New Model of Progressive Non-Insulin-Dependent Diabetes Mellitus in Mice Induced by Streptozotocin

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This study was designed to clarify the relationship between streptozotocin (STZ) dosage (100, 150 and 200 mg/kg i.p.) and the resulting diabetogenic response in mice (8-week-old male ICR). In this experiment, we found that a single i.p. injection of 100 mg/kg STZ is able to induce progressive diabetes mellitus, in which non-fasting serum glucose levels begin to rise from 3 weeks and continue to rise throughout the experimental period until 9 weeks. The non-fasting serum insulin levels of 100 mg/kg STZ-treated mice were normal during the experimental period. In addition, the population of insulin-immunoreactive cells (beta cells) in the islets of pancreata was slightly less than in normal mice at 9 weeks. In 200 mg/kg STZ-treated mice, on the other hand, the insulin levels were below measurable values and insulin-immunoreactive cells were not observed. It is concluded from these results that the progressive diabetic mouse model induced by a single i.p. injection of 100 mg/kg STZ, unlike 200 mg/kg STZ-induced diabetes which is insulin-dependent, is non-insulin-dependent.

Key words streptozotocin diabetes; non-insulin-dependent diabetes; mouse

Streptozotocin (STZ) is widely used to induce diabetes in animals as a model of insulin-dependent diabetes mellitus (IDDM).1) The i.v. injection of 25 to 100 mg/kg STZ can produce a dose-dependent diabetogenic action in rats. The present study was designed to determine the dose-dependent diabetogenic action of STZ in mice.

MATERIALS AND METHODS

Male ICR mice (Nippon SLC, Shizuoka) (8-week-old) used in the experiment were housed in an air-conditioned room at 23±1°C and fed a standard diet (MM-3 pellets) (Funahashi Farm, Chiba). After fasting for 20h, animals of the STZ-treated groups received a single i.p. injection of 100, 150 or 200 mg STZ/kg of body weight (Sigma, St. Louis, MO, U.S.A.), freshly dissolved in 0.05 mol/l citrate buffer, pH 4.5. Normal animals were injected with an equivalent volume of citrate buffer. Blood samples of STZ-treated and normal animals were withdrawn from the cavernous sinus with a capillary at 1, 3, 5, 7 and 9 weeks after treatment with STZ to determine serum glucose and insulin. At 9 weeks, pancreata were taken to stain insulin-immunoreactive cells (beta cells) immediately after collecting blood samples. Serum glucose and insulin were determined using commercial reagents of Glucose ClII-test Wako (Wako Pure Chemical Industries, Ltd., Osaka) and ELISA Insulin kit (Seikagaku Industries, Ltd., Tokyo), respectively. Beta cells were immuno-histochemically stained using a Histofine SAB kit (Nichirei, Tokyo). Data are represented as mean±S.E. Statistical analysis was done by ANOVA followed by the Bonferroni/Dunn test. p<0.05 was considered significant.

RESULTS

The non-fasting serum glucose levels of normal groups were about 200 mg/dl throughout the 9-week observation period after treatment with STZ (Fig. 1). In 150 and 200 mg/kg STZ-treated groups, the serum glucose levels rose markedly from 1 week after treatment with STZ and high glucose levels of 500 to 700 mg/dl and 700 to 800 mg/dl, respectively, were maintained for 1 to 9 weeks (Fig. 1). However, the glucose levels of 100 mg/kg STZ-treated group began to rise from 3 weeks and continued to rise until 9 weeks (about 700 mg/dl) (Fig. 1). The non-fasting insulin levels of the 100 mg/kg STZ-treated group were similar to those of the normal group, although the levels of 200 mg/kg STZ-treated group were below measurable values (Fig. 2). The population of insulin-immunoreactive cells (beta cells) in the islets of pancreas was slightly less in 100 mg/kg STZ-treated mice than in normal mice (Fig. 3). However, these cells were not observed in the 200 mg/kg STZ-treated group (Fig. 3).

DISCUSSION

STZ has been commonly used to induce not only animal models of IDDM,1) but also lean type of non-insulin-dependent diabetes (NIDDM) with hypoinsulinemia by neonatal (1 or 2-day-old mice or rats) STZ administration.2–5) Recently, Luo et al. succeeded in making an animal model of NIDDM by a single i.p. injection of low dose (100 mg/kg) STZ to 6-week-old insulin-resistant mice with hyperinsulinemia fed either a high-fat or high-fructose diet, although these diet-induced insulin-resistant animals did not develop hyperglycemia without the STZ injection.6) In this experiment, they failed to produce hyperglycemia in mice fed the standard diet by low-dose STZ in the 4-week-observation after STZ treatment.

In the present study, we succeeded in producing slowly progressive diabetes mellitus by a single i.p. injection of 100 mg/kg STZ to 8-week-old male ICR mice fed a standard diet. The reason Luo et al. failed to develop diabetes mellitus in their animals fed the standard diet may have been due to differences in the age of mice when STZ was injected and/or the observation period following that treatment. Interestingly, in our experiment, non-fasting serum glucose was still within normal levels at 1 week after treatment with STZ and thereafter continued to rise until 9 weeks when the experiment was terminated. It has been shown that injection of various diabetogenic doses of STZ is able to produce from mild to severe diabetes mellitus in rats.1) When 7-week-old male

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Sprague-Dawley rats received a single i.v. injection of the minimum diabetogenic dose STZ (15 mg/kg), there was a temporary rise in blood glucose levels at 1 week after treatment and then a spontaneous return to normal levels by 2 weeks. Therefore, it is noteworthy that only a single i.p. injection of 100 mg/kg STZ developed progressive diabetes mellitus in adult mice from 3 weeks. In addition, in this model, non-fasting serum insulin was maintained at normal levels for the 9-week-observation period and insulin-immunoreactive cells in pancreata (beta cells) were well preserved even at 9 weeks.

These results suggest that the 100 mg/kg STZ-induced mouse model is non-insulin-dependent and the elevation of blood glucose levels may be due to the increase in insulin resistance rather than to impaired insulin secretion. On the other hand, in 200 mg/kg STZ-treated animals, high glucose levels were maintained for 1 to 9 weeks. Furthermore, the serum insulin levels of these animals were below measurable values and insulin-immunoreactive cells were not detected.

Thus, the 200 mg/kg STZ-injected mouse exhibited the characteristics of IDDM. This new model may be useful to identify the mechanism of induction and progression of NIDDM in human or to screen antidiabetic substances.

REFERENCES