

## Strain Differences in the Diabetogenic Activity of Streptozotocin in Mice

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We have already reported that slowly progressive non-insulin-dependent diabetes mellitus (NIDDM) is produced by a single i.p. injection of a subdiabetogenic dose (100 mg/kg) of streptozotocin (STZ) to 8-week-old male ICR mice. The aim of the present study was to clarify whether or not the progressive NIDDM is induced in ddY, BALB/c, C57BL/6 and ICR mice by the administration of STZ. Eight-week-old male mice of the 4 different strains were administered a single i.p. injection of STZ at various doses (ICR, ddY and BALB/c: 100—200 mg/kg; C57BL/6: 75—150 mg/kg). Among the ddY, BALB/c and C57BL/6 mice, a time course-related rise in non-fasting serum glucose levels throughout the observation period of 1—12 weeks after STZ administration was only induced in the 125 mg/kg STZ ddY and 100 mg/kg STZ ICR mice. In contrast with serum glucose levels, the area of islets and the percentage of the relative number of insulin-immunoreactive cells ( $\beta$ -cells) to glucagon-immunoreactive cells ( $\alpha$ -cells) in the 100 mg/kg STZ ICR and 125 mg/kg STZ ddY mice continued to decrease gradually over time. In addition, in low dose STZ mice of both strains, the insulin response to glucose stimulation was extremely impaired over time, although non-fasting serum insulin levels were maintained near normal levels. The rate of the progression of diabetes was faster in the 125 mg STZ ddY mice than in the 100 mg/kg STZ ICR mice.

**Key words** streptozotocin; slowly progressive diabetes; ICR; ddY

Diabetes mellitus is classified into two types, insulin-dependent (IDDM, type 1) and non-insulin-dependent diabetes mellitus (NIDDM, type 2). NIDDM is a disease characterized by peripheral insulin resistance and impaired insulin secretion. NIDDM patients are divided into 2 categories, obese type with hyperinsulinemia and non-obese type with hypoinsulinemia.

Streptozotocin (STZ; *N*-nitroso derivative of glucosamine) is a broad-spectrum antibiotic extracted from *Streptomyces acromogenes*.<sup>1)</sup> It is a pancreatic  $\beta$ -cell toxin that induces rapid and irreversible necrosis of  $\beta$ -cells<sup>2)</sup> and is widely used in making experimental animal models of IDDM.<sup>3—6)</sup> Junod *et al.*<sup>5)</sup> reported that the i.v. injection of 25 to 100 mg/kg STZ to rats was able to produce a dose-dependent hyperglycemia.

Recently, we succeeded in producing a new mouse model of slowly progressive NIDDM by only a single i.p. injection of a subdiabetogenic dose (100 mg/kg) of STZ to 8-week-old male ICR mice.<sup>7,8)</sup> However, there is yet no experimental evidence about whether or not the slowly progressive NIDDM is produced in mice of other strains, except for ICR, by a single i.p. injection of a subdiabetogenic dose of STZ. Therefore, in the present study, we examined the dose-dependent diabetogenic action of STZ in 8-week-old male ddY, BALB/c, and C57BL/6 mice and compared it with that in 8-week-old male ICR mice. We found that slowly progressive NIDDM was produced in ddY mice as well as ICR mice by a single i.p. injection of a subdiabetogenic dose (125 mg/kg) of STZ. In order to clarify the characteristics of the progressive diabetes in ddY mice, we also followed-up the changes in islet morphology (the number of  $\alpha$ - and  $\beta$ -cells) and serum glucose and insulin responses to oral glucose load after STZ administration.

### MATERIALS AND METHODS

**Animals** Eight-week-old male ICR, ddY, BALB/c and C57BL/6 mice (Nippon SLC, Shizuoka, Japan) were housed

in an isolator caging system in air-conditioned animal room at 23±1°C. All experimental procedures described were approved by the Experimental Animal Research Committee of Meijo University, Faculty of Pharmacy.

**Induction of Diabetes** Mice were fasted for 20 h before diabetes was induced with STZ. ICR and ddY mice received a single i.p. injection of 100, 150 or 200 mg/kg and 100, 125, 150 or 200 mg/kg, respectively, of STZ (Sigma, St. Louis, MO, U.S.A.) freshly dissolved in 0.05 M citrate buffer, pH 4.5. BALB/c and C57BL/6 mice received a single i.p. injection of 100, 150, 175 or 200 mg/kg and 75, 100 or 150 mg/kg, respectively, of STZ. Normal mice of each strain were injected with the equivalent volume of citrate buffer.

**Serum Glucose, Insulin and Total Cholesterol Levels, Body Weight, Urine Volume, and Drinking Water and Food Consumption** In the first experiment, blood samples from normal ICR, ddY, BALB/c and C57BL/6 mice and mice administered various doses of STZ were taken from the cavernous sinus with a capillary tube under ether anesthesia at 1, 3, 5, 7, 9 and 12 weeks after STZ administration for the determination of serum glucose, insulin (100 and 200 mg/kg STZ ICR and 125 and 200 mg/kg STZ ddY mice and normal mice of both strains) and total cholesterol. Body weight was measured immediately before blood collection. After collection of blood samples, the animals were kept in individual metabolic cages for 24 h and drinking water (distilled water) and food consumption per 24 h were measured.

In the second experiment, blood samples from the 100 and 200 mg/kg STZ ICR and 125 and 200 mg/kg STZ ddY mice were obtained at 20 (non-fasting time) and 0.5 h (fasting time) before and 2, 7, 24, 48 and 72 h after STZ administration in order to measure serum glucose and insulin. Serum glucose, insulin and total cholesterol were determined using commercial reagents: Glucose CII-test Wako (Wako Pure Chemical Industries, Ltd., Tokyo), ELISA Insulin kit (Moriguchi Seikagaku Industries, Ltd., Tokyo) and Detamina TC-5 (Kyowa Medix Co., Ltd., Tokyo), respectively.

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**Immunocytochemistry** For glucagon and insulin immunostainings, the 100 and 200 mg/kg STZ ICR and 125 and 200 mg/kg STZ ddY mice and normal mice of both strains were killed with an overdose of ether and their pancreata were removed 1 d, and 1, 4, 9 and 12 weeks after STZ administration. The pancreata were fixed in 10% buffered formalin and then embeded in paraffin. Two consecutive sections (section thickness, 1- $\mu$ m) were cut from the paraffin block.

One of the two sections was stained with anti-glucagon monoclonal antibody (Histofine SAB-PO kit) (Nichirei, Tokyo, Japan) to observe glucagon immunoreactive cells ( $\alpha$ -cells). The other section was stained with anti-insulin monoclonal antibody (Histofine SAB-PO kit) (Nichirei, Tokyo, Japan) to observe insulin immunoreactive cells ( $\beta$ -cells).

The glucagon- and insulin-immunoreactive cell populations in the islets were counted.

**Oral Glucose Tolerance Test** Oral glucose tolerance test in the 100 mg/kg STZ ICR and 125 mg/kg STZ ddY mice and normal mice of both strains was carried out on 3 d and 1, 2, 4, 6 and 8 weeks after STZ administration. These animals were fasted for 18 h before the test and then given 2 g/kg glucose solution orally. Blood samples were taken from the cavernous sinus with a capillary under ether anesthesia at 0.5 h before and at 0.5, 1, 2 and 4 h after glucose loading. Serum glucose and insulin levels (only at 3 d and at 1, 2, 6 weeks) were determined as described above.

