

Blood Glucose Level and Survival in Streptozotocin-Treated Human Chymase Transgenic Mice

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Abstract

A growing body of evidence suggests the potential role of chymase in organ injury in diabetes. We investigated blood glucose levels and survival in transgenic mice carrying the human chymase gene (Tg). Intraperitoneal injections of streptozotocin (STZ) (200, 100, 75 and 50 mg/kg in total, i.p.) were given to uninephrectomized Tg mice and wild-type C57BL/6 (BL) mice. Before STZ injection, the Tg mice had significantly lower body weights and slightly higher systolic blood pressure as compared with the BL mice. STZ-treated Tg mice showed significantly higher postprandial blood glucose levels as compared with the STZ-treated BL mice. The survival prevalence of STZ-treated Tg mice was zero, whereas BL mice showed a value of 40% until 42 days. STZ (100, 75 or 50 mg/kg, i.p.)-treated Tg mice also showed a similar pattern as compared with the STZ-treated BL mice. These data suggest that human chymase contributes to blood glucose levels and mortality during the progression of diabetes.

Key Words: streptozotocin, human chymase transgenic mice, diabetes, survival

Introduction

Blockade of the renin-angiotensin system (RAS) with angiotensin-converting enzyme (ACE) inhibitors or angiotensin II (Ang II) antagonists has preventive effects against the onset of diabetes and organ injury in diabetes (11, 12, 16). Recent studies have suggested that chymase-dependent pathways play an important part in local production of Ang II in the kidney, heart and arteries in humans, dogs and hamsters (2, 5, 14, 18).

Huang *et al.* (5) reported that the expression of chymase is upregulated in the glomeruli of patients with diabetic nephropathy. This suggested the po-

tential role of human chymase in intrarenal production of Ang II in diabetes. Similarly, chymase expression in humans is increased in the coronary and renal arterioles of diabetic patients (8). *In vitro* studies have also indicated the potential roles of chymase in high glucose-induced generation of Ang II in human mesangial cells (1) and in rat vascular smooth muscle cells (10). In addition to the Ang II-forming activity of human chymase, involvement of mammalian chymase in local inflammation has also been suggested by several *in vitro* (15, 24) and *in vivo* (3) studies.

Recently, Takai *et al.* (23) showed that treatment with a chymase inhibitor resulted in the protection of pancreatic islets in streptozotocin (STZ)-induced

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diabetes in hamsters. The results stated above suggest that hamster chymase contributes to injury to pancreatic islets in hamsters. However, the role of human chymase in the pathophysiology of diabetes has not been investigated. The present study was conducted to investigate the survival and blood glucose levels responding to STZ administration in transgenic (Tg) mice carrying the human chymase gene (7).

Materials and Methods

All experimental procedures were carried out according to the guidelines for the care and use of animals established by Kagawa University (Kagawa, Japan).

Experiments were carried out in male Tg mice carrying the human chymase gene (7) and male wild-type C57BL/6 mice age-matched (10 weeks) at the beginning of the experiment. Mice were housed and maintained in a pathogen-free facility under controlled temperature ($23 \pm 2^\circ\text{C}$) and humidity ($55 \pm 5\%$) with a 12-h light/dark cycle. Throughout the experimental period, mice had free access to standard laboratory food and tap water.

Human Chymase Transgene and Production of Tg Mice

Preparation of the human chymase transgene and production of Tg mice have been described previously in detail (7). In brief, isolated human chymase cDNA (25) was subcloned into pCAGGS, a pKCR-3-based plasmid that contains a CMV-IE fragment and lies upstream of the chicken β -actin promoter (13). Human chymase sequences were confirmed by the dideoxy sequencing method. The pCAGGS-h-chymase plasmid DNA was digested with SalI and KpnI, and the isolated human chymase transgene fragment was gel-purified and used for microinjection (4).

The C57BL/6 mouse strain was used to produce human chymase Tg mice. Fertilized eggs were collected from the oviducts of superovulated C57BL/6 females that had been mated to males of the same genetic background. Several hundred molecules of the human chymase transgene fragment were microinjected into the fertilized eggs of C57BL/6 mice according to a previously described method (21). Koga *et al.* (7) showed that human chymase transgene expression and immunoreactivity were detected in all organs (7).

