KGA-2727, a Novel Selective Inhibitor of a High-Affinity Sodium Glucose Cotransporter (SGLT1), Exhibits Antidiabetic Efficacy in Rodent Models

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ABSTRACT

The high-affinity sodium glucose cotransporter (SGLT1) plays a critical role in glucose absorption from the gastrointestinal tract. We have developed 3-(3-(4-[3-[(3-[(3-((R,S)-2,3-dihydroxy-4-methyl-6-[[2S,3R,4S,6R]-3,4,5,6-tetrahydro-2H-pyran-2-yl]oxy)phenyl]propylamino)propionamide]; T-1095A, 3-(1-benzofuran-5-yl)-1-(2-hydroxy-4-methyl-6-[[2S,3R,4S,6R]-3,4,5,6-tetrahydro-2H-pyran-2-yl]oxy)phenyl)propan-1-one.

Efficacy in Rodent Models

Sodium Glucose Cotransporter (SGLT1), Exhibits Antidiabetic Activity in Rodent Models

KGA-2727, a Novel Selective Inhibitor of a High-Affinity Sodium Glucose Cotransporter (SGLT1), Exhibits Antidiabetic Efficacy in Rodent Models

Type 2 diabetes mellitus is characterized by hyperglycemia caused by pancreatic β-cell dysfunction and is a lifestyle-related disease connected to obesity and lack of exercise. Large clinical trials have confirmed that it is important to practice long-term tight control of blood glucose levels to avoid the development of diabetic complications [The Diabetes Control and Complications Trial Research Group, 1993; UK Prospective Diabetes Study (UKPDS) Group, 1998]. Diet, exercise, and drugs are treatment modalities used on patients with diabetes to control their blood glucose levels. For this control, it is important to attenuate postprandial hyperglycemia and reduce fasting hyperglycemia (Woerle et al., 2007). Epidemiology studies have shown that postprandial hyperglycemia has a strong association with cardiovascular risks (DECODE Study Group and the European Diabetes Epidemiology Group, 2001), endothelial dysfunction (Williams et al., 1998), and retinopathy (Shiraia et al., 2005). Although many clinical agents improve overall glycemic control including postprandial hyperglycemia, several therapeutics specifically target postprandial blood glucose. For example, α-glucosidase inhibitors, such as acarbose, voglibose, and miglitol, are prescribed in clinical practice to control postprandial blood glucose. Those inhibitors inhibit the digestion of carbohydrates, delay the absorption of carbohydrates from the gastrointestinal tract, and consequently suppress the postprandial elevation of the blood glucose level (Göke and Herrmann-Rinke, 1998). It has also been reported that acarbose is effective in preventing or delaying the incidence of diabetes when applied to patients with impaired glucose tolerance (Chiaisson et al., 2002). Recently, the Inter-
national Diabetes Federation (2011) launched a new global guideline for the management of postprandial glucose. From these viewpoints, modification of postprandial carbohydrate absorption is a target for the development of new antidiabetic agents.

The high-affinity sodium glucose cotransporter (SGLT1) plays a critical role in the absorption of glucose (Wright et al., 2007) and is expressed in the small intestines and encoded by the SLC5A1 gene. Patients who have defective mutations in SLC5A1 suffer from gastrointestinal symptoms caused by the impaired absorption of monosaccharides (Turk et al., 1991). Based on pathogenetic studies on diabetes, it was reported that SGLT1 played an important role in the accelerated absorption of glucose. It was confirmed that the mRNA and protein levels of SGLT1 increased, and the absorption of glucose was accelerated in Otsuka Long Evans Tokushima Fatty and streptozotocin-induced diabetic rats (Dyer et al., 1997; Fujita et al., 1998). Also in patients with diabetes, the mRNA and protein levels of SGLT1 are highly increased in the small intestine (Dyer et al., 2002). Therefore, it is reasonable to expect that delaying glucose absorption by SGLT1 inhibition would be effective in normalizing postprandial hyperglycemia.