**Statistical Analysis** The results obtained are expressed as the mean  $\pm$  S.E.M. The data were analyzed using one-way analysis of variance and Duncan's multiple range test or non-parametric statistics. In all cases,  $p < 0.05$  was considered significant.

**RESULTS**

**Changes in Serum Glucose, Total Cholesterol and Insulin Levels after STZ Administration in ICR, ddY,**

**BALB/c and C57BL/6 Mice** The non-fasting serum glucose levels of the normal groups of ICR, ddY, BALB/c and C57BL/6 mice were 176—225 mg/dl, 206—252 mg/dl, 133—180 mg/dl and 196—228 mg/dl, respectively, throughout the observation period of 1—12 weeks (Fig. 1).

In the 150 and 200 mg/kg STZ ICR mice, the serum glucose levels rose markedly from 1 week after STZ administration and the high glucose levels were thereafter maintained until 12 weeks (termination of the experiment) (150 mg/kg STZ: 585—835 mg/dl; 200 mg/kg STZ: 644—849 mg/dl). The glucose levels in the 100 mg/kg STZ ICR mice were within a normal range at 1 week, but continued to rise thereafter till 12 weeks when the experiment terminated (1 week:  $270 \pm 22$  mg/dl; 12 weeks:  $706 \pm 38$  mg/dl). In the 150 and 200 mg/kg STZ ddY mice as well as high doses of STZ in ICR mice, the high glucose levels were maintained throughout the observation period of 1—15 weeks (150 mg/kg STZ: 489—755 mg/dl; 200 mg/kg STZ: 520—772 mg/dl). In the 100 mg/kg STZ ddY mice, no apparent rise of the glucose levels was observed throughout the observation period. However, in the 125 mg/kg STZ ddY mice, the time course-related rise in serum glucose levels was seen throughout the observation period (1 week:  $305 \pm 39$  mg/dl; 12 weeks:  $728 \pm 41$  mg/dl). In the 175 and 200 mg/kg STZ BALB/c mice, the high serum glucose levels were observed throughout 1—12 weeks (175 mg/kg STZ: 406—483 mg/dl; 200 mg/kg STZ: 501—611 mg/dl). In the 150 mg/kg STZ BALB/c mice, a slight but significant increase in the glucose levels was seen from 1 week, but a time course-related rise of the glucose levels thereafter was not seen. The glucose levels in the 100 mg/kg STZ BALB/c mice were within a normal range even at 12 weeks. In the 150 mg/kg STZ C57BL/6 mice, high glucose levels over 950 mg/dl were recognized at 1 week but all mice died of severe weakness by 5 weeks. In the 100 mg/kg STZ C57BL/6 mice, high glucose levels were maintained throughout 1—12 weeks (406—541 mg/dl).

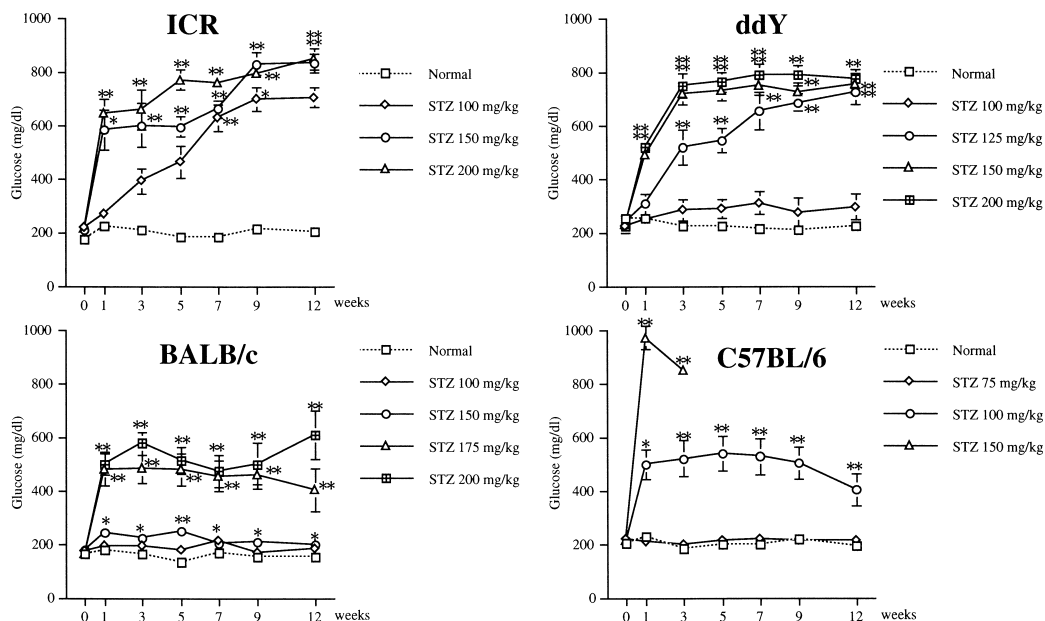


Fig. 1. Changes in Non-fasting Serum Glucose Levels in ICR, ddY, BALB/c and C57BL/6 Mice after a Single i.p. Injection of Various Doses of Streptozotocin (STZ)

Each plot denotes the mean  $\pm$  S.E.M. for 8 mice. Significantly different from respective normal, \*  $p < 0.05$ , \*\*  $p < 0.01$ .

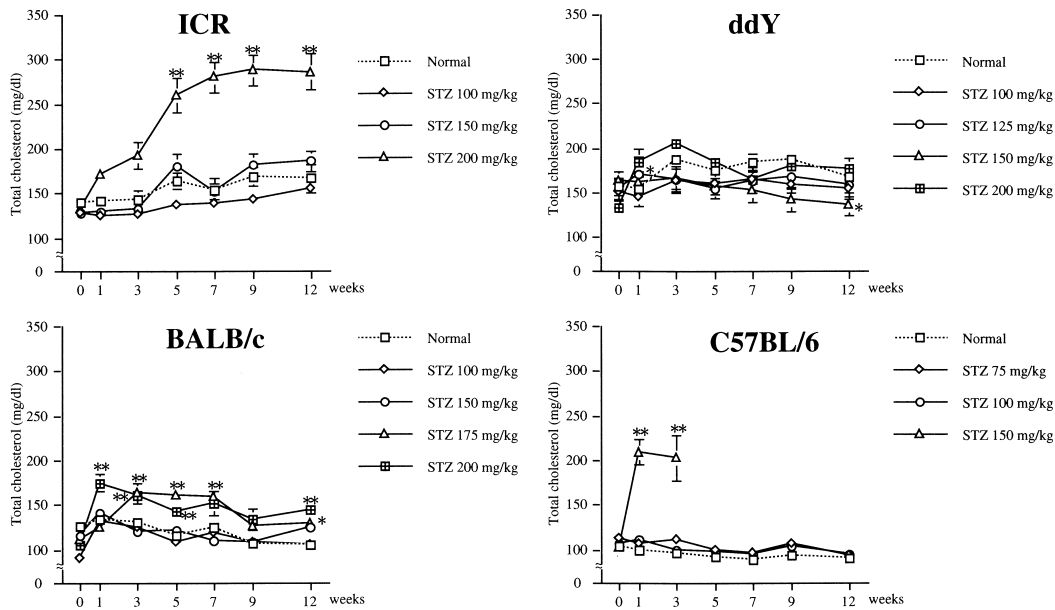


Fig. 2. Changes in Non-fasting Serum Total Cholesterol Levels in ICR, ddY, BALB/c and C57BL/6 Mice after a Single i.p. Injection of Various Doses of Streptozotocin (STZ)

Each plot denotes the mean±S.E.M. for 8 mice. Significantly different from respective normal, \* $p < 0.05$ , \*\* $p < 0.01$ .

