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Inhibition of Postprandial Hyperglycemia Prevents the Incidence of Diabetes in Spontaneously Diabetic Torii (SDT) Rats

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Abstract: The Spontaneously Diabetic Torii (SDT) rat shows Impaired Glucose Tolerance (IGT) before the onset of diabetes. The present study investigated the effects of an improvement of IGT on the incidence of diabetes in male SDT rats. Voglibose, α-glucosidase inhibitor was used to improve glucose intolerance. In SDT rats at 10 weeks of age, a single dose of voglibose (0.03, 0.1 and 0.3 mg kg^{-1}) improved the glucose tolerance dose-dependently. Moreover, voglibose was administered as a dietary mixture to SDT rats from 10-20 weeks of age. As a result, voglibose suppressed the incidence of diabetes in SDT rats. In voglibose-treated rats at 20 weeks of age, pancreatic weights were increased and the histopathological pancreatic changes such as irregular boundaries, atrophy, fibrosis and decrease of insulin-immunoreactivity in islets were improved. In conclusion, inhibition of postprandial hyperglycemia prevented the incidence of diabetes in SDT rats. Also, SDT rat is a useful model for development of a preventive drug on diabetes.

Key words: Pancreas, postprandial hyperglycemia, SDT rat, insulin, diabetes, dose

INTRODUCTION

Type 2 diabetes mellitus is a major health problem associated with excess morbidity and mortality (Szoke *et al*., 2008; Ranjbar *et al*., 2011). Patients who develop type 2 diabetes mellitus pass through a phase of Impaired Glucose Tolerance (IGT) (Weyer *et al*., 1999). The prevalence of type 2 diabetes and IGT increases with aging (Goran *et al*., 2008; Chang and Halter, 2003). With the anticipated further aging of population, the burden of type 2 diabetes and IGT on health-care system will continue to grow (Szoke *et al*., 2008). Defects in insulin secretion or insulin action are the major abnormalities leading to development of glucose intolerance (Weyer *et al*., 1999; Kushwah *et al*., 2010; Rafiq and Mitra, 2010).

Glucose intolerance develops when insulin secretion fails to compensate for insulin resistance, resulting in postprandial hyperglycemia (Xu *et al*., 2008). Intervention in IGT stage that reduces insulin resistance or protects the β cells in pancreas prevents onset of diabetes or delays the progression (Chiasson *et al*., 2002). The Spontaneously Diabetic Torii (SDT) rat is a model of non-obese spontaneous diabetes which was developed by Torii Pharmaceutical Co., Ltd. (Tokyo, Japan) (Masuyama *et al*., 2004; Shinohara *et al*., 2000). Male SDT rats develop diabetes mellitus from about 20 weeks of age and the diabetic complications such as ocular lesion and nephropathy, from about 40 weeks of age (Ohta *et al*., 2007; Sasase *et al*., 2006). Moreover, SDT rats show glucose intolerance before onset of diabetes. The IGT was observed at 12 and 16 weeks of age (Matsui *et al*., 2009; Ohta *et al*., 2011).

In this study, α-glucosidase inhibitor voglibose was administered to male SDT rats in a pre-diabetic stage and the effects of voglibose on the glucose intolerance and the development of diabetes were investigated. The effects on voglibose were examined by a single dose and by chronic administration.

MATERIALS AND METHODS

Animals: Male SDT rats (Clea Japan, Tokyo, Japan) were used for the study. Rats were housed in suspended

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bracket cages and given a standard laboratory diet (CRF-1, Oriental Yeast Co., Ltd. Tokyo, Japan) and water *ad libitum* in a controlled room for temperature, humidity and lightning.

Acute effects of voglibose on SDT rats: SDT rats at 10 weeks of age were used for this study. Over night-fasted SDT rats were divided into four groups, vehicle (0.5% methyl cellulose solution), voglibose 0.03, 0.1 and 0.3 mg kg^{-1}, respectively. Sprague-Dawley (SD) rat was used as a control rat. Voglibose and starch solution (2 g/5 mL/kg) administered to the rats simultaneously. Blood samples were collected from the tail vein before and 60, 120 and 180 min after starch-loading. Serum glucose and insulin levels were examined at each point. Blood samples were collected from the tail vein of overnight-fasted rats. The glucose level was measured using commercial kits (Roche Diagnostics, Basel, Switzerland) and automatic analyzer (Hitachi, Tokyo, Japan). Serum insulin level was measured with a rat-insulin Enzyme-Linked Immunosorbent Assay (ELISA) kit (Morinaga Institute of Biological Science, Yokohama, Japan). Glucose Area under the Curve (AUC) after starch-loading was also calculated.

