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OBTAINING A MOUSE MODEL OF STREPTOZOTOCIN-INDUCED TYPE 1 DIABETES MELLITUS

G.N. Skaletskaya, N.N. Skaletskiy, G.N. Bubentsova, L.A. Kirsanova, Yu.B. Basok, V.I. Sevastianov

Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

Objective: to obtain a stable mouse model of type 1 diabetes mellitus (T1DM) using streptozotocin (STZ), which has a toxic effect on pancreatic beta cells. **Materials and methods.** Experiments were performed on 30 white non-diabetic male mice of the SHK colony, which were injected intraperitoneally with STZ at a dose of 200 mg/kg by two methods: 15 animals (group 1) once and 15 animals (group 2) intermittently – 5 consecutive days at 40 mg/kg per day. **Results.** In group 1, one mouse died after 2 days due to hypoglycemic coma, 4 mice developed hyperosmolar hyperglycemia (>33.3 mmol/l), 3 mice had spontaneous remission of diabetes, and 7 mice had stabilized hyperglycemia at levels close to 20 mmol/l. In group 2, only one mouse showed spontaneous remission of diabetes, while the remaining 14 animals showed stable diabetes with average hyperglycemia levels moderately above 20 mmol/L until the end of the 4-week follow-up. A histological study of the pancreas of these animals confirmed the destructive effect of STZ on islets in the form of mass death of insulin-producing β-cells. **Conclusion.** Split-dose intraperitoneal injection of STZ provides a stable experimental T1DM in 93% of laboratory mice.

Keywords: diabetes mellitus, streptozotocin, glycemia.

INTRODUCTION

Diabetes mellitus (DM) remains one of the major challenges of modern medicine and public health due to its high prevalence and steadily increasing incidence. Inadequate prevention and treatment of specific complications leads to reduced work capacity and to premature death among patients with DM. Therefore, development of new, more effective approaches to antidiabetic treatment is highly important. New DM therapies should typically undergo a required cycle of preclinical trials, primarily laboratory animal experiments. In this regard, the use of laboratory models that are adequate for DM in humans becomes crucial. At the same time, it is essential to determine the etiopathogenetically appropriate type of diabetes – type 1 diabetes (T1DM) or type 2 diabetes (T2DM) – for a given experimental model [1–3]. The most commonly used diabetic models are mice and rats.

T1DM in mice can be broadly divided into two main types: spontaneous and induced. A characteristic example of spontaneous T1DM is its experimental model in non-obese diabetic (NOD) mice [4]. About 1 month after birth, signs of pancreatic islet inflammation occur in these animals. This is accompanied by destruction of pancreatic insulin-producing beta cells. Such a destructive process happens most intensively at the age of 11–14 weeks against the background of infiltration of the pancreatic islets by immune cells. Mass death of beta cells leads to absolute insulin deficiency and formation of a characteristic diabetic syndrome. However, the use of this model, as well as other mice with spontaneous T1DM, has shown to be inadequate, as many drugs successfully used in NOD mice were ineffective in clinical trials. Since achieving stable diabetes in mice with spontaneous β-cell destruction is often impossible to guarantee, extrapolation of antidiabetic treatment results using such experimental DM models in the preparation of clinical trials is problematic [5–8].

Since insulin insufficiency is a hallmark of T1DM, it can be achieved experimentally by administering chemicals that kill beta cells. With the development of different protocols for injection of beta-cytotoxic drugs, it is possible to obtain T1DM models that meet the criteria for the experimental studies performed. The most widely used agent that can induce β-cell destruction is streptozotocin (STZ).