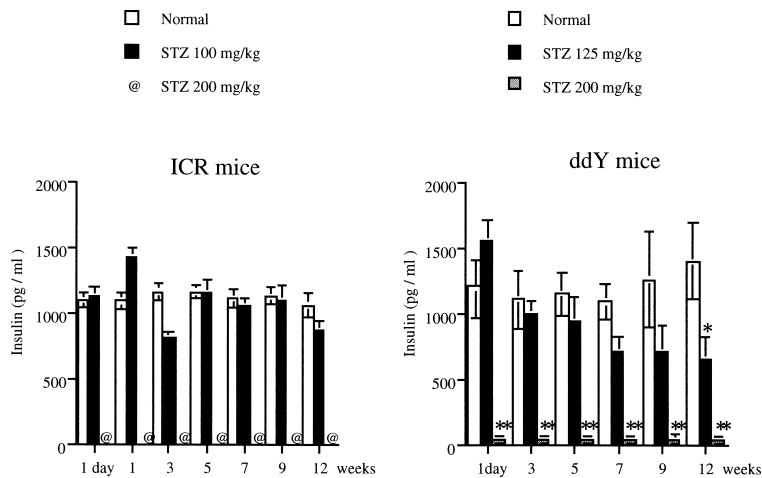


Fig. 3. Changes in Non-fasting Serum Insulin Levels after a Single i.p. Injection of Streptozotocin (STZ) in 100 and 200 mg/kg STZ ICR and 125 and 200 mg/kg ddY Mice

Serum insulin levels in 200 mg/kg STZ ICR mice were below measurable values. Each plot denotes the mean±S.E.M. for 8 mice. Significantly different from respective normal at \* $p < 0.05$ , \*\* $p < 0.01$ .

However, the glucose levels in the 75 mg/kg STZ C57BL/6 mice were within the normal range for the observation period. Thus, slowly progressive diabetes was induced in the 125 mg/kg STZ ddY mice as well as the 100 mg/kg STZ ICR mice.

In only the highest dose STZ ICR (200 mg/kg) and C57BL/6 mice (150 mg/kg STZ), non-fasting serum total cholesterol levels were markedly higher than those in respective normal mice (Fig. 2). However, the total cholesterol levels of the 100 and 150 mg STZ ICR and 125, 150 and 200 mg/kg STZ ddY mice were within the normal range, although hyperglycemia was observed in these mice.

The non-fasting serum insulin levels were determined only in the 100 and 200 mg/kg STZ ICR and 125 and 200 mg/kg STZ ddY mice. The serum insulin levels in the 100 mg/kg STZ ICR mice, which induced the progressive diabetes, were

within a normal range from 1 to 12 weeks when the experiment was terminated after STZ administration (Fig. 3. left). On the other hand, the insulin levels in the 125 mg/kg STZ ddY mice, which slowly progressive diabetes was induced, decreased slightly over time after STZ administration and were significantly lower than the normal levels at 12 weeks (Fig. 3. right). However, the insulin levels in the 200 mg/kg STZ ICR and ddY mice, in which hyperglycemia was induced from 1 week after STZ administration, were extremely low.

**Changes in Serum Glucose and Insulin Levels within 72 h after STZ Administration in ICR and ddY Mice**  
The serum glucose and insulin levels within 72 h after STZ administration were determined only in the 100 and 200 mg/kg STZ ICR and 125 and 200 mg/kg STZ ddY mice. In the 100 mg/kg STZ ICR and 125 mg/kg STZ ddY mice, a

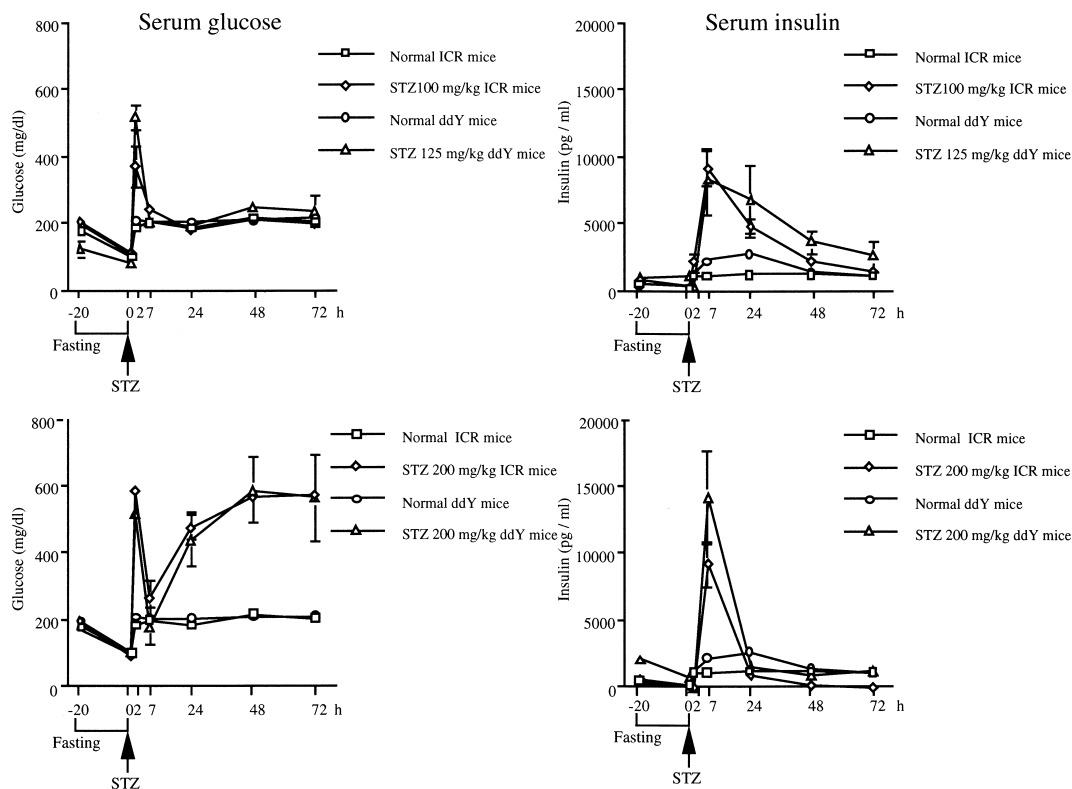


Fig. 4. Changes in Serum Glucose and Insulin Levels within 72 h after a Single Injection of Streptozotocin (STZ) in ICR and ddY Mice  
Each plot denotes the mean  $\pm$  S.E.M. for 8 mice.

transient rise of serum glucose levels was observed at 2 h after STZ administration (Fig. 4, left-top panel). All mice of both strains given low dose STZ returned to normoglycemia within 24 h. On the other hand, in the 200 mg/kg STZ ICR and ddY mice, a transient rise in the serum glucose levels was seen at 2 h after STZ administration, following which they returned to near normal levels by 7 h (Fig. 4, left-bottom panel). Thereafter, the glucose levels in the high dose STZ ICR and ddY mice again rose and stable hyperglycemia was observed at 24, 48 and 72 h. Serum insulin levels in the 100 mg/kg STZ ICR and 125 mg/kg STZ ddY mice showed a remarkable rise at 7 h after STZ administration (Fig. 4, right-top panel). Thereafter, the insulin levels in both strains of mice given low dose STZ slowly decreased. On the other hand, in the 200 mg/kg STZ ICR and ddY mice, a transient rise of serum insulin levels was recognized at 7 h after STZ administration (Fig. 4, right-bottom panel). Thereafter, the insulin levels in both strains of mice given a high dose of STZ rapidly decreased and were under measurable values by 48 h.

