7-O-Galloyl-d-sedoheptulose Is a Novel Therapeutic Agent against Oxidative Stress and Advanced Glycation Endproducts in the Diabetic Kidney

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Diabetes is the leading cause of end-stage renal failure, since glucose-dependent metabolic factors are synergistically activated within the diabetic kidney. Accordingly, in Japan, there is much debate over the health benefits of natural therapies to reduce these risk factors. In our previous study, we reported that Cornus officinalis Sieb. et Zucc. possessed an antidiabetic effect via ameliorating glucose-mediated metabolic disorders as well as aminoguanidine, an inhibitor of advanced glycation endproduct (AGE) formation, with a renoprotective effect. The aim of the present study was to investigate the effect of 7-O-galloyl-d-sedoheptulose (GS) against diabetic oxidative stress and AGE formation. Streptozotocin-induced diabetic rats were orally administered GS for 20 d, and the changes in serum glucose levels, as well as those of body weight every 10 d were evaluated. In addition, glucose, fluorescent AGE, methylglyoxal, glycolaldehyde (GA), and immunoblotting analyses for heme oxygenase-1, receptor for AGE, N^2-(carboxymethyl)lysine, N^ε-(carboxyethyl)lysine, and GA-pyridine were performed in the kidney at the end of the experiment. The results obtained in this study demonstrated that 20 d of treatment with GS had beneficial effects on hypoglycemic and renal metabolic abnormalities, including renal glucose, oxidative stress, and AGE formation. Together, our data help to elucidate its potential therapeutic value against diabetic kidney disease.

Key words 7-O-galloyl-d-sedoheptulose; Cornus officinalis; diabetic kidney disease; oxidative stress; heme oxygenase-1; advanced glycation endproduct

Materials and Methods

General Experimental Procedures Optical rotations were measured with a Jasco™ DIP-370 digital polarimeter. 1H-, 13C-NMR, 1H-1H COSY, NOESY, HSQC, and HMBC spectra were recorded at 27 °C with a Varian Unity plus 500 spectrometer operating at 500 MHz for 1H and 125 MHz for 13C. Mass spectra (MS) were recorded on a JEOL JMS-700N spectrometer, and glycerol was used as the matrix for FAB-MS measurements. Column chromatography was performed with Diaion ™ HP20SS, MCI-gel CHP 20P (75—150 μm) (Mitsubishi Chemical Co., Japan), Sephadex ™ LH-20 (25—100 μm) (GE Healthcare, Piscataway, NJ, U.S.A.), and Chromatorex ODS (Fuji Siylsa Chemical Ltd., Aichi, Japan). TLC was performed on 0.2 mm precoated Kieselgel 60 F254 plates (Merck & Co., Inc., Whitehouse Station, NJ, U.S.A.) with benzene—ethyl formate—formic acid (1:7:1, v/v) or chloroform—methanol—water (14 : 6 : 1, v/v). Spots were de-
ected via UV illumination and by spraying with 2% ethanoic FeCl₃ or 10% sulfuric acid reagent, followed by heating. Analytical HPLC was performed on a 250×4.6 mm i.d. Cosmosil 5C₁₈-AR II column (Nacalai Tesque Inc., Kyoto, Japan) with gradient elutions of CH₃CN in 50 mM H₃PO₄ from 10—30% in 30 min and 30—75% in 15 min at a flow rate of 0.8 ml/min and detection with a Jasco™ MD-910 photodiode array detector.