Induction of Diabetes

In rats, a single dose of STZ is effective for inducing type-1 diabetes. In mice, multiple injections of low doses of STZ are effective for maintaining mouse viability and inducing pancreatic dysfunction

which is similar to that observed at the onset of type-1 diabetes in humans (22). In the present study, to induce type-1 diabetes in mice, single or multiple doses of STZ were used as described below.

Ten-week-old male Tg mice and BL mice were allowed to acclimatize for one week. They then underwent uninephrectomy (UNX). They were given one week to recover, and then given a daily intraperitoneal injection of freshly prepared STZ (50 mg/kg body weight in 0.1 M citrate buffer, pH 4.5; Sigma-Aldrich, St. Louis, MO, USA) for 4 consecutive days or 2 days (17, 22), or a single dose of freshly prepared STZ (75 and 50 mg/kg body weight in 0.1 M citrate buffer, pH 4.5) to induce diabetes. Citrate buffer was prepared with 0.1 M of citric acid solution mixed with 0.1 M of citrate sodium solution by 3:5 of volume with pH 4.5. Control mice were injected with citrate buffer alone. Blood glucose levels were examined 4 and 7 days after the final STZ injection. One drop of blood was collected from the tip of the tail by needle puncture for measuring blood glucose concentration.

Experimental Design

After one week of acclimatization, Tg and BL mice were subjected to right UNX under sodium pentobarbital anesthesia (40 mg/kg, i.p.). One week after UNX, UNX-Tg mice were randomly divided into five groups and treated with one of the following combinations: group-I (n = 7): UNX + vehicle (citrate buffer) injection (Tg-Con); group-II (n = 15): UNX + STZ (50 mg/kg, i.p., 4 consecutive days) injection (Tg-200 mg); group-III (n = 12): UNX + STZ (50 mg/kg, i.p., 2 consecutive days) injection (Tg-100 mg); group-IV (n = 7): UNX + STZ (75 mg/kg, i.p., a single dose) injection (Tg-75 mg); and group-V (n = 7): UNX + STZ (50 mg/kg, i.p., a single dose) injection (Tg-50 mg). UNX-BL mice were randomly divided into five groups and treated with one of the following combinations: group-VI (n = 7): UNX + vehicle (citrate buffer) injection (BL-Con); group-VII (n = 15): UNX + STZ (50 mg/kg, i.p., 4 consecutive days) injection (BL-200 mg); group-VIII (n = 12): UNX + STZ (50 mg/kg, i.p., 2 consecutive days) injection (BL-100 mg); group-IX (n = 7): UNX + STZ (75 mg/kg, i.p., a single dose) injection (BL-75 mg); and group-X (n = 7): UNX + STZ (50 mg/kg, i.p., a single dose) injection (BL-50 mg). The experiment was continued for 42 days after the final STZ injection.

Systolic Blood Pressure (SBP), Heart Rate (HR) and Postprandial Blood Glucose (PPBG)

SBP and HR were measured in conscious mice by tail-cuff plethysmography (BP-98A; Softron Company, Tokyo, Japan). SBP and HR were measured

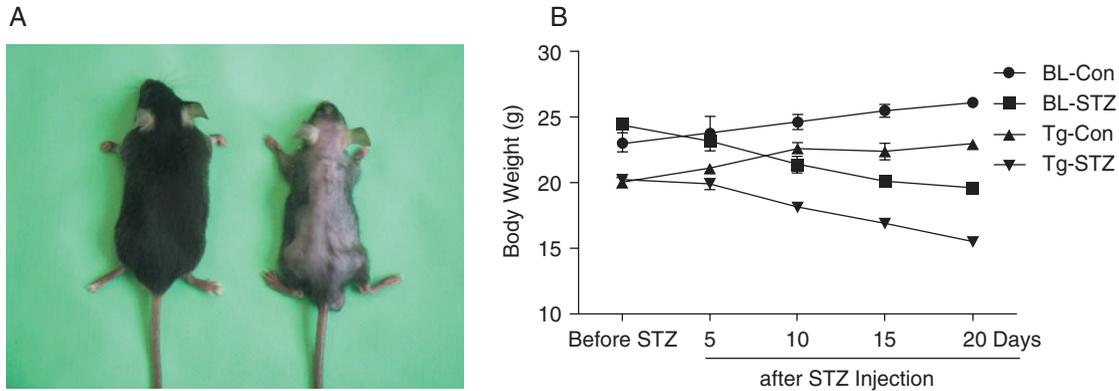


Fig. 1. (A), phenotypic characteristics of human chymase-Tg and BL mice. (B), changes in body weight during the experiment. Data are means \pm SEM.