**Changes in Body Weight, Urine Volume, and Drinking Water and Food Consumption after STZ Administration in ICR and ddY Mice** Body weight in the normal groups of ICR and ddY mice increased gradually during the 12-week-observation period. Body weight in the 150 mg/kg or 200 mg/kg STZ ICR and ddY mice with severe hyperglycemia from 1 week after STZ administration was significantly lower than that in the respective normal mice throughout the observation period of 1–12 weeks (Fig. 5A). On the other hand, body weight in the 100 mg/kg STZ ICR and 125 mg/kg ddY mice, in which a time course-related rise of serum glucose levels was induced, was not significantly dif-

ferent from that in the respective normal mice for the observation period.

Urine volume per 24 h in the 150 or 200 mg/kg STZ ICR and ddY mice was markedly greater than that in the respective normal mice throughout 3–12 weeks after STZ administration (Fig. 5B). In addition, drinking water (Fig. 5C) and food consumption (Fig. 5D) per 24 h at the same doses in STZ ICR and ddY mice were also markedly greater than those in the respective normal mice throughout the same period. On the other hand, urine volume and drinking water and food consumption in the 100 mg/kg STZ ICR and 125 mg/kg STZ ddY mice steadily increased for 1–12 weeks, although the degrees of increases were less than those found in mice of both strains given 150 or 200 mg/kg STZ.

**Changes in Pancreatic Islet Morphology after STZ Administration in ICR and ddY Mice** There was a marked involution of pancreatic islets in the 200 mg/kg STZ ICR and ddY mice, and the area of islets in both strains markedly decreased from 1 week after STZ administration (ICR: Fig. 6; ddY: Fig. 7). On the other hand, in the 100 mg/kg STZ ICR and 125 mg/kg STZ ddY mice, the area of islets decreased gradually as time passed after STZ administration. However, the degree of involution of the islets was greater in the 125 mg/kg STZ ddY mice than in the 100 mg/kg STZ ICR mice at all stages after STZ administration.

The percentage of the number of glucagon ( $\alpha$ -cells)- and insulin-immunoreactive cells ( $\beta$ -cells) in pancreatic islets in normal ICR and ddY mice was about 20% and 80%, respectively, throughout the observation of 1–12 weeks (ICR: Figs. 6, 8A; ddY: Figs. 7, 8B). The percentage of the number of  $\beta$ -cells in 200 mg/kg STZ ICR and ddY mice decreased markedly, by less than 10% and 14%, respectively, from 1

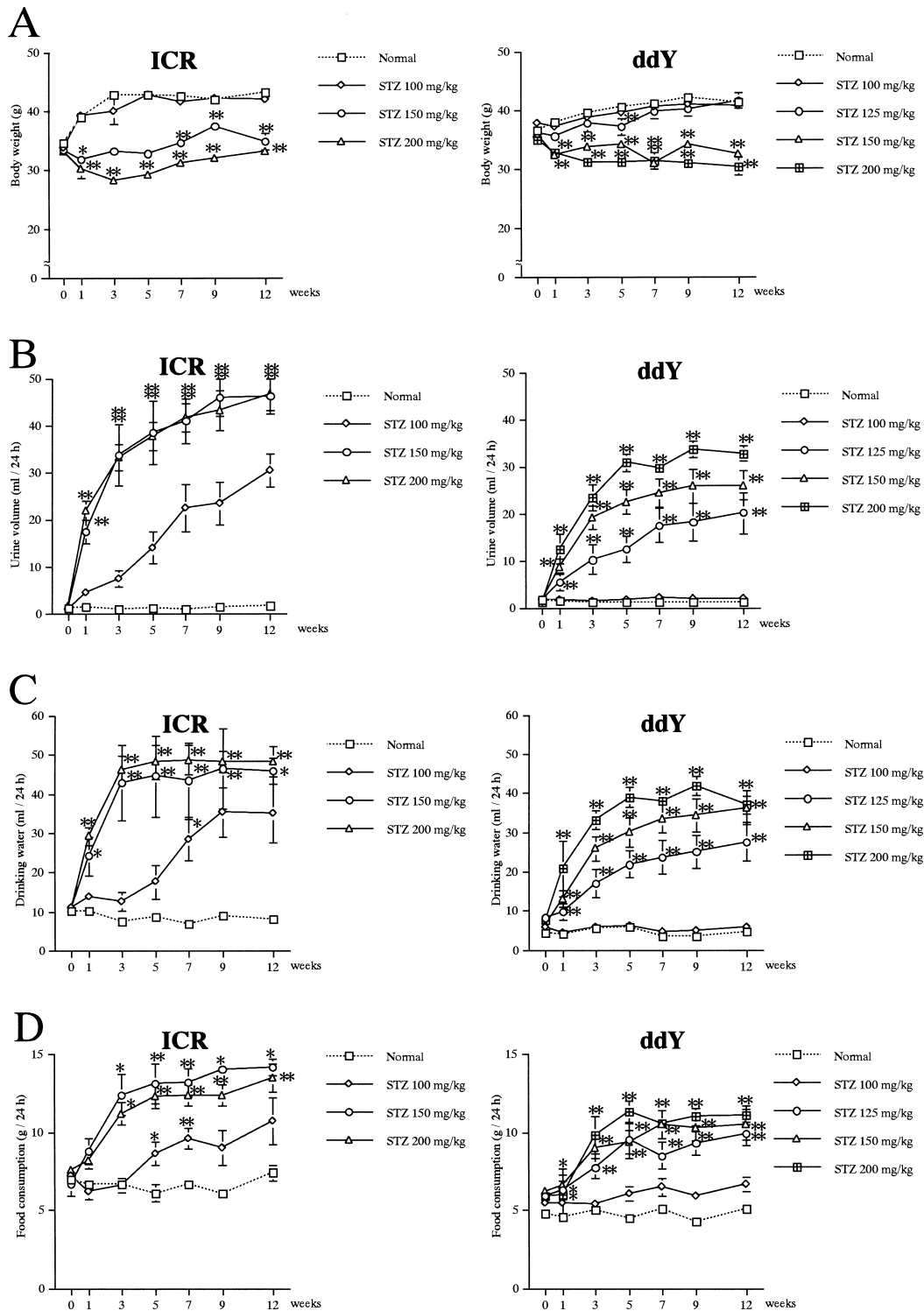


Fig. 5. Changes in Body Weight (A), Urine Volume (B), and Drinking Water (C) and Food Consumption (D) in ICR and ddY Mice after a Single i.p. Injection of Various Doses of Streptozotocin (STZ)

Each plot denotes the mean  $\pm$  S.E.M. for 8 mice. Significantly different from respective normal, \*  $p < 0.05$ , \*\*  $p < 0.01$ .

week. On the other hand, the percentage of the number of  $\beta$ -cells in the 100 mg/kg STZ ICR mice continued to decrease slowly over time, but was still preserved by 64% even at 12 weeks. The number of  $\beta$ -cells in 125 mg/kg STZ ddY mice also decreased slowly over time, but the degree of the decrease was greater in mice of this strain (percentage of the number of  $\beta$ -cells at 12 weeks: 33%).