Preparation of Cornus officinalis Fractions and Purification of 7-O-Galloyl-D-sedoheptulose (GS) The extract of Cornus officinalis (100 g), which was produced by Tsumura & Co. (Tokyo, Japan) was fractionated by Sephadex™ LH-20 column chromatography (32 cm×5 cm) with water containing increasing proportions of methanol (0—100%, 10% stepwise gradient elution) and finally 60% acetone to give four fractions: S1 (94.52 g), S2 (1.20 g), S3 (2.15 g), and S4 (1.55 g). The fraction S1 was further separated by Diaion™ HP-20SS column chromatography (28 cm×5 cm) with water–methanol (0—100%, 10% stepwise gradient elution) to give S1D1 (85.64 g) and S1D2 (7.88 g). TLC and HPLC analysis, which were performed as mentioned above, showed that S1D1 and S1D2 mainly contained sugars and iridoid glycosides, and S2, S3, and S4 contained phenolic substances (Fig. 1A). A portion of S2 (150 mg) was further purified by MCI-gel CHP20P column chromatography (28 cm×2 cm) with 0—10% MeOH to give GS (98 mg), as shown in Figs. 1B—D. GS (4.8 g) was also obtained on a larger scale from a MeOH extract of Cornus officinalis (1.5 kg) (purchased from Uchida Wakanyaku, Tokyo, Japan) in a manner similar to that described above.

7-O-Galloyl-D-sedoheptulose (GS) A white amorphous powder, HR-FAB-MS m/z: 363.0903, C₁₄H₁₉O₁₁ [M+H] requires 363.0927. ¹H-NMR (acetone-d₆/D₂O) of major anomer δ: 7.13 (s, galloyl-H), 4.36 (m, H-4, H-7a), 4.23 (dd, J=6.6, 11.7 Hz, H-7b), 4.09 (d, J=6.4 Hz, H-3), 4.05 (m, H-6), 3.88 (t, J=5.5 Hz, H-5), 3.50 (2H, brs, H-1). ¹H-NMR (acetone-d₆+D₂O) of major anomer δ: 167.0 (galloyl C-7), 145.9 (galloyl C-3,5), 138.7 (galloyl C-4), 121.5 (galloyl C-1), 109.8 (galloyl C-2,6), 103.7 (C-2), 83.3 (C-5), 78.0 (C-3), 77.1 (C-4), 71.1 (C-6), 66.2 (C-7), 64.4 (C-1). Other anomeric carbon signals are observed at δ 98.2, 103.7, and 109.0. Assignments of the signals were achieved by COSY, HSQC, and HMBC spectral analysis. The structure was further confirmed by the formation of an osazone derivative: a mixture of compound (10 mg), phenylhydrazine hydrochloride (20 mg), and sodium acetate (30 mg) in water (0.5 ml) was heated at 80 °C for 25 min and the resulting precipitates were collected by filtration. The ¹H-NMR spectral data (in DMSO-d₆) and [α]D value was coincided with the data of the osazone derivative of 7-O-sedoheptulose gallate.

Experimental Protocol Male Wistar rats (4—5 weeks age, 120—130 g) were obtained from Japan SLC, Inc. (Hamamatsu, Japan), kept in wire-bottomed cages, and exposed to a 12-h light/dark cycle. The room temperature and humidity were maintained automatically at about 25 °C and 60%, respectively. These animals had ad libitum access to a laboratory diet (CLEA Japan Inc., Tokyo, Japan; comprising 24.0% protein, 3.5% lipid, and 60.5% carbohydrate) and water. After several days of adaptation, the rats were randomly separated into normal control (n=5) and diabetic groups. The diabetic groups were given an intraperitoneal (i.p.) injection of STZ (Sigma-Aldrich, St. Louis, MO, U.S.A.) (50 mg/kg body weight (BW)) in 10 mM citrate buffer (pH 4.5). Rats receiving an injection of citrate buffer were used as a normal control. After 10 d of the STZ or vehicle injection, the body weights were measured and blood glucose levels were determined.
samples were obtained from the tail vein between 10:00 am and 11:00 am, avoiding the influence of food consumption, and used to determine glucose levels. Rats with a blood glucose level higher than 400 mg/dl were used as diabetic rats and randomly divided into three experimental groups (diabetic (D), D+GS20, and D+GS100 (n=8, respectively)). Diabetic rats were treated orally with GS dissolved in water at doses of 20 or 100 mg/kg/d via gavage, while diabetic control rats were given water. The dose of GS was determined considering its content in Cornus officinalis and previous report.7,8) After 20 d of the administration period, 24-h urine samples were collected using metabolic cages and blood samples were obtained between 10:00 am and 11:00 am from the abdominal aorta under pentobarbital anesthesia (50 mg/kg, i.p.), and then the serum was immediately separated from the blood samples by centrifugation (1000×g at 4 °C). After renal perfusion through the renal artery with ice-cold physiological saline, the kidneys were removed from each rat and frozen at −80°C until analysis. All experiments were approved by the Guidelines for Animal Experiments of the University of Toyama.