Chronic effects of voglibose on SDT rats: Voglibose (0.03%) in a dietary mixture was administered to SDT rats from 10-20 weeks of age. Non-fasted serum glucose, insulin, Triglyceride (TG) and Total Cholesterol (TC) levels were examined every 2 weeks. The glucose and insulin levels were measured as described. TG and TC levels were measured using commercial kits (Roche Diagnostics, Basel, Switzerland) and automatic analyzer (Hitachi, Tokyo, Japan). Necropsy was performed at 20 weeks of age. After the pancreatic weights were measured, the pancreas was fixed in 10% neutral buffered formalin. After resection, the tissue was paraffin-embedded by standard techniques and thin-sectioned (3-5 μm). The sections were stained with Hematoxylin and Eosin (HE). Immunohistochemistry (IHC) was performed on sections of pancreas with an anti-insulin antibody (Dako, Kyoto, Japan) by indirect staining using peroxidase-conjugated anti-guinea pig immunoglobulin (Dako).

Statistical analysis: Results of biological parameters and pancreatic weights were expressed as the mean±standard deviation. In acute experiment, statistical analysis of differences between mean values was performed with one-way Analysis of Variance (ANOVA) followed by Dunnett's two-tailed test. In chronic experiment, statistical analysis of differences between mean values was performed using the F-test followed by the Student's t-test or Aspin-Welch's t-test. Differences were defined as significant at $p<0.05$.

RESULTS AND DISCUSSION

Acute effects of voglibose were shown in Fig. 1 and 2. Serum glucose levels on voglibose administration (0.03, 0.1 and 0.3 mg kg^{-1}) decreased at 60 and 120 min after starch-loading (Fig. 1a). The glucose AUC levels significantly decreased dose-dependently (Fig. 2). Changes in serum insulin levels of voglibose were not observed at 60 and 120 min after starch-loading. The level at voglibose 0.03 mg kg^{-1} increased at 180 min after starch-loading whereas the levels at 0.1 and 0.3 mg kg^{-1}

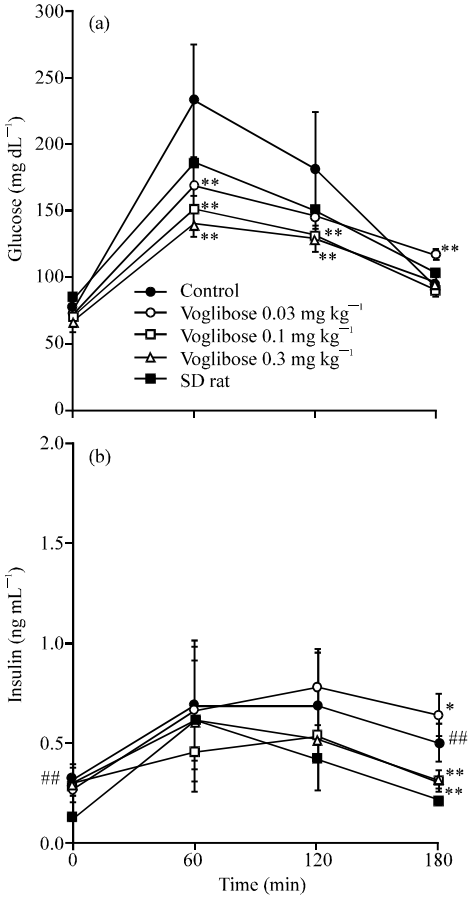

Fig. 1: a) Effect of voglibose on serum glucose and b) serum insulin levels in starch-loaded SDT rats at 10 weeks of age. Voglibose was administered immediately before starch-loading. Data represent mean±standard deviation (n = 5). *$p<0.05$, **$p<0.01$; significantly different from the control. ##$p<0.01$; significantly different from SD rat

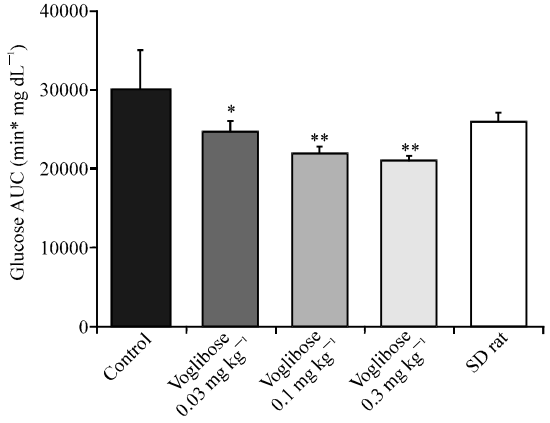

Fig. 2: The bar graphs show the area under the response curve from 0-180 min. Glucose areas were calculated on Fig. 1a. Data represent mean±standard deviation (n = 5). *p<0.05, **p<0.01; significantly different from the control