**Serum Glucose and Insulin Responses to Oral Glucose**

**Loading after STZ Administration in Low Dose STZ ICR and ddY Mice** Glucose tolerance testing in the 100 mg/kg STZ ICR and 125 mg/kg STZ ddY mice in comparison with the respective normal mice was carried out at various intervals from 3 d to 8 weeks after STZ administration. The fasting serum glucose levels in the 100 mg/kg STZ ICR and 125 mg/kg STZ ddY mice at 0.5 h before oral glucose loading was already significantly higher than those in the respective

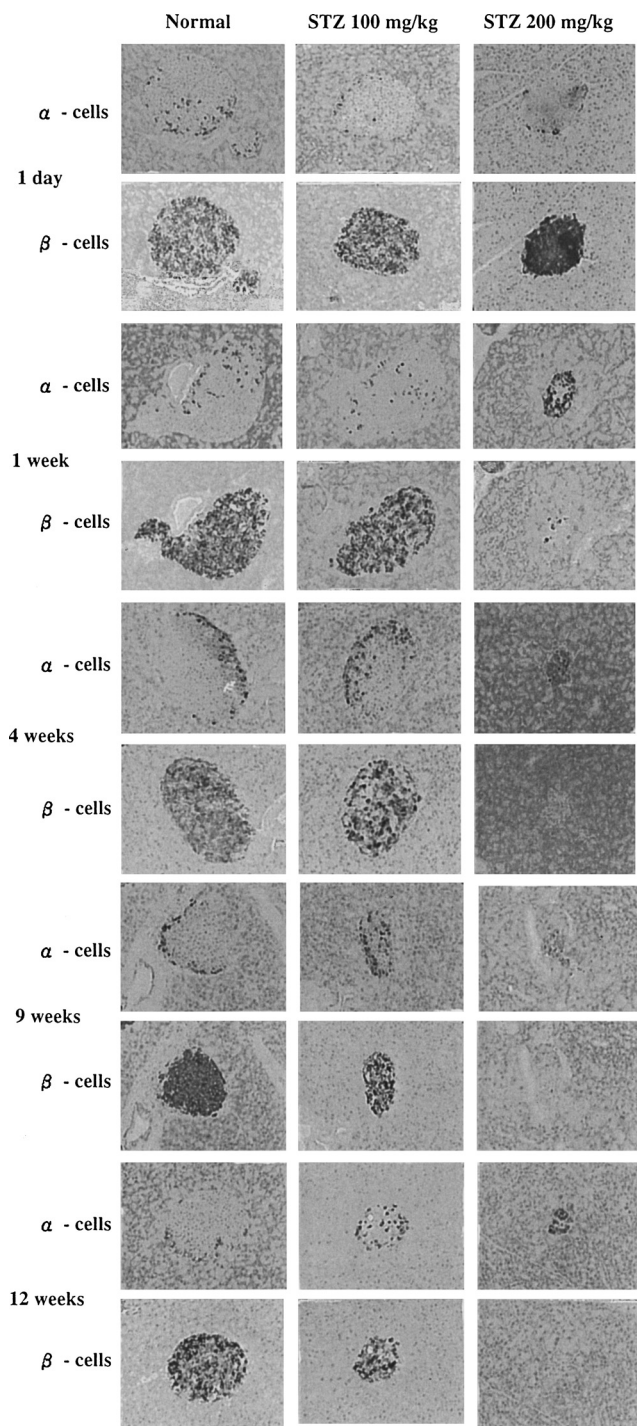


Fig. 6. Insulin-Immunostaining of  $\alpha$ - and  $\beta$ -Cells in the Islets of Pancreas at 1 d, and 1, 4, 9 and 12 Weeks after a Single i.p. Injection of Streptozotocin (STZ) in Normal, 100 and 200 mg/kg STZ ICR Mice  
Original magnification  $\times 100$ .

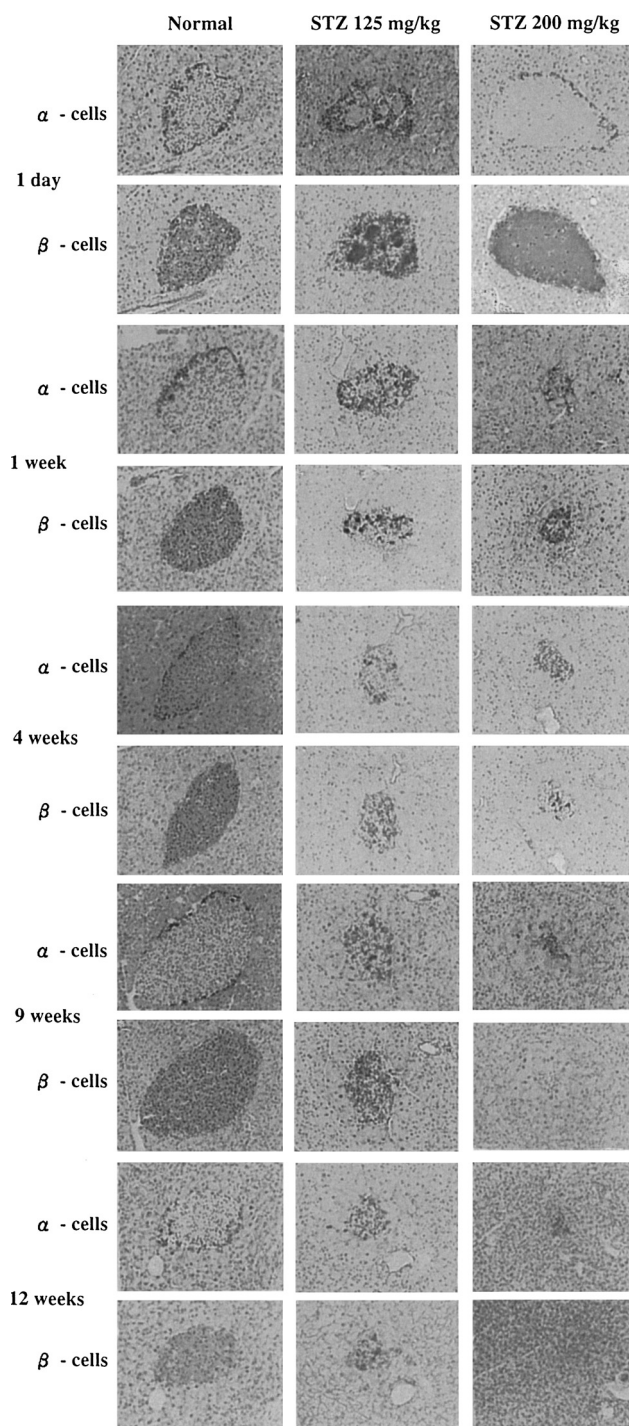


Fig. 7. Insulin-Immunostaining of  $\alpha$ - and  $\beta$ -Cells in the Islets of Pancreas at 1 d, and 1, 4, 9 and 12 Weeks after a Single i.p. Injection of Streptozotocin (STZ) in Normal, 125 and 200 mg/kg STZ ddY Mice  
Original magnification  $\times 100$ .

normal groups throughout the observation period of 3 d—8 weeks after STZ administration (ICR: Fig. 9A; ddY: Fig. 10A). The degree of hyperglycemia under fasting was greater in the diabetic ddY mice (2 weeks: diabetic:  $239 \pm 39$  mg/dl vs. normal,  $100 \pm 2$  mg/dl,  $p < 0.01$ ) than in the diabetic ICR mice (2 weeks: diabetic,  $129 \pm 3$  mg/dl vs. normal,  $87 \pm 6$  mg/dl,  $p < 0.01$ ). In the diabetic ICR and ddY mice and respective normal mice, the serum glucose levels after oral glucose loading (2 g/kg) reached a peak at 0.5 h and then gradu-

ally decreased. After glucose loading, the serum glucose levels in the diabetic ICR and ddY mice were significantly higher than those in their normal mice at all points of measurement from 2 weeks and 3 d, respectively. Thereafter, the degree of glucose tolerance in diabetic mice of both strains gradually reduced with time. In addition, the time course-related reduction in glucose tolerance was greater in the diabetic ddY mice than the diabetic ICR mice.