To date, several groups of researchers have reported that SGLT inhibitors can be considered a novel class of antidiabetic drugs. However, most of them inhibit SGLT2, the low-affinity sodium glucose cotransporter, which plays a role in renal glucose reabsorption (Isaji, 2011; Kinne and Castaneda, 2011). Some of the SGLT2 inhibitors are now under investigation in clinical trials. Sergliflozin and remogliflozin, reported from our laboratory at the Kissei Pharmaceutical Co., Ltd., are also SGLT2 inhibitors (Katsuno et al., 2007, 2009; Fujimori et al., 2008, 2009). Those SGLT2 inhibitors reduce the blood glucose level by inhibiting renal glucose reabsorption and increasing urinary glucose excretion. However, no inhibitor selective for SGLT1 had been reported. In this article, we describe the efficiency of a novel selective SGLT1 inhibitor for the treatment of diabetes.

Materials and Methods

Chemicals. KGA-2727 [%3-{3-[4-[3-(β-D-glucopyranosyl oxy)-5-isopro pyl-1H-pyrazol-4-ylmethyl]-3-methylphenox y}propylamino]propionamide] was synthesized by Kissei Pharmaceutical Co., Ltd. Methyl-α-D-glucopyranoside (AMG) and phlorizin dihydrate were purchased from Sigma-Aldrich (St. Louis, MO). Methyl-D-[U-14C]glucopyranoside was obtained from GE Healthcare (Little Chalfont, Buckinghamshire, UK). Starch was purchased from Nacalai Tesque (Kyoto, Japan). Acarbose was prepared by extraction from Glucobay purchased from Bayer Yakuhin (Osaka, Japan). Dipeptidyl peptidase IV inhibitor was purchased from Linco Research (St. Charles, MO). Other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan).

Animals. Male Wistar, Zucker diabetic fatty (ZDF) fa/fa (ZDF/Gmi Crl-fa/fa), and ZDF lean (ZDF/Gmi Crl-lean) rats were purchased from Charles River Japan, Inc. (Yokohama, Japan). All rats were housed under a 12-h light cycle (lights on 8:00 AM to 8:00 PM) under controlled room conditions (room temperature, 20–26°C; humidity, 35–65%), fed a laboratory Chow diet (CE-2 pellets; CLEA Japan, Tokyo, Japan), and provided water ad libitum. All animal experiments were performed in accordance with the guidelines approved by the Laboratory Animal Committee of Kissei Pharmaceutical Co., Ltd.

Inhibitory Effects of KGA-2727 on Human and Rat SGLTs. Human and rat SGLT expression plasmids were constructed as de-
KGA-2727, 10, 30, and 100 ppm; and acarbose, 100 ppm. ZDF-lean rats of the same age served as the lean control group. The day when the chronic treatment was started was defined as day 0. Body weight, plasma glucose, and glycated hemoglobin (GHB) were measured periodically in the fed state. The plasma glucose concentration was determined as described above. GHB was determined with an HLC-723GHB device (Tosoh, Tokyo, Japan). The weight of powdered diet was measured periodically.

On day 37, the oral glucose tolerance test was performed for the evaluation of insulin secretion. After overnight fasting, the plasma glucose and insulin concentrations were measured at different time points after oral glucose loading (2 g/kg body weight). The plasma insulin concentration was measured with an insulin enzyme-linked immunosorbent assay kit (Seikagaku Corporation, Tokyo, Japan).

On days 2, 19, and 40, the rats were relocated to metabolic cages, and urine samples were collected for 24 h. The urine volume and glucose concentration were measured.

At the end of the chronic treatment study, the rats were anesthetized with ether, and blood was collected via the portal vein with syringes containing aprotinin and a dipeptidyl peptide IV inhibitor. The plasma concentration of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) were measured with a glucagon-like peptide-1 (active) enzyme-linked immunosorbent assay kit (Linco Research) and GIP radioimmunoassay kit (Peninsula Laboratories, Belmont, CA), respectively.