STZ was originally isolated from actinobacteria Streptomyces achromogenes in 1960 as an antibiotic with a putative antitumor effect. In preclinical tests in laboratory animals, the diabetogenic property of STZ was discovered and described in 1963 [9]. In subsequent years, it was shown [10] that the occurrence of a characteristic diabetes after STZ injection is due to selective destruction of pancreatic beta cells and development of absolute insulin deficiency, characteristic of insulin-dependent (type I) DM in humans. This finding has been

Corresponding author: Galina Skaletskaya. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (903) 771-18-13. E-mail: Skalink@mail.ru

successfully used in the development of experimental models of DM using STZ, primarily in rats and mice, which have natural endurance and good tolerance to even severe diabetes. An argument in favor of the induced DM model (in addition to STZ, alloxan, a mesoxalic acid ureide, is sometimes used, but it is less cytospecific and more toxic), compared with expensive races of rats and mice with spontaneous DM, is its much lower cost, especially since these drugs can be successfully administered even to outbred animals.

In our earlier article [11], we outlined two basic protocols for creating an STZ model of stable DM in laboratory rats. STZ was administered by a simple and safe method – intraperitoneal – which is not inferior to intravenous injection in terms of diabetogenic efficacy [12]. It was shown that a single injection of STZ at a dose of 70 mg per 1 kg body weight in Wistar rats produced a mixed diabetogenic effect, ranging from a slight increase and spontaneous reversal of hyperglycemia to a very high level of hyperglycemia and the death of some rats. Moreover, split-dose injection of STZ in the same dose (70 mg/kg) but divided into 5 consecutive days (14 mg/kg each), did not lead to animal death and there were almost no cases of spontaneous reversion of experimental DM, ensuring its stable course.

For several reasons (lower cost of animals, including their maintenance, saving diabetogenic drug and tested antidiabetic agent, etc.) it is reasonable to use laboratory mice as a diabetic model. In this regard, we decided to determine the STZ administration regimen that would ensure stable diabetes in these animals, considering the experience of obtaining experimental DM in rats, but using an STZ dose that is more accepted when dealing with mice (200 mg/kg) [13].

MATERIALS AND METHODS

Experiments were performed on 30 SHK white non-diabetic male mice with an initial weight of 25–30 g. The animals were obtained from a specialized nursery at the Research Center for Biomedical Technologies, Federal Biomedical Agency (Russia).

All animal manipulations were performed in compliance with the bioethical principles approved by the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes, 2005 and in accordance with the Rules of Laboratory Practice (Order 7080 approved by the Russian Ministry of Health, dated August 23, 2010.

STZ from Sigma (USA) was used as a diabetogenic agent. The agent was dissolved ex tempore in saline and injected in the animals intraperitoneally at the rate of 200 mg per 1 kg body weight by two methods: as a single injection in 15 mice (group 1) and intermittently in the remaining 15 mice, for five consecutive days, at a dose of 40 mg/kg each day (group 2). Glycemia in mice was determined at 12:00–12:30 noon in fasting capillary blood using test strips by Accu-Chek Performa glucometer (Roche), measuring range 0.6–33.3 mmol/L. To assess the nature and degree of morphological lesion of islets by STZ, we performed histological examination of the pancreas of mice with DM euthanized at the end of the experiment, as well as intact animals (control). Paraffin blocks were prepared from formalin-fixed pancreatic tissue samples, and 4–5 μm thick sections were stained with hematoxylin and eosin, and immunohistochemically for insulin and glucagon to detect islet beta cells and alpha cells.

Statistical data processing was performed using Microsoft Excel (2016) software. Descriptive statistics indicators – number of observations, arithmetic mean and standard deviation – were calculated. Student's t-test was used to determine the statistical significance of differences between means. Differences were considered statistically significant if significance level p did not exceed 0.05.

RESULTS AND DISCUSSION

The first visible signs of change in condition among the majority of the experimental animals were noted after 3–4 days of follow-up: thirst (increased volume of water intake) and polyuria appeared. In both groups, diabetes was confirmed by determination of hyperglycemia (Fig. 1); however, its magnitude and dynamics differed significantly depending on how STZ was administered – once or intermittently.