The rise in serum insulin levels stimulated by glucose load

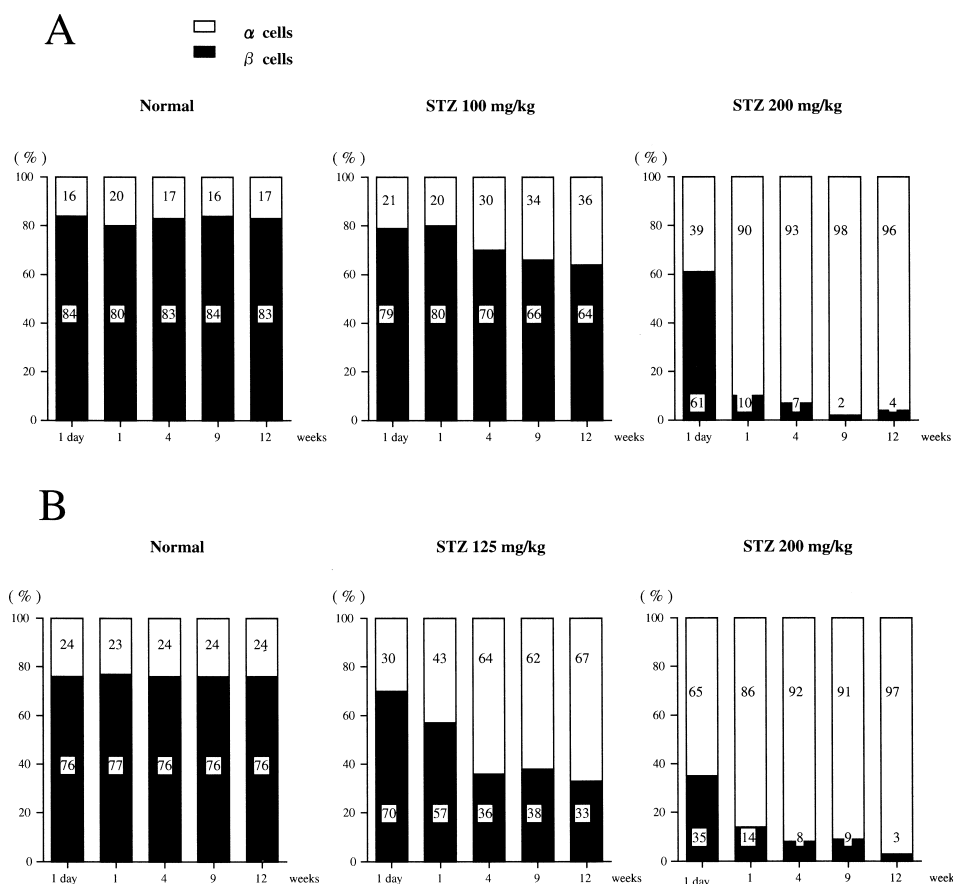


Fig. 8. Changes in the Percentage of the Number of Glucagon ( $\alpha$ -Cells)- and Insulin-Immunoreactive Cells ( $\beta$ -Cells) in the Islets of Pancreas after a Single i.p. Injection of Streptozotocin (STZ) in 100 and 200 mg/kg STZ ICR (A) and 125 and 200 mg/kg STZ ddY Mice (B)

in the 100 mg/kg STZ ICR mice and 125 mg/kg ddY mice were similar to those in the respective normal mice up to 1 week and 3 d, respectively, after STZ administration but then subsided gradually as time passed (ICR: Fig. 9A; ddY: Fig. 10A). The rise in the insulin levels due to glucose stimulation in the low dose STZ ddY mice had already been completely lost at 1 week, although marked increases in the insulin levels in the low dose STZ ICR mice and normal ICR mice were still observed at this time.

## DISCUSSION

STZ has been commonly used to induce not only animal models of IDDM,<sup>3-6</sup> but also NIDDM with hypoinsulinemia by neonatal (1- or 2-d-old mice or rats) STZ administration.<sup>9-11</sup> It has been reported that STZ is capable of producing mild to severe types of diabetes according to the dosage used, when it is given to adult rats by either single i.v. or i.p. injection.<sup>5</sup>

In our previous studies, when STZ was injected i.p. in 8-week-old male ICR mice at doses ranging from 75 to 200 mg/kg, only 100 mg/kg STZ induced slowly-progressive diabetes mellitus.<sup>8</sup> In other words, in the mice administered 100 mg/kg STZ, non-fasting serum glucose levels continued to increase gradually after STZ administration without affecting the non-fasting serum insulin levels. In contrast to the 100 mg/kg STZ mice, non-fasting serum glucose levels in the 200 mg/kg STZ mice rose sharply from 1 week after STZ ad-

ministration and a long-term stable hyperglycemia with low serum insulin levels below measurable values was then observed. Furthermore, in previous studies, we examined the effect of troglitazone, an antidiabetic agent that improves insulin resistance, on glucose tolerance at 12 weeks after STZ injection in the 100 and 200 mg/kg STZ ICR mice. Troglitazone (100 mg/kg, *p.o.*) was significantly effective at lowering the glucose levels in the 100 mg/kg STZ mice, although it failed to exhibit a hypoglycemic action in the 200 mg/kg STZ mice. Fujiwara *et al.*<sup>12</sup> have reported that troglitazone (CS-045) is effective in insulin-resistant diabetic animal models such as the KK- $A^y$  mouse, the ob/ob mouse, and the Zucker fatty rat. They also have shown that this agent is ineffective in STZ-induced insulin-dependent or normal animals. These results strongly suggest that the 100 mg/kg STZ-induced diabetic mouse model is non-insulin dependent, while the 200 mg/kg STZ-induced one is insulin-dependent. Furthermore, the results obtained by troglitazone suggest that the 100 mg/kg STZ-induced diabetic mouse model is insulin-resistant.

In the present study, in order to clarify whether or not the progressive NIDDM is induced in mice of other different strains as well as ICR mice by STZ administration, we injected STZ i.p. at various doses in 8-week-old male ICR, ddY, BALB/c and C57BL/6 mice. In this experiment, the smallest dose of STZ to induce diabetes in ICR, ddY, BALB/c and C57BL/6 mice was 100 mg/kg, 125 mg/kg, 150 mg/kg and 100 mg/kg, respectively. However, a time course-