The pancreas and kidney were fixed with 10% phosphate-buffered formalin and then embedded in paraffin to prepare tissue sections. The pancreatic sections were stained with azan stain and immunostaining for insulin and quantitatively analyzed with an image processor (LUZEX-3; Nikon, Tokyo, Japan) as follows. For assessment of the insulin index (Ins-I) or fibrosis index (Fib-I), 10 islets larger than 1 mm² were randomly selected from each section, and the percentage of insulin-positive area or fibrous area within each selected islet was calculated, respectively. The kidney sections were stained with hematoxylin-eosin, periodic acid-Schiff, and periodic acid methenamine silver stain and morphologically analyzed. For the assessment of glomerulosclerosis, 50 glomeruli were randomly selected, and the percentage of sclerotic area within glomerular area was scored as follows: normal, 0; −0 to 25%, 1; −25 to 50%, 2; −50 to 75%, 3; >75%, 4. The average of these scores was used as a glomerulosclerosis score of each animal. Glycogen deposition in distal tubules was assessed by counting the number of periodic acid-Schiff-positive tubules in 20 microscopic fields (total area = approximately 14.5 mm²) and was scored as follows: 0, negative; −1 to 20, minimal, ±21 to 60, slight; ±60 to 80, moderate; ±80, severe. A change in dilatation of distal tubules was scored as follows: negative, −; there were a few changes, minimal, ±; the changes were focally observed, slight, +; the changes were diffusely observed, moderate, ++.

Statistical Analysis. Data were presented as the mean ± S.E.M. for each group. Statistical analyses were performed with SAS Systems version 8.2 (SAS Institute, Cary, NC). In numerical data analysis, statistical significance was determined with one-way analysis of variance, Dunnett’s multiple comparison, nonparametric multiple comparison, univariate repeated-measures analysis as a split-plot design, multiplex comparison by each time period, or t test as appropriate. In categorical data analysis, statistical significance was determined with Wilcoxon test, Kruskal-Wallis test, and nonparametric Dunnett’s multiple comparison test as appropriate. Differences assessed by each test were considered statistically significant at p < 0.05.

Results

Structure of KGA-2727. The structure of KGA-2727 is shown in Fig. 1A, and the structure of phlorizin, a nonselective SGLT inhibitor, is shown in Fig. 1B. KGA-2727 has an O-glucoside structure similar to that of phlorizin. The aglycon portion of KGA-2727 is a pyrazole derivative.

Potency and Selectivity of KGA-2727 in Inhibiting SGLT1. A Dixon plot analysis for KGA-2727 displayed good linearity for human SGLT1 and SGLT2 (Fig. 2, A and B, respectively). The results of the Dixon plot show that KGA-2727 inhibited these SGLTs in a competitive manner. KGA-2727 dose-dependently inhibited AMG uptake by SGLT1 and SGLT2. The Kᵢ values for KGA-2727 and phlorizin against human and rat SGLTs are shown in Table 1. KGA-2727 more potently inhibited SGLT1 than did phlorizin and vice versa for SGLT2. The selectivity ratios (Kᵢ for SGLT2/Kᵢ for SGLT1) of KGA-2727 were 140 (human) and 390 (rat), whereas those of phlorizin were 0.084 (human) and 0.195 (rat). These data suggest that KGA-2727 was a potent and selective SGLT1 inhibitor.

Effects of KGA-2727 on Saccharide Absorption Rate in Rat Small Intestine. The percentage of sucrose recovery was approximately 78%, as calculated from the initial control value. In the control group, 80.6% of the sucrose was digested after 15 min. The effects of KGA-2727 on glucose and fructose absorption rates are shown in Fig. 3. KGA-2727 decreased the absorption rate of glucose in a dose-dependent manner. Phlorizin tended to decrease this rate, but its effect was not statistically significant. On the other hand, KGA-2727 and phlorizin had no effect on the absorption rate of fructose after sucrose injection. Thus, KGA-2727 inhibited the absorption of only glucose.

Effects of KGA-2727 on Gastrointestinal Carbohydrate Content after Administration of Starch and Inhibitor to Normal Rats. Figure 4 shows the effects of
KGA-2727 (A and C) and acarbose (B and D) on carbohydrate and glucose contents in the gastrointestinal tract. Residual carbohydrate (Fig. 4, A and B) was calculated from the amount of glucose in the hydrolyzed sample and indicates the content of carbohydrate (i.e., monosaccharide plus polysaccharides). Residual glucose (Fig. 4, C and D) was calculated from the amount of glucose in the nonhydrolyzed sample and indicates the content of monosaccharide glucose. The amount of residual carbohydrate in the control was approximately 5% at 3 h after oral starch administration. KGA-2727 increased the total amount of residual carbohydrate in a dose-dependent manner. Three hours after administration, the total amount of residual carbohydrate was 6.7% in the control group but 14.0, 26.2, and 45.5% in the presence of 0.1, 0.3, and 1.0 mg/kg KGA-2727, respectively (Fig. 4A). More than half of the residual carbohydrate in the KGA-2727-treated groups was monosaccharide glucose (Fig. 4C). KGA-2727 increased the residual glucose in the lower intestine. Acarbose also increased the content of residual carbohydrate (Fig. 4B); but in this case, the residual carbohydrate was not glucose (Fig. 4D). The amount of residual carbohydrate in ~0.1 to 0.3 mg/kg KGA-2727-treated groups was similar to that in the 2 mg/kg acarbose-treated group. These data indicate that KGA-2727 delayed carbohydrate absorption in the gastrointestinal tract and increased the amount of glucose in the lower intestine.