In group 1 (single injection at a dose of 200 mg/kg), one mouse died on day 2, apparently from hypoglycemic coma (glycemia of 1.3 mmol/L). Four of the remaining 14 mice had a rise in blood glucose, from moderate to

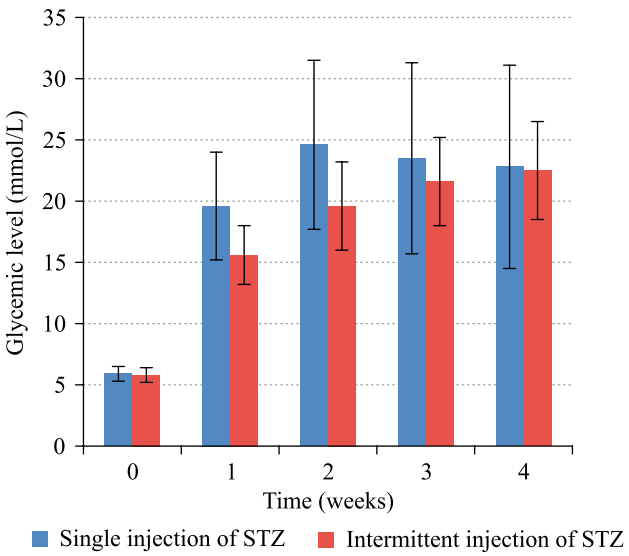

Fig. 1. Changes in glycemia after single and split-dose injection of streptozotocin in non-diabetic mice over a 4-week follow-up period

hyperosmolar hyperglycemia (>33.3 mmol/L) within 1–2 weeks. Three of them had a moderate rise in glycemia in the first 2 weeks after STZ injection (up to 12.8–17.9 mmol/L, mean 15.3 mmol/L); in the next 2 weeks, it spontaneously mean to subnormal (not fasting) levels, and by the end of follow-up it ranged from 8.9 to 12.8 mmol/L (average 11.0 mmol/L). In the remaining 7 group 1 mice, hyperglycemia was quite severe and persistent, ranging from 19.1 to 24.8 mmol/L (mean 22.3 mmol/L) at the end of follow-up.

So, 1 out of 15 experimental mice had fatal hypoglycemia following a single (200 mg/kg) intraperitoneal injection of STZ. The most likely explanation of this was the massive amounts of insulin released into the bloodstream following the mass death of islet beta cells as a result of the cytotoxic action of STZ. The remaining 14 mice also experienced hyperglycemia, but its height and dynamics of changes were ambiguous, and their features made it possible to identify three variants of STZ-induced DM after a single STZ injection. The first of them can be attributed to the maximum diabetogenic effect noted in 4 animals, where hyperglycemia reached an exceedingly elevated level (>33.3 mmol/l), which persisted practically until the end of a 4-week follow-up.

At the same time, the animals' overall health was not terrible, confirming that mice can tolerate induced DM relatively well, even in cases where very high glycemia is reached. However, the use of animals with prohibitive levels of hyperglycemia is hardly advisable because this makes it impossible to accurately assess the dynamics of glycemic changes during studies of a specific sugar-lowering medication.

The second variant of the course of STZ-induced DM can be attributed to changes in glycemia in 3 other mice. Following a period of moderate hyperglycemia, there was a gradual but significant drop in blood sugar levels, accompanied by disappearance of severe clinical signs of diabetes. This may be considered a spontaneous reversion of T1DM which appears to have occurred because the animals' pancreatic β-cells are not sensitive enough to the toxic effect of STZ.

In the remaining 7 mice from group 1, fairly severe hyperglycemia was stable and most often exceeded 20 mmol/L. The proportion of these variants of hyperglycemia dynamics in mice from this group was 29%, 21%, and 50%, respectively (Fig. 2).

Significantly more favorable results were obtained in group 2 mice, where STZ was injected into the peritoneal cavity also at 200 mg/kg, but in small equal doses (40 mg/kg/day) for 5 consecutive days.