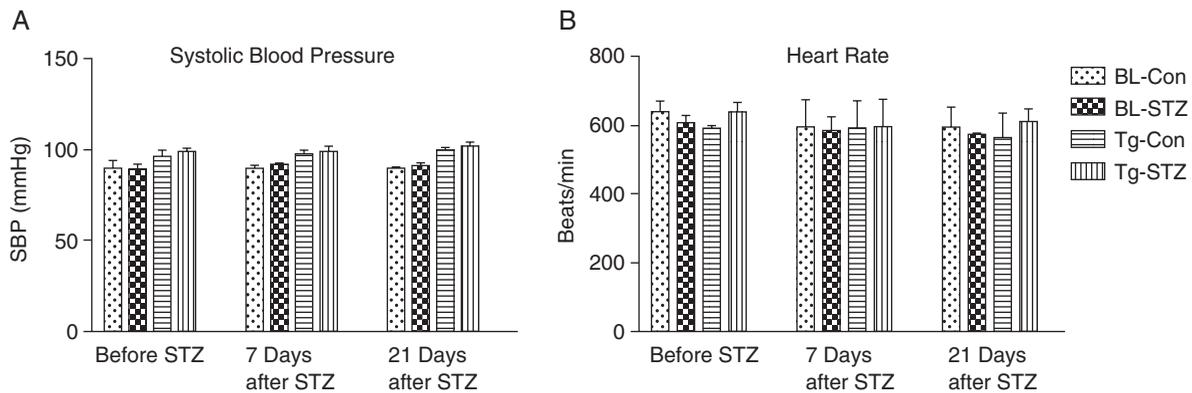


Fig. 2. (A), SBP and (B), HR of human chymase-Tg and BL mice during the experiment. Data are means \pm SEM.

in Tg mice and BL mice before and 7 and 21 days after the final STZ injection. PPBG was measured with a glucose analyzer (Sanwa-Kagaku, Company Limited, Nagoya, Japan) after 4 and 7 days after the final STZ injection.

Statistical Analysis

Values are the means \pm SEM. Statistical comparisons of differences between groups were done using one-way analysis of variance combined with the Newman-Keuls post-hoc test. $P < 0.05$ was considered significant.

Results

Phenotypic Characteristics of Tg Mice

Genetically, human chymase-Tg mice show reduced fat in the skin and viscera, oligotrichia and alopecia (Fig. 1A) (7). This phenotypic change was, therefore, used as a positive marker to discriminate Tg mice from non-Tg wild-type mice. The mean body weight of the Tg mice was significantly lower than

that of the BL mice at 10 weeks of age (20 ± 3 g and 24 ± 3 g, respectively, $P < 0.001$). A significant reduction in body weight was observed 20 days after the final STZ injection in Tg mice and BL mice (Fig. 1B, BL-Con vs. BL-STZ and Tg-Con vs. Tg-STZ, $P < 0.01$, respectively). Daily food intake in Tg mice was higher than that of BL mice (4.3 ± 0.65 and 3.4 ± 0.80 g/day/mouse, respectively), but this difference was not significant.

SBP and HR

The SBP of Tg mice tended to be higher than that of BL mice at 10 weeks of age (103 ± 2 and 96 ± 3 mmHg, respectively), but the difference was not significant. A significant difference in HR between Tg mice and BL mice (620 ± 12 and 571 ± 16 bpm) was not observed. Treatment with different doses of STZ affected neither SBP nor HR in Tg mice and BL mice (Fig. 2, A and B).