Effects of KGA-2727 on Plasma Glucose Concentration after Oral Glucose Loading in Streptozotocin-Induced Diabetic Rats. Streptozotocin-induced diabetic rats showed hyperglycemia after glucose loading compared with normal rats. In those diabetic rats, KGA-2727 significantly inhibited, in a dose-dependent manner, the elevation of plasma glucose after glucose loading (Fig. 5). The plasma glucose elevation in diabetic rats in the presence of 0.3 mg/kg KGA-2727 was similar to that in the normal group. Acarbose at a dose of 30 mg/kg did not inhibit the rise in plasma glucose after glucose loading (Fig. 5).

Effects of Chronic Treatment of ZDF Rats with KGA-2727. The changes in body weight of ZDF rats chronically treated with KGA-2727 or acarbose are shown in Table 2. The body weight of the obese rats was significantly higher than that of the lean rats throughout the observation period. The increase in body weight of the obese control slowed down after day 38, but not that of the KGA-2727 or acarbose-treated obese groups. As a result, rats treated with KGA-2727 or acarbose gained more weight than the obese control rats at the endpoint (Table 2). KGA-2727 reduced food consumption, and acarbose tended to reduce food consumption on day 44 (Table 2). The amounts of drug intake, calculated from food consumption from days 23 to 44, were as follows: KGA-2727 at 10, 30, and 100 ppm, 0.7, 1.8, and 6.0 mg/kg/day, respectively and acarbose at 100 ppm, 6.7 mg/kg/day. The levels of alanine aminotransferase and aspartate aminotransferase, markers of hepatic damage, were not elevated in the KGA-2727-treated groups or the acarbose-treated group (data not shown).

The plasma glucose level (Fig. 6A) and HbA1c (Fig. 6B) in the obese control group were significantly higher than that in the

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TABLE 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>SGLT1</th>
<th>SGLT2</th>
<th>SGLT1</th>
<th>SGLT2</th>
</tr>
</thead>
<tbody>
<tr>
<td>KGA-2727</td>
<td>97.4 ± 42.3</td>
<td>13,600 ± 7400</td>
<td>43.5 ± 26.0</td>
<td>17,100 ± 9900</td>
</tr>
<tr>
<td>Phlorizin</td>
<td>233 ± 83</td>
<td>19.6 ± 4.3</td>
<td>210 ± 64</td>
<td>41.0 ± 11.9</td>
</tr>
</tbody>
</table>

Fig. 3. Effects of KGA-2727 or phlorizin on saccharide absorption rates in the closed loop of the rat small intestine. Absorption rates of glucose (empty bars) and fructose (filled bars) were calculated from the amounts of glucose and fructose in the closed loop contents after the injection of sucrose and test compound solution. Data are presented as the means ± S.E.M. (n = 5). ***, p < 0.001 versus control.

Fig. 4. Effects of KGA-2727 (A and C) or acarbose (B and D) on residual gastrointestinal carbohydrate and glucose after oral starch administration to normal rats. A and B, the residual carbohydrate indicates the ratio of polysaccharides and monosaccharides to the dosage of starch. C and D, the residual glucose indicates the ratio of monosaccharides glucose to the dosage of starch. Data are presented as the means ± S.E.M. (n = 4). *, p < 0.05; ***, p < 0.01 versus control. Int., intestine.
In the present work, we described the efficacy of a novel selective SGLT1 inhibitor, KGA-2727, as an antidiabetic drug. Recently, SGLTs have attracted attention as targets for antidiabetic drugs. Many of the reported SGLT inhibitors have high affinity for SGLT2, which is the predominant transporter that mediates renal glucose reabsorption in the proximal tubule. However, there are no reports characterizing SGLT1-selective inhibitors. We focused on SGLT1, which plays a critical role in glucose absorption from the gastrointestinal tract, as a molecular target based on the concept that inhibition of accelerated glucose absorption by SGLT1 would lead to control of the blood glucose level.