Firstly, there was no case of animal death, which can be primarily down to exclusion of hypoglycemic coma due to split-dose administration of less toxic doses of STZ. For the same reason, there was no rise in glycemia to an ultra-high level. Secondly, only in one case was there a tendency to spontaneous reversal of hyperglycemia, most likely due to individual low sensitivity of pancreatic β-cells in this animal to STZ. Finally, an overwhelming number of mice in group 2 (14 out of 15) showed chronic hyperglycemia, which most often exceeded 20 mmol/L, but did not reach a very high level, averaging 23.4 mmol/L at the end of follow-up. Such stability of diabetes in split-dose STZ injection is quite probably facilitated by the development of an autoimmune process in the pancreatic islets of the experimental mice, leading to irreversible death of beta cells; this process has been identified by several researchers [7, 8, 10, 12, 13].

Thus, in 14 of the 15 experimental mice, or 93% of the cases, split-dose STZ stabilized experimental T1DM without causing extreme elevations in hyperglycemia; just one mouse had a spontaneous reversion of its diabetic status (Fig. 3).

Upon completion of the experiments, histological examinations of the experimental animals' pancreas de-

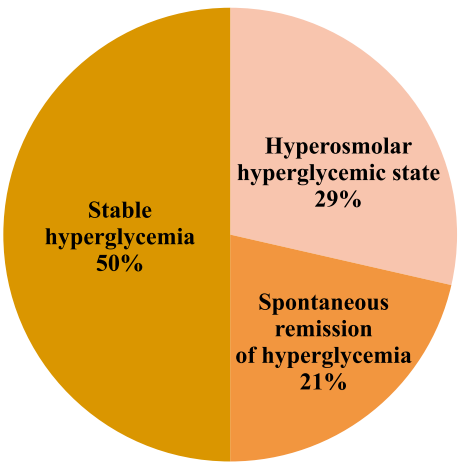

Fig. 2. Different degrees and dynamics of hyperglycemia in mice after a single intraperitoneal injection of streptozotocin at a dose of 200 mg/kg

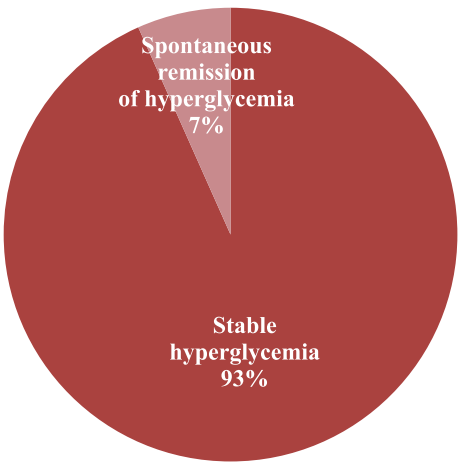

Fig. 3. Different degrees and dynamics of hyperglycemia in laboratory mice after split-dose intraperitoneal injection of streptozotocin at a total dose of 200 mg/kg

monstrated some structural alterations typical of STZ-induced T1DM. When compared to the morphologic image of the pancreas of intact nondiabetic mice (Fig. 4, a, 5), the destructive changes in the islets (Fig. 4, b, 6) were particularly noticeable in group 2 rats with stable diabetes. At the same time, almost all insulin-producing islet beta cells died after split-dose STZ injection (Fig. 6, a), while glucagon-producing alpha cells were not exposed to toxic effects and remained in the form of compact groups (Fig. 6, b), as if filling the vacant spaces occupied by β-cells earlier. Preservation of α-cells in STZ-induced DM mice has also been reported in a number of relevant studies [12, 13].

CONCLUSION

Both single and split-dose injections of STZ can stabilize a T1DM model in mice. However, split-dose injection is considerably more rational. Although after a single intraperitoneal STZ injection at a dose of 200 mg/kg, the proportion of mice with marked hyperglycemia was quite high (78.6%), but a significant proportion of them (28.6%) had unacceptably high (>33.3 mmol/L) blood glucose levels. At the same time, 21.4% of animals in this group showed signs of spontaneous reversion of diabetes. After a 4-week follow-up of group 1 mice, only half of them could be selected as experimental animals with experimental DM.