decreased (Fig. 1b). In SDT rats at 10 weeks of age, a single dose of voglibose improved the glucose intolerance dose-dependently. It is reported that voglibose inhibited the postprandial hyperglycemia in normal rats (Matsuo *et al*., 1992; Luo *et al*., 2001). In SD rats, a single dose of voglibose 0.43 mg kg^{-1} improved the glucose tolerance after carbohydrate (sucrose, maltose or starch) loading (Matsuo *et al*., 1992). When the intestine in Wistar rat was perfused in the recircular mode with 10 mmol L^{-1} maltose plus voglibose, voglibose 2 μmol L^{-1} showed inhibitory effect on the absorption of maltose (Luo *et al*., 2001). In SDT rats, voglibose did not affect TG and TC levels after starch-loading. When the effect on postprandial hypertriglycemia was investigated in obese Zucker fatty rats or non-obese Goto-Kakizaki (GK) rats, voglibose did not show a significant effect on the increase of triglyceride after fat-loading (Mine *et al*., 2002). In SDT rats at 10 weeks of age, voglibose showed an improvement of glucose tolerance at 0.03 mg kg^{-1} or higher (Fig. 1a). The pharmacological potency in SDT rats was similar with that in SD rats (Matsuo *et al*., 1992).

Chronic effects of voglibose were shown in Fig. 3 and 4. Non-fasted serum glucose levels did not change among each group from 10-16 weeks of age. SDT rats in voglibose-untreated (control) group developed diabetes from 18 weeks of age and three SDT rats developed diabetes at 20 weeks of age (Fig. 3a). Development of diabetes (>250 mg dL^{-1} in non-fasted glucose level) was not observed in voglibose-treated group. The non-fasted serum insulin, TG and TC levels did not change during the experimental period (Fig. 3a-c). Also, voglibose treatment did not affect the body weight

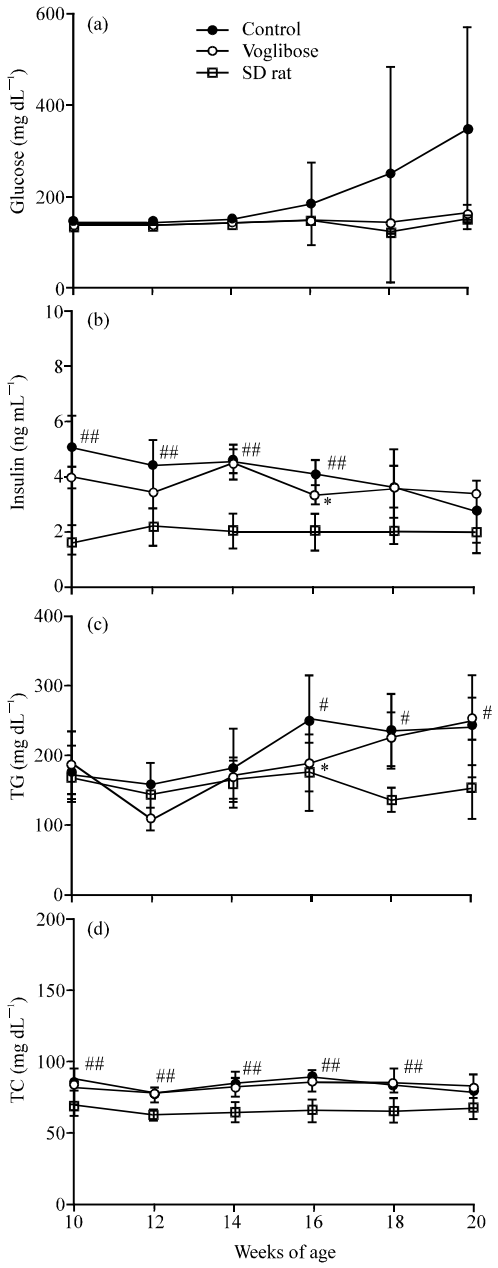

Fig. 3: a) Effects on serum glucose; b) insulin; c) TG and d) TC levels in SDT rats with chronic treatment of voglibose. Data represent mean±standard deviation (n = 5). *p<0.01; significantly different from the control. #p<0.05, ##p<0.01; significantly different from SD rat

and the food intake in SDT rats during the experimental period. Chronic treatment with voglibose prevented the development of diabetes in SDT rats. Chronic effects of α-glucosidase inhibitor, voglibose or acarbose were investigated in other diabetic models.