To develop a selective SGLT1 inhibitor, we selected a pyrazole-\(O\)-glucoside structure as the basal scaffold structure. We evaluated the potency of KGA-2727 against human and rat SGLTs in cells transiently expressing these transporters and observed dose-dependent inhibition by the compound. Based on \(K_p\) values, we confirmed KGA-2727 to be a potent and highly selective inhibitor of SGLT1. The selectivity ratios of KGA-2727 were far higher than those of phlorizin (Table 1). According to Oku et al. (1999), the selectivity ratio (human SGLT2/human SGLT1) of 3-(1-benzofuran-5-yl)-1-(2-hydroxy-4-methyl-6-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl](oxy)phenyl)propan-1-one (T-1095A), a nonselective SGLT inhibitor, is 0.25, and that of phlorizin is 1. Other SGLT inhibitors reported so far are selective SGLT2 inhibitors. For instance, dapagliflozin and remogliflozin, respectively, have 1200- and 365-fold specificity for SGLT2 versus normal. In the present work, we described the efficacy of a novel selective SGLT1 inhibitor, KGA-2727, as an antidiabetic drug.
KGA-2727 inhibits SGLT1 but not GLUT5. In cells transiently expressing GLUT5, KGA-2727 had no effect on fructose uptake (data not shown). Although phlorizin showed significant decreasing trend in glucose absorption from the small intestine (Fig. 3). This is probably because phlorizin is rapidly hydrolyzed to phloretin and glucose in this tissue (Ehrenkranz et al., 2005).

The residual glucose in the gastrointestinal tract was elevated in the KGA-2727-treated groups, but not in the acarbose-treated group (Fig. 4). In the oral glucose tolerance test, KGA-2727 suppressed postprandial hyperglycemia in streptozotocin-induced diabetic rats, but acarbose could not (Fig. 5). These differences between KGA-2727 and acarbose are accounted for by the above-mentioned mechanisms; KGA-2727 inhibits glucose absorption, whereas acarbose inhibits metabolism to glucose in the lumen of the gastrointestinal tract. In the oral glucose tolerance test, the loading carbohydrate is glucose. Therefore, acarbose could not inhibit the absorption of glucose, and the plasma glucose was elevated after glucose loading (Fig. 5). High-fructose corn syrup and other forms of sugar that are rich in glucose are routinely added to processed food and many types of beverages (Bray et al., 2004; Malik et al., 2010). Thus, diabetic patients have many opportunities to take in carbohydrates as glucose. Therefore, KGA-2727, which suppresses postprandial hyperglycemia after glucose intake, may have an advantage over α-glucosidase inhibitors as an antidiabetic medication.

The most common adverse effects of α-glucosidase inhibitors are abdominal distension and flatulence (Chiasson et al., 2002; Kawamori et al., 2009). If the dosage is too high relative to the amount of carbohydrate in the meal, undigested carbohydrates pass in to the large bowel. Carbohydrates fermented by the intestinal flora cause the abdominal symptoms. In view of this, KGA-2727 also may cause the abdominal distension and flatulence. In view of this, KGA-2727 also may cause the adverse effects as well as α-glucosidase inhibitors. However, the obvious adverse effects were not observed at the dose that used in this study.

In diabetes, hyperglycemia on its own contributes to the development of disturbed insulin secretion by pancreatic β-cells and insulin resistance (Rossetti et al., 1987). Hyperglycemia causes progressive damage to pancreatic β-cells through endoplasmic reticulum stress and oxidative stress and consequently diminishes insulin secretion (Robertson et al., 2003; Prentki and Nolan, 2006; Eizirik et al., 2008). The blood insulin level in the ZDF rat increases to compensate for insulin resistance until 10 weeks of age and then decreases along with the progressive dysfunction of pancreatic β-cells (Sugimoto et al., 2008). Several studies have shown that