PPBG

There was no significant difference in PPBG

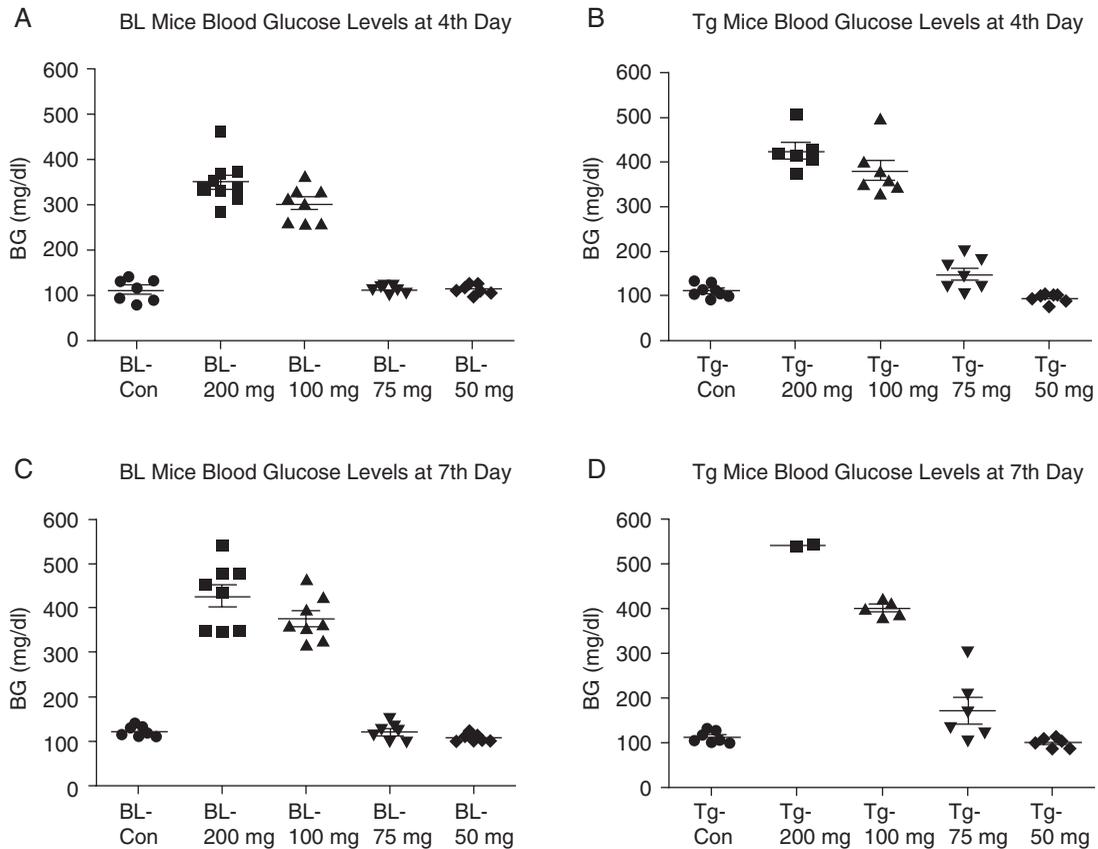


Fig. 3. PPBG levels of BL mice and human chymase-Tg mice at 4 days (A, B respectively) and 7 days (C, D respectively) after the final STZ injection. Data are means \pm SEM.

levels between Tg-Con mice and BL-Con mice (Fig. 3). Four days after the final STZ injection, BL-200 mg mice and BL-100 mg mice showed hyperglycemia whereas BL-75 mg mice and BL-50 mg mice did not (Fig. 3A). Similarly, Tg-200 mg mice and Tg-100 mg mice showed hyperglycemia whereas Tg-75 mg mice and Tg-50 mg mice did not (Fig. 3B). Four days after the final STZ injection, Tg-200 mg and Tg-100 mg mice showed significantly higher PPBG levels than BL-200 mg and BL-100 mg mice ($P < 0.01$, respectively). In BL mice and Tg mice, a similar pattern of PPBG level was observed 7 days after the final STZ injection (Fig. 3, C and D). Seven days after the final STZ injection, Tg-200 mg mice showed significantly higher PPBG levels than BL-200 mg mice ($P < 0.05$). PPBG levels were not statistically different between Tg-100 mg mice and BL-100 mg mice.

Survival

Mice survival proportions were evaluated 42 days after the final STZ injection and the data were analyzed by a Kaplan-Meier survival curve (Fig. 4). BL mice showed 100% survival except for the BL-200 mg group. BL-200 mg mice showed 33.3% sur-

vival at 42 days after STZ injection (Fig. 4A). All mice of the Tg-200 mg group died within 8 days after STZ injection, and the survival was, therefore, zero (Fig. 4B). Tg-100 mg mice showed 20% survival 42 days after STZ injection (Fig. 4B).