Since no experimental animals died and a significantly higher proportion of them had stable experimental T1DM in the absence of excessive hyperglycemia, the induction of T1DM by split-dose STZ injection should be viewed as more significant. The number of cases of spontaneous reversion of diabetic status was negligible. This type of STZ-induced DM is the one that can be suggested for use in an objective assessment of the out-

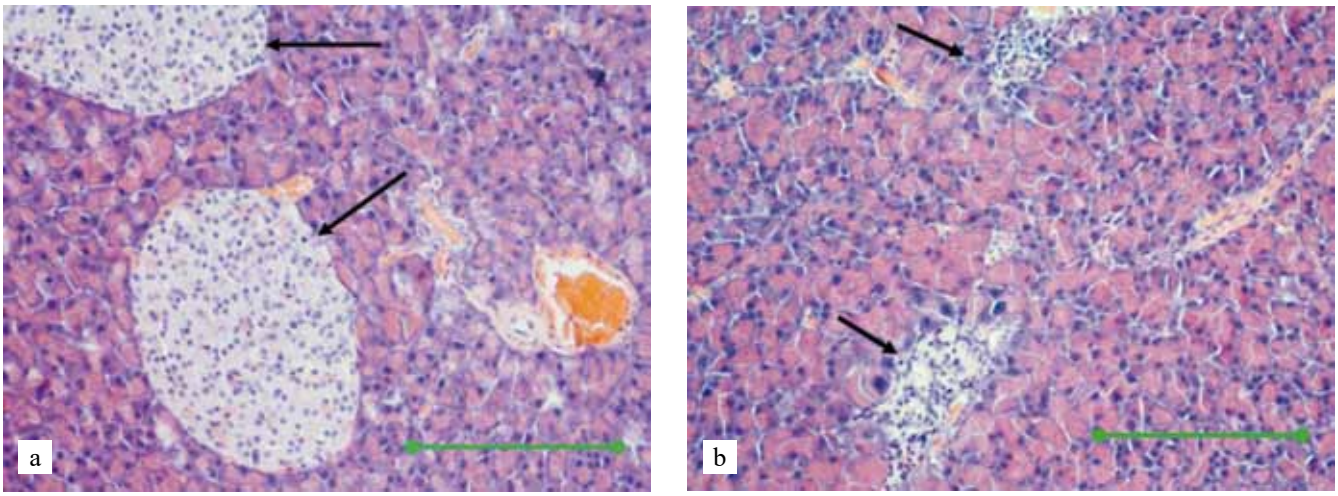

Fig. 4. Morphological pictures. a, islets in the pancreas of an intact non-diabetic mouse; b, pancreas of a mouse with streptozotocin-induced T1DM – severe destruction of islets. H&E stain. Scale bar, 200 μm