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Lean Control</th>
<th>KGA-2727 10 ppm</th>
<th>KGA-2727 30 ppm</th>
<th>KGA-2727 100 ppm</th>
<th>Acarbose 10 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight, g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>166 ± 5</td>
<td>230 ± 8†††</td>
<td>220 ± 6</td>
<td>227 ± 3</td>
<td>225 ± 5</td>
</tr>
<tr>
<td>Day 48</td>
<td>283 ± 11</td>
<td>361 ± 10†††</td>
<td>403 ± 8**</td>
<td>406 ± 4***</td>
<td>381 ± 7</td>
</tr>
<tr>
<td>Day 1</td>
<td>24.7 ± 5</td>
<td>40.8 ± 5.0</td>
<td>45.8 ± 5.6</td>
<td>34.3 ± 4.2</td>
<td>34.1 ± 5.5</td>
</tr>
<tr>
<td>Day 44</td>
<td>18.0 ± 0.9</td>
<td>30.8 ± 2.6†††</td>
<td>28.5 ± 2.3</td>
<td>23.8 ± 0.7*</td>
<td>23.4 ± 0.9*</td>
</tr>
</tbody>
</table>

*, p < 0.05; †††, p < 0.01; ††††, p < 0.001 vs. the lean control group.
†††, p < 0.001 vs. the obese control group.

Fig. 6. Changes in plasma glucose and GHb during chronic treatment of Zucker diabetic fatty rats with KGA-2727 or acarbose. The plasma glucose concentration (A) and GHb (B) were measured in the ZDF rats and the ZDF lean rats fed a powder diet containing the indicated test compound in the fed state. Data are presented as the mean ± S.E.M. (n = 8).

Fig. 7. Changes in urinary glucose excretion and urine volume in Zucker diabetic fatty rats during chronic treatment with KGA-2727 or acarbose. On days 2, 19, and 40, the urine samples were collected for 24 h. The urinary glucose excretion (A) was calculated from the urine volume (B) and glucose concentration. Data are presented as the means ± S.E.M. (n = 8). *, p < 0.05; †, p < 0.01; †††, p < 0.001 versus the obese control group. *, p < 0.05; ††††, p < 0.001 versus the lean control group.
pharmacological treatment to control hyperglycemia results in preservation of pancreatic β-cells in rodent models (Koyama et al., 2000; Fukaya et al., 2009). For example, a β-glucosidase inhibitor preserves pancreatic β-cells in Goto-Kakizaki rats, a model for type 2 diabetes (Goda et al., 2007).

In our results, chronic treatment with KGA-2727 also exhibited protective effects on the insulin contents or fibrotic changes of pancreatic β-cells (Table 3) through the improvement of hyperglycemia (Fig. 6) in the ZDF rats. Furthermore, the chronic treatment of ZDF rats with KGA-2727 preserved

**TABLE 3**

Chronic effects of KGA-2727 or acarbose on Ins-I and Fib-I of the pancreas in Zucker diabetic fatty rats

Data are presented as the means ± S.E.M. (n = 8).

<table>
<thead>
<tr>
<th>Lean Control</th>
<th>Obese (fa/ fa)</th>
<th>KGA-2727</th>
<th>Acarbose, 100 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ins-I, %</td>
<td>46.9 ± 1.9</td>
<td>17.4 ± 1.8†††</td>
<td>19.0 ± 1.8</td>
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<tr>
<td>Fib-I, %</td>
<td>3.25 ± 0.83</td>
<td>10.41 ± 1.50†††</td>
<td>8.47 ± 0.96</td>
</tr>
</tbody>
</table>

†††, p < 0.001 vs. the obese control group.

**TABLE 4**

Chronic effects of KGA-2727 or acarbose on morphometric changes in the kidneys of Zucker diabetic fatty rats

Data are presented as the means ± S.E.M. or as the number of animals in each grade (n = 8).

<table>
<thead>
<tr>
<th>Lean Control</th>
<th>Obese (fa/ fa)</th>
<th>KGA-2727</th>
<th>Acarbose, 100 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerulosclerosis score</td>
<td>0.17 ± 0.12</td>
<td>0.80 ± 0.03</td>
<td>0.75 ± 0.02</td>
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<tr>
<td>Glycogen deposition in distal tubules</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>8</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>±</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>‡</td>
<td>0</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Significance</td>
<td>†††</td>
<td>N.S.</td>
<td>***</td>
</tr>
<tr>
<td>Dilatation of distal tubule</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>8</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>±</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>‡</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Significance</td>
<td>†††</td>
<td>N.S.</td>
<td>***</td>
</tr>
</tbody>
</table>

†, p < 0.05; †††, p < 0.001 vs. the lean control group.

