, injected twice every other week. Ishiko et al²² concluded that diabetes was induced in tree shrews only when the dose exceeded 300 mg/kg. These results are consistent with our studies, suggesting that tree shrews are not as sensitive to STZ as rats. STZ (methyl-1-nitrosyl-C2-D glucose) enters β -cells through the GLUT2 receptor, and binds to DNA via alkylation, inducing DNA strand breaks, which trigger repair mechanisms. The levels of nicotinamide adenine dinucleotide (NAD) and ATP decrease below the physiological levels, resulting in cell death. STZ is associated with the expression of the low-affinity glucose transporter GLUT2, and the varied toxicity of STZ in different animal species is mainly associated with the expression of GLUT2.^{26,27} In addition, a large number of ultra-small islets were found in tree shrew tissue, most of which were cell clusters consisting of a few cells instead of typical islet-like structures. These cell clusters were not adequately vascularized, and their deconstruction may require a sufficiently large dose of STZ.

Type 1 diabetes animal models were induced with a single application of STZ and blood glucose levels were altered over three phases. The first phase starts from 2-4 h after administration, during which blood glucose elevations occur following injury to the islet β -cells by STZ, leading to a decline in insulin secretion. The second phase (hypoglycemia) occurs 5-24 h after administration, during which islet β -cells become necrotic, and large amounts of insulin are released into the blood. The final phase of persistent hyperglycemia follows the hypoglycemic period, as a result of serious injury to islet β -cells. Generally, necrosis of β -cells mainly occurs during hypoglycemia. In this study, we found that 7-21 days after STZ treatment cell degeneration and necrosis were visible in islets. This was especially pronounced in rat tissue, which may be due to inter-species differences in sensitivity of islet β -cells to STZ toxicity.^{28,29}

Our study showed that islets in rats were significantly restored after injury by STZ, which mainly occurred via regeneration of islet cells, proliferation of pancreatic centro-acinar cells and transformation of pancreatic ductal cells. Seven days after STZ treatment, significant islet cell regeneration was observed, and scattered cell regeneration was still visible at 14 days. Islets appeared to collapse from day 3 post injury, and were not significantly recovered by day 21, which indicated that the regenerated cells were limited in number, and were unable to completely replace the cells lost due to necrosis and absorption of β -cells. In addition, the islet α -cells were

initially distributed in the peripheral areas of islets in the control group, while after STZ treatment, they increased in number and were distributed throughout the islets, which lasted until day 21 after administration, further confirming the above conclusion.

Insulin-positive cells (β -cells) in the islets significantly decreased after STZ application. However, the variation in number of cells was small during the subsequent observation periods. At the same time, glucagon-positive cells (α -cells) significantly increased after STZ treatment, although the variation in numbers was insignificant during the subsequent periods of observation. We speculate that the regenerated cells in islets are mainly interstitial cells. A very limited number of regenerated β -cells were found. In addition, the analysis of PDX-1, which is mainly expressed in β -cells secreting insulin and δ -cells secreting somatostatin, showed that PDX-1 expression in islets was consistent with the expression of insulin, which further reinforces the above conclusion.

After STZ-induced injury of islets in rats, regeneration and transformation of extra-islet insulin-secreting cells were significant. Regeneration mainly occurred in centro-acinar exocrine cells. Three days after STZ treatment, insulin-positive centro-acinar cells were visible, and cell masses were observed on days 14 to 21. At the same time, transformation mainly occurred in the pancreatic tubules of exocrine cells, where the tubular endothelial cells were positive for insulin 3 days after STZ treatment. Extensive insulin-positive staining was visible in the tubular lumen on days 14 to 21, which indicated widespread regeneration and transformation of insulin-secreting cells. Early studies concluded that islet β -cells in adult animals mainly originated from self-replicating β -cells.³⁰ Lee et al developed a partial pancreatectomy model and a tracer method of BrdU uptake in animals to confirm the origin of regenerated β -cells via self-replication.⁴ Chung et al used pancreatic duct ligation and simultaneous application of alloxan to show that the regenerated β -cells originated mainly from transformation of islet α -cells.⁵ Bonner-Weir et al reported that replication was the main mechanism in adult animals, while regeneration of β -cells was also very common in young animals.³¹ Other studies showed that, after injury of islet β -cells, the regenerated insulin-secreting cells derived mainly from centro-acinar cells of exocrine pancreas, which was first confirmed in zebrafish studies.^{32,33} Beer et al further established the central role of centro-acinar cells at the end of the pancreatic duct in islet β -cell regeneration.³⁴

In this study, we found that after STZ application the proportion of islet α -cells increased up to day 21 after administration, while insulin-positive cells in the islets were not significantly restored during the experiment. These results do not support the conclusion that the transformation of α -cells is the main mechanism of post-injury proliferation of islet β -cells. However, in terms of overall function, the blood glucose levels in rats increased persistently after STZ treatment. The glucose tolerance test revealed that exogenous glucose-stimulated secretion of insulin was clearly inadequate. Therefore, regeneration and transformation of insulin-secreting cells do not result in accurate control of blood glucose levels. Insulin secreted by regenerated and transformed cells is confined to the pancreatic duct of exocrine cells, limiting its role in glucose

metabolism. In summary, repair of injured islet β -cells in rats is dominated by regeneration and transformation of extra-islet cells, while replicated and regenerated intra-islet β -cells are limited in number.

Repair of the STZ-injured islets in tree shrews was not as extensive as in rats. On day 7 after STZ treatment, scattered regenerated cells were observed, which were not as obvious as in rats. Further, no regeneration of centro-acinar cells was observed. In addition, transformation of the insulin secretory function, visible in ductal epithelial cells, was also not as conspicuous as in rats. These results were similar to regeneration of islet β -cells in humans,³⁵ suggesting that the repair mechanism of islet β -cells injured by STZ in tree shrews may be similar to that in humans.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; YQZ, YQL, JFY, XD, MMN, LX, Yunxiao Jia, XMS, DXK, WGW, PFT and NL conducted the experiments, YQZ analyzed the data and wrote the manuscript, and JJD and HC conceived and designed the experiments. All authors read and approved the final manuscript.

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