When voglibose was administered to GK rats from 12-36 weeks of age, fasting blood, non-fasting blood and Hemoglobin A1c (HbA1c) levels were significantly decreased (Koyama *et al*., 2000).

Moreover, chronic treatment with acarbose improved the hyperglycemia in Long-Evans Tokushima Otsuka (LETO) rats, obese diabetic Wistar rats and Otsuka Long-Evans Tokushima Fatty (OLETF) rats (Yamamoto and Otsuki, 2006; Carrascosa *et al*., 2001; Yamamoto *et al*., 1999). It is considered that a chronic treatment with α-glucosidase inhibitor induced the sufficient glycemic control by daily inhibition of postprandial hyperglycemia.

In pancreas of SDT rats at 20 weeks of age, histopathological abnormalities such as irregular boundaries, atrophy, fibrosis and decrease of insulin-immunoreactivity in islets were observed. However, those changes in pancreas were inhibited by chronic treatment of voglibose (Fig. 4).

Although, pancreatic weight in SDT rats was decreased as compared with that in SD rats, voglibose treatment diminished the reduction of pancreatic weight (Table 1). Voglibose inhibited the postprandial hyperglycemia and prevented the development of diabetes, resulting in an improvement of pathological abnormalities in pancreas of SDT rats.

Chronic treatment with glucosidase inhibitor showed a pancreatic protection effect in diabetic models. Islet of GK rat treated with voglibose for 24 weeks showed a good preservation of β cells (Koyama *et al*., 2000). In LETO rat treated with acarbose for 60 weeks, the pancreatic weight was increased and the pancreatic atrophy was inhibited (Yamamoto and Otsuki, 2006). Pancreatic abnormalities such as a decrease of the number and size of islets and the connective tissue proliferation in OLETF rat were improved by chronic treatment with acarbose (Yamamoto *et al*., 1999).

In both obese diabetic and non-obese diabetic models, chronic administration with an α-glucosidase inhibitor induced good glycemic control and pancreatic protection. In clinical study, α-glucosidase inhibitor such as voglibose and acarbose, showed a prevention of type 2 diabetes mellitus (Kawamori *et al*., 2009; Chiasson *et al*., 2002).

The pharmacological intervention delayed progression of IGT to diabetes. The results showed that pharmacological intervention with voglibose in SDT rats with IGT can delay progression to type 2 diabetes. SDT rat is considered to be useful for development of a preventive drug on type 2 diabetes.

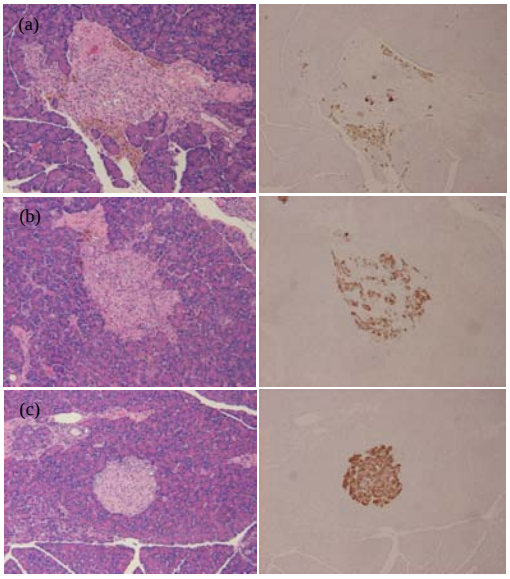

Fig. 4: a) Histopathological analysis of pancreas; b) control rat and c) voglibose-treated SDT rat at 20 weeks of age and SD rat at 20 weeks of age. Original magnification x200. HE stain (Left panel). Immunostained with anti-insulin (Right panel)

Table 1: Effect of voglibose treatment on pancreatic weights in SDT rats at 20 weeks of age

Weight	Control	Voglibose	SD rat
Absolute (g)	1.06±0.13$^{\#\#}$	1.32±0.06**	1.59±0.13
Relative (mg g^{-1})	1.88±0.14$^{\#\#}$	2.52±0.18**	3.05±0.28

Data represents mean±standard deviation (n = 5). $^{\#\#}p<0.01$; significantly different from SD rat. **p<0.01; significantly different from control

CONCLUSION

This study shows that inhibition of postprandial hyperglycemia prevented the incidence of diabetes in SDT rats. Also, SDT rat is a useful model for development of a preventive drug on diabetes.

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