Discussion

The present study investigated the survival of human chymase-Tg mice in response to hyperglycemia induced by STZ injection. In the Tg mice, the group treated with 200 mg/kg of STZ showed hyperglycemia and all mice died within 8 days after STZ injection. Tg mice treated with 100 mg/kg of STZ also showed hyperglycemia, and their survival for 42 days after STZ injection was 20%. The survival in BL mice treated with 200 and 100 mg/kg STZ were 33.3% and 100%, respectively. The finding that human chymase-Tg mice elicit an obviously low survival prevalence during hyperglycemia induced by STZ indicates possible role of human chymase in the pathophysiology of diabetes.

The reasons why human chymase-Tg mice elicit a low prevalence of survival in response to STZ administration remain unclear. However, human

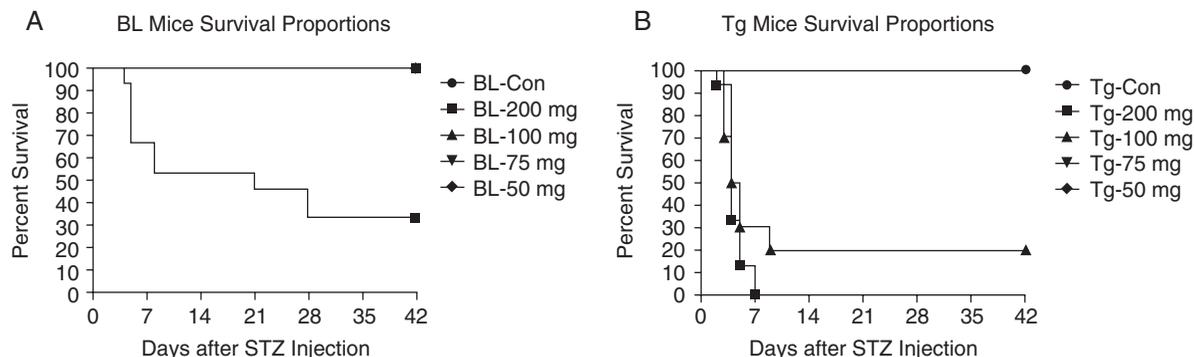


Fig. 4. Kaplan-Meier survival curve of BL mice (A) and human chymase-Tg (B) mice.

chymase-Tg mice showed significantly higher PPBG levels than the wild-type mice 4 and 7 days after injection. These data suggest that the augmentation of STZ-induced hyperglycemia contributes to the low survival rate in the human chymase-Tg mice. That is, STZ-induced pancreatic islet injury may be augmented by over-expression of the human chymase gene. This concept is supported in a recent study by Takai *et al.* (23) who showed that treatment with a chymase inhibitor resulted in pancreatic islet protection in STZ-induced diabetic hamsters. However, the mechanisms responsible for human chymase-induced pancreatic islet injury remain undetermined.

In agreement with a previous study (7), the present study showed that human chymase-Tg mice elicit lower body weight and slightly elevated SBP as well as alopecia. Rat chymase transgenic expression in vascular smooth muscle cells is also associated with a moderate increase in blood pressure (6) suggesting the role of chymase in SBP elevation *in vivo*. Koga *et al.* (7) observed that elevated blood pressure in human chymase-Tg rats was completely normalized by Ang II AT1 receptor antagonists. These data suggest possible contribution of chymase-dependent Ang II formation to the development of hypertension. The cause of alopecia in Tg mice is unclear. Clinical studies have shown that mast cells are involved in the pathogenesis of various types of hair loss and alopecia. In scarring alopecias (19, 20) and androgenetic alopecia (9), the number of skin mast cells is remarkably increased. However, determination of the infiltration of mast cells in the skin of human chymase-Tg mice was beyond the scope of this study and was not carried out. Another limitation of the present study is that we did not measure Ang II levels and did not evaluate renal parameters due to the low prevalence of survival of STZ-treated Tg mice. Further studies are needed to investigate the STZ-induced changes in Ang II levels and in renal injury in Tg mice.

In conclusion, human chymase-Tg mice showed

hyperglycemia and a lower prevalence of survival in response to STZ administration. These data suggest the possible role of human chymase in the pathophysiology of diabetes. However, further studies are needed to determine the precise mechanisms by which human chymase contributes to the pathogenesis of diabetes.

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