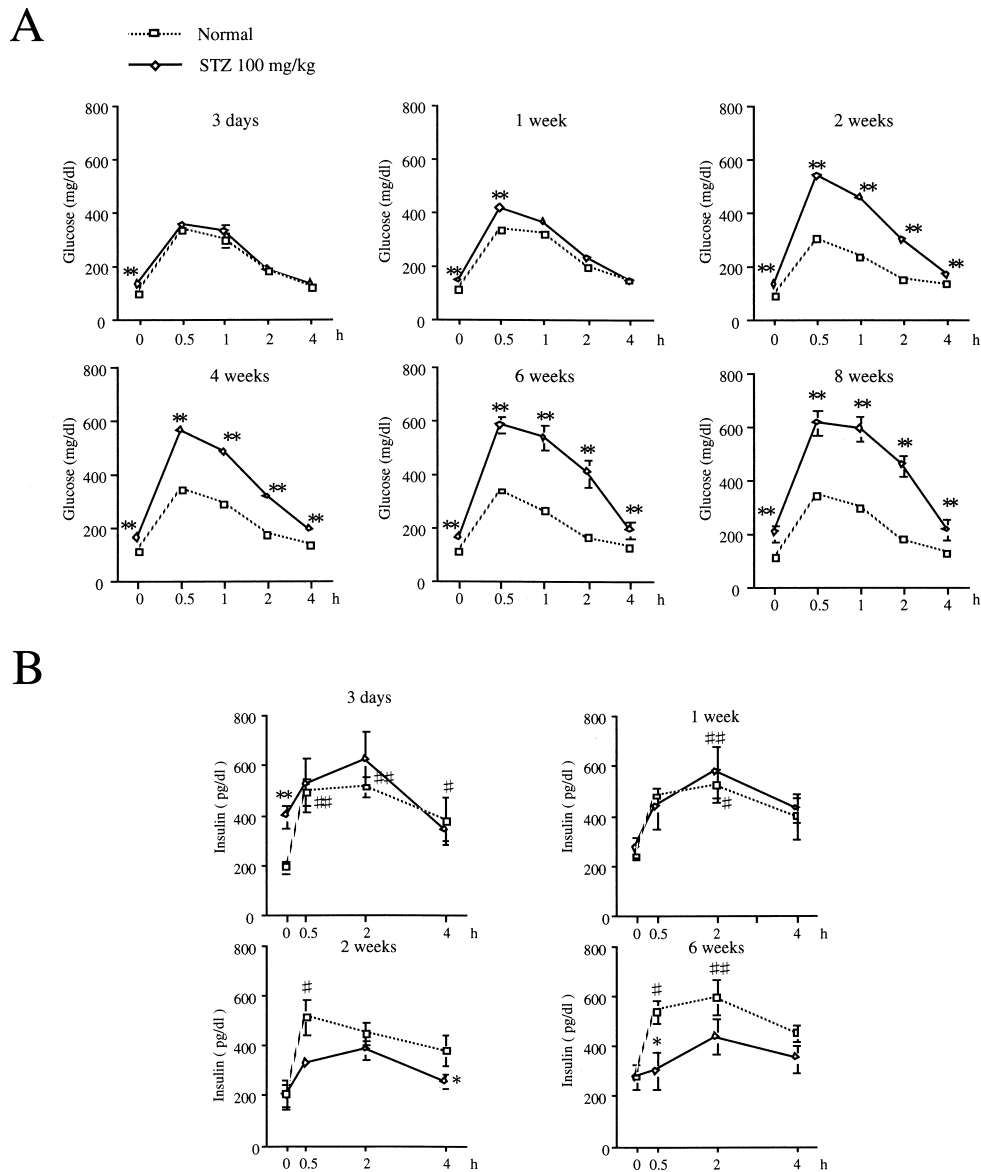


Fig. 9. Changes in Serum Glucose and Insulin Responses to Oral Glucose Loading (2 g/kg) after a Single i.p. Injection of Streptozotocin (STZ) in 100 mg/kg STZ ICR Mice

Blood samples were collected at 0.5 h (0 h in figure) before and at 0.5, 1, 2 and 4 h after glucose loading for determination of serum glucose. Each plot denotes the mean  $\pm$  S.E.M. for 8 mice. Significantly different from respective normal, \* $p$ <0.05, \*\* $p$ <0.01. Significantly different from respective level at 0 h, # $p$ <0.05, ## $p$ <0.01 (B).

related rise of non-fasting serum glucose levels throughout the observation period of 1–12 weeks after STZ administration was induced only in ICR and ddY mice given an i.p. injection of STZ at 100 mg/kg and 125 mg/kg, respectively. In addition, the non-fasting serum insulin levels in the 100 mg/kg STZ ICR mice were within a normal range throughout the observation period. The insulin levels in the 125 mg/kg STZ ddY mice decreased slightly as the days passed after STZ administration. On the other hand, both ICR and ddY mice receiving an i.p. injection of STZ at 200 mg/kg showed a markedly high serum glucose levels with markedly low serum insulin levels during the observation period of 1–12 weeks. Thus, relatively higher serum insulin levels in the 125 mg/kg STZ ddY mice as well as in the 100 mg/kg STZ ICR mice, which induced the time course-related increases in the serum glucose levels, may be, in part, due to the result of the progression of insulin-resistance mentioned above.

STZ at doses of 125 mg/kg in ddY mice and 100 mg/kg in

ICR mice induced time course-related increases in 24 h urine volume as well as 24 h drinking water and food consumption. In addition, body weight in the 125 mg/kg STZ ddY mice and 100 mg/kg STZ ICR mice was not different from that in their respective normal mice throughout the observation period of 1–12 weeks after STZ administration. However, body weight in the animals of both strains given 200 mg/kg STZ was markedly lower than that in the respective normal animals throughout the observation period from 1 week. Of the ddY and ICR mice given various doses of STZ, only the highest dose of STZ (200 mg/kg) in ICR mice produced hypercholesterolemia.

Morphological observation of pancreatic islets indicated that the area of islets and the percentage of the relative number of insulin-immunoreactive cells ( $\beta$ -cells) to glucagon-immunoreactive cells ( $\alpha$ -cells) in the 100 mg/kg STZ ICR and 125 mg/kg STZ ddY mice continued to decrease slowly with time throughout the 12-week-observation period. The rate of