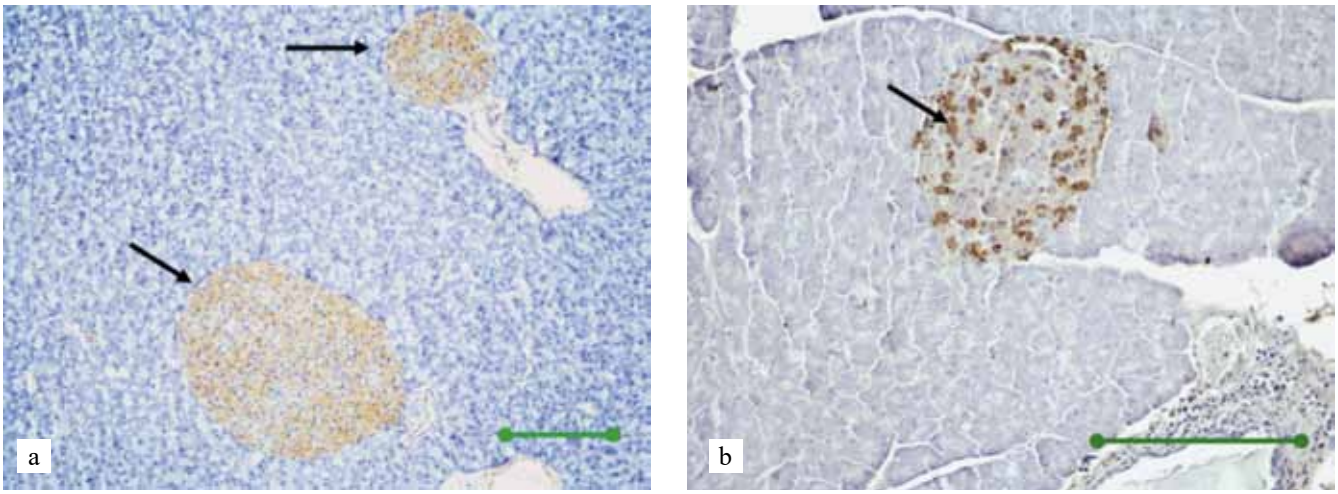

Fig. 5. Pancreas of an intact nondiabetic mouse. a, identification of beta cells in the islets. Immunohistochemical staining for insulin. Scale bar, 100 μm; b, identification of alpha cells in the islets. Immunohistochemical staining for glucagon. Scale bar, 200 μm

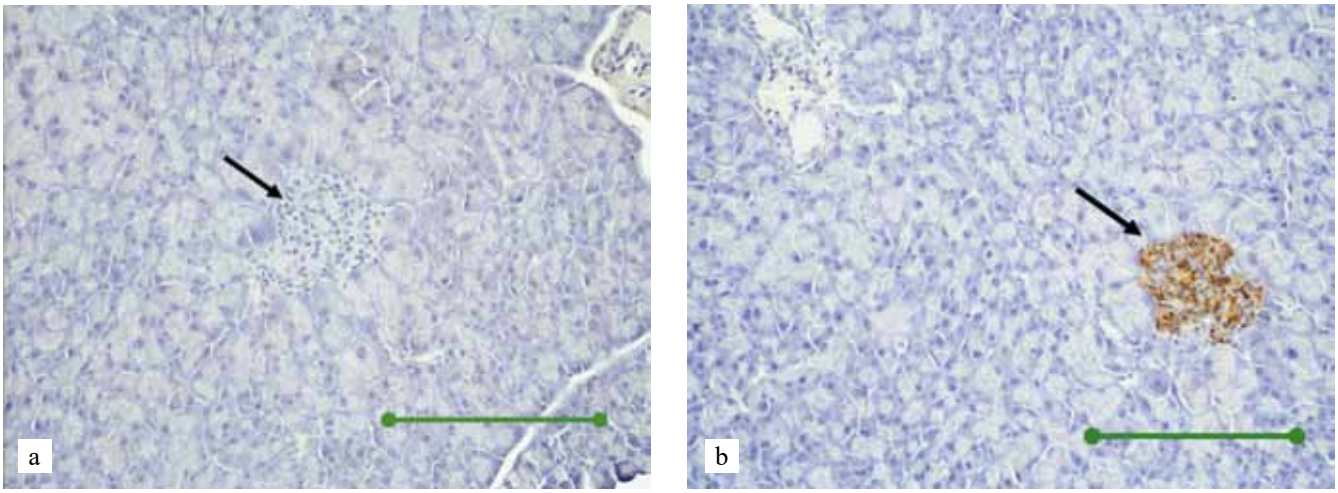

Fig. 6. Pancreas of a mouse with streptozotocin-induced T1DM. a, no beta cells in the islet. immunohistochemical staining for insulin; b, compactly located alpha cells in the islet. Immunohistochemical staining for glucagon. Scale bar, 200 μm

comes of various islet cell transplantation choices and other antidiabetic treatment methods.

The authors declare no conflict of interest.

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