†††, p < 0.001 vs. the lean control group.
their ability to secrete insulin from pancreatic β-cells through improving hyperglycemia in the ZDF rats (Fig. 8). The lean rats, without insulin resistance, would be able to control their plasma glucose with a much lower amount of insulin than the obese rats with insulin resistance. Therefore, no increase in the plasma insulin after glucose loading was observed in the lean control group (Fig. 8). Our results suggest that the amelioration of hyperglycemia with KGA-2727 prevents the development of pancreatic β-cells exhaustion and improves glucose metabolism.

Hyperglycemia causes damages to the kidneys and pancreatic β-cells (Yamagishi et al., 2007). According to Coimbra et al. (2000), glomerulosclerosis was first noted in 18-week-old ZDF rats. In our study, the assessment of glomerulosclerosis was performed in 13-week-old ZDF rats. Therefore, the sclerotic changes of renal glomerulus were almost mild (Table 4).

On the other hand, the morphometric changes in the distal tubules were observed in the obese control group (Table 4). KGA-2727 ameliorated these changes in the distal tubules and decreased urinary glucose and volume (Table 4; Fig. 7). Therefore, the decreases in urinary glucose and volume induced by the chronic treatment with KGA-2727 were associated with the morphometric changes in the distal tubules rather than the glomerulosclerosis.

Thus, like chronic treatment with acarbose, that with KGA-2727 suppressed the development of diabetic conditions such as dysfunction of pancreatic β-cells and the kidneys, and that suppression was a consequence of attenuation of the postprandial hyperglycemia. These results show that KGA-2727 had efficacy in the treatment of diabetes comparable with that of acarbose, a widely used antidiabetic agent.

It has been reported that α-glucosidase inhibitors enhance and prolong GLP-1 secretion in human and rodent models (Qualmann et al., 1995; Moritoh et al., 2009). In our results, chronic treatment with KGA-2727 increased the portal GLP-1 concentration in ZDF rats (Fig. 9A). GLP-1 is secreted from intestinal endocrine L-cells, which are located mainly in the distal ileum and colon, and exerts glucoregulatory actions. GLP-1 acts on pancreatic β-cells, and that action leads to glucose-dependent insulin secretion, induction of β-cell proliferation, and enhanced resistance to apoptosis (Baggio and Drucker, 2007). GLP-1 also reduces food intake by direct action on central nervous systems that regulate ingestive behavior and by inhibiting gastric emptying (Baggio and Drucker, 2007). In our results, the reduction of food consumption was observed in the drug-treated groups (Table 2) that showed higher concentrations of GLP-1 (Fig. 9A). This enhanced GLP-1 secretion may have contributed in part to the antidiabetic effects of KGA-2727. According to Moritoh et al. (2009), delayed absorption of carbohydrates caused by α-glucosidase inhibitors might have been responsible for the increased GLP-1 observed. GLP-1 release can be stimulated by nutrients including glucose and other sugars (Baggio and Drucker, 2007; Gorboulev et al., 2012). KGA-2727 increased the amount of glucose in the distal part of the small intestine (Fig. 4), which possesses many GLP-1-secreting cells. The glucose increased in the distal intestine could thus stimulate GLP-1 secretion.

In clinical studies, pharmacological interventions have been used to investigate whether drugs delay the onset of type 2 diabetes. α-Glucosidase inhibitors (acarbose and voglibose) prevent the progression of impaired glucose tolerance to type 2 diabetes by improving postprandial hyperglycemia (Chiasson et al., 2002; Kawamori et al., 2009). Likewise, KGA-2727 may delay the onset of type 2 diabetes. We suggest that KGA-2727 represents an attractive therapeutic candidate for the treatment of diabetes and impaired glucose tolerance.

**Authorship Contributions**

**Participated in research design:** Itoh and Isaji.

**Conducted experiments:** Shibazaki, Tomae, Ishikawa-Takemura, and Itoh.

**Contributed new reagents or analytic tools:** Fusimi.

**Performed data analysis:** Shibazaki, Tomae, Ishikawa-Takemura, and Itoh.

**Wrote or contributed to the writing of the manuscript:** Shibazaki, Tomae, Ishikawa-Takemura, Fusumi, Itoh, Yamada, and Isaji.

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