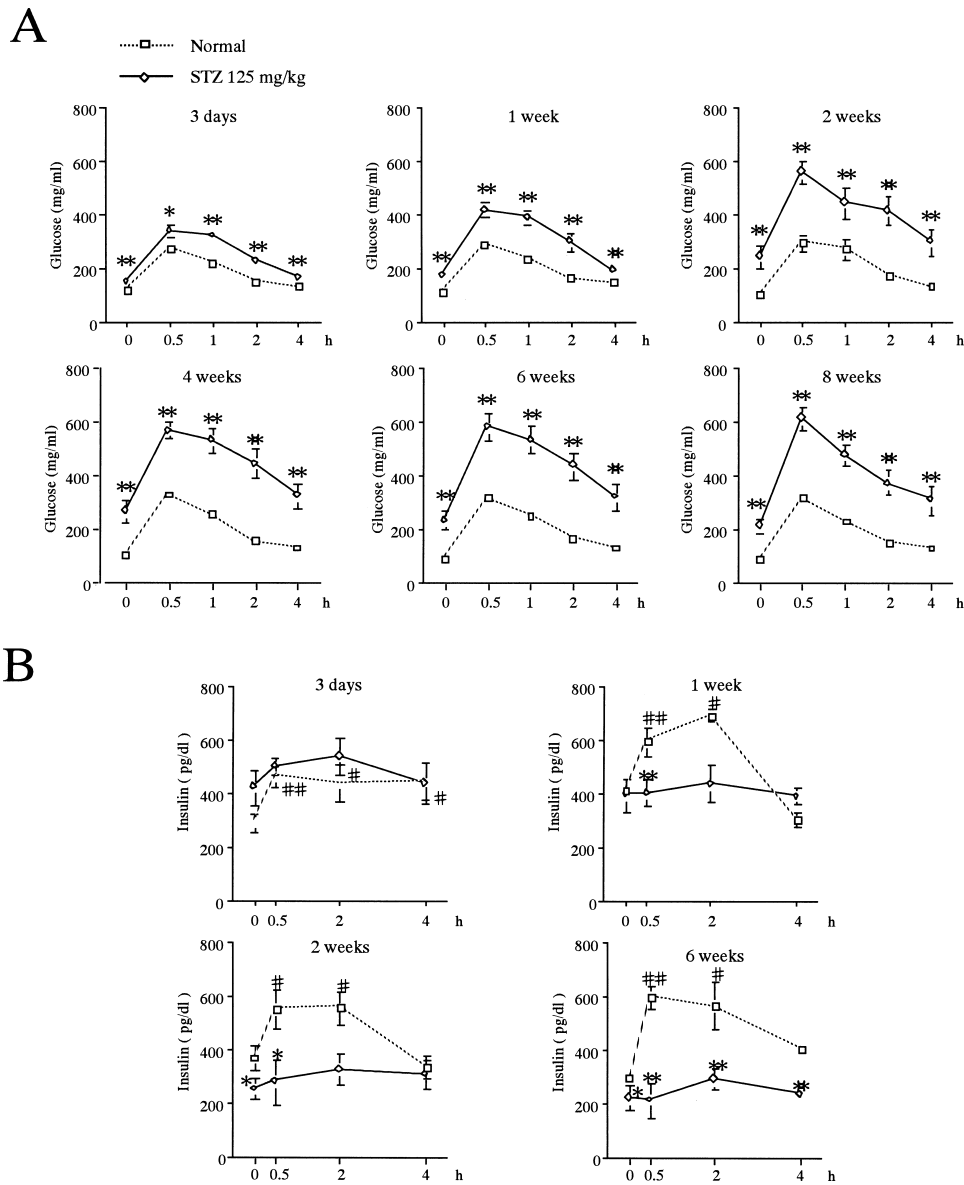


Fig. 10. Changes in Serum Glucose and Insulin Responses to Oral Glucose Loading (2 g/kg) after a Single i.p. Injection of Streptozotocin (STZ) in 125 mg/kg STZ ddY Mice

Blood samples were collected at 0.5h (0h in figure) before and at 0.5, 1, 2 and 4 h after glucose loading for determination of serum glucose. Each plot denotes the mean  $\pm$  S.E.M. for 8 mice. Significantly different from respective normal, \* $p$ <0.05, \*\* $p$ <0.01. Significantly different from respective level at 0h, # $p$ <0.05, ## $p$ <0.01 (B).

decrease in both morphological parameters in the 125 mg/kg STZ ddY mice was greater than that in the 100 mg/kg STZ ICR mice. Morphological observation in the 200 mg/kg STZ ICR and ddY mice revealed a marked involution of pancreatic islets and a marked reduction in the number of islet insulin-immunoreactive cells ( $\beta$ -cells) from 1 week after STZ administration. Thus, a marked reduction in  $\beta$ -cells in the 200 mg/kg STZ ICR and ddY mice was observed. However, in contrast,  $\alpha$ -cells were not affected even by high doses of STZ. These results suggest that slowly progressive diabetes mellitus induced in ddY mice receiving 125 mg/kg STZ as well as that induced in ICR receiving 100 mg/kg STZ is non-insulin dependent, while that induced in mice of both strains receiving 200 mg/kg STZ is insulin-dependent. The reason why slowly progressive diabetes mellitus was induced only in ICR and ddY mice by a single i.p. injection of a subdiabetogenic dose of STZ is unclear. In addition, the exact mecha-

nism of the decrease in the number of  $\beta$ -cells during the progression of this diabetes is also unclear. It is interesting, however, that in the 100 mg/kg STZ ICR mice, non-fasting serum insulin levels were maintained at normal levels throughout the observation period, although there was a time course-related reduction in the number of  $\beta$ -cells. These results suggest that, in the 100 mg/kg STZ ICR mice, the  $\beta$ -cells remaining that escaped attack by STZ may cause an oversecretion of insulin to maintain normoglycemia. Therefore, it is reasonable to assume that the reduction in the number of  $\beta$ -cells may be attributed to the inhibition of their proliferation due to the excess activity of the remaining  $\beta$ -cells. The percentage of the reduction in the number of  $\beta$ -cells was higher in the 125 mg/kg STZ ddY mice than in the 100 mg/kg STZ ICR mice in all weeks after STZ administration. These results indicate that the speed of the progression of diabetes mellitus induced by the low dose STZ is faster in ddY mice

than in ICR mice. These results also suggest that both slowly progressive NIDDM mouse models may convert into IDDM after long-term chronic hyperglycemia.

In the next experiment, we conducted glucose tolerance tests at various intervals after STZ administration to further clarify the characteristics of low dose STZ-induced diabetes mellitus in ICR and ddY mice. The serum glucose levels after glucose loading in the 100 mg/kg STZ ICR and 125 mg/kg ddY mice were significantly higher than those of normal mice at 2 weeks and 3 d, respectively, after STZ administration, and thereafter the degree of glucose tolerance in mice of both strains gradually decreased with time. In this case, the degree of the time course-related reduction of glucose tolerance was greater in ddY mice than in ICR mice. In addition, the insulin response to glucose loading in the low dose STZ ICR and ddY mice became weaker in direct proportion to the reduction in glucose tolerance. The degree of the insulin response to glucose loading was weaker in the 125 mg/kg STZ ddY mice than in the 100 mg/kg STZ ICR mice. It has also been demonstrated that insulin responses to glucose loading are impaired during the progression of diabetes in STZ-injected neonatal mice<sup>13)</sup> or rats.<sup>10)</sup> Thus, the insulin response to glucose loading in low dose STZ-induced diabetic ICR or ddY mice was similar to that in neonatal STZ-induced diabetic mice or rats.

It is conceivable that the impairment of glucose-stimulated insulin secretion in low dose STZ-induced diabetic ICR and ddY mice may be in part related to the decrease in the number of islet  $\beta$ -cells. As it has been reported that hyperglycemia<sup>14,15)</sup> and hyperlipidemia<sup>16)</sup> impair  $\beta$ -cell functions, such as the inhibition of glucose-stimulated insulin secretion or islet glucose metabolism, the abnormalities of glucose or lipid metabolism may further promote impairment of the function and proliferative capacity of  $\beta$ -cells.

In the present study, it is of interest that only in ICR and ddY mice, and not BALB/c and C57BL/6 mice, was slowly progressive NIDDM induced by a single i.p. injection of a subdiabetogenic low dose of STZ (100 and 125 mg/kg, respectively). It has been shown that there is a temporary rise of blood glucose levels followed by spontaneous recovery within a week, when a low dose (30 or 40 mg/kg, i.v.) of STZ was injected in non-fasted male Sprague-Dawley rats weighing approximately 250 g.<sup>17)</sup> It is not clear the reason why slowly progressive NIDDM was not induced in mice of other different strains or animals of different species. These two slowly progressive diabetic mouse models closely resemble slowly progressive type 1 diabetes in humans, which is

induced as NIDDM and then converts into IDDM after 5–6 years. In slowly progressive diabetes in human, serum antibody titer against pancreatic islets is positive and lymphocytic infiltration is recognized in the islets.<sup>18)</sup> However, the islet antibody and lymphocytic infiltration were not detected in the islets of STZ-induced diabetic mice.

In summary, the results of the present study indicate that of ICR, ddY, BALB/c and C57BL/6 mice, slowly progressive NIDDM can only be induced in ICR and ddY mice by a single i.p. injection of a subdiabetogenic low dose of STZ. It has already been reported that low molecular weight chitosan, a health food, prevents the progression of low dose STZ-induced slowly progressive NIDDM in ICR mice.<sup>19)</sup> Therefore, these two new diabetic mouse models may be useful for screening antidiabetic substances and clarifying the mechanism of the progression of NIDDM in humans.

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