Biochemical Analysis Serum glucose, creatinine (Cr), albumin, total protein, total cholesterol, and triglyceride levels were examined using commercial reagents (Glucose CII-Test WakoTM, A/G B-Test WakoTM, Cholesterol E-Test WakoTM, and Triglyceride E-Test WakoTM obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); CRE-EN Kainos obtained from Kainos Laboratories, Inc. (Tokyo, Japan)). Urine component levels were determined as follows: for protein analysis using a commercial reagent (MicroTP-Test WakoTM from Wako Pure Chemical Industries, Ltd.) and for Cr using a commercial reagent (CRE-EN Kainos). Cr clearance (Ccr) was calculated on the basis of urinary Cr, serum Cr, urine volume, and body weight using the following equation: Ccr (ml/min/kg BW) = [urinary Cr (mg/dl) × urinary volume (ml)/serum Cr (mg/dl)] × [1000/BW (g)] × [1/1440 (min)].

Thiobarbituric Acid-Reactive Substance (TBARS) Level Determination TBARS levels in serum were determined using the methods of Naito and Yamakawa.11) Mitochondria were prepared from kidney homogenate by differential centrifugation (800×g and 12000×g, respectively) at 4 °C according to the methods of Johnson and Lardy12) and Jung and Pergande,13) respectively, with minor modifications for the determination of mitochondrial TBARS levels. Each pellet was resuspended in preparation medium, and the concentration of TBARS was determined by the method of Mi- hara and Uchiyama.14) The protein level was examined by the method of Itzhaki and Gill15) with bovine serum albumin (BSA) as the standard.

Glucose and Fluorescent AGE Levels in Kidney The renal glucose level was determined by the method of Momo- se et al.16) with some modifications. In brief, frozen kid- ney tissue was homogenized with ice-cold physiological saline, and, after being deproteinized, the content of glucose was determined using the Wako kit described above. The renal AGE level was determined by the method of Nakayama et al.17) Minced kidney tissue was delipidated with chloro- form and methanol (2:1, v/v) overnight. After washing with methanol and distilled water, the tissue was homogenized in 0.1 N NaOH, followed by centrifugation at 8000×g for 15 min at 4 °C. The amounts of AGEs in these alkali-soluble samples were determined by measuring fluorescence at an emission wavelength of 440 nm and an excitation wavelength of 370 nm. A native BSA preparation (1 mg/ml of 0.1 N NaOH) was used as a standard, and its fluorescence intensity was defined as one unit of fluorescence. The fluorescence values of samples were measured at a protein concentration of 1 mg/ml and expressed in arbitrary units (AU) compared with the native BSA preparation.

Methylglyoxal (MG) and Glycolaldehyde (GA) Levels in Kidney The frozen kidney tissue was homogenized with ice-cold physiological saline, and MG and GA values in homogenates were evaluated. Determination of MG was performed by applying the principle that MG is quantitatively converted with glutathione (GSH) and glyoxalase I to s-lactoyl-GSH, which can be directly measured by the change in extinction (Δ230).18) The renal GA value was determined by applying the principle that the GA formed is reduced by NADH, with the reduction being catalyzed by alcohol dehydrogenase (ADH), and measured by the change in extinction (Δ240) caused by the addition of ADH.19) The protein level was examined as described above, and each sample value was calculated at a protein concentration of 1 mg/ml and represented as the % of normal.

Protein Preparation and Immunoblotting Analyses The analysis of protein expression was determined by the method of Yamabe et al.20) Samples (30 μg protein/lane) were electrophoresed on 10 or 12% sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE), separated proteins were electrophoretically transferred to a nitrocellulose membrane, and blocked with 5% (w/v) skim milk solution for 1 h at room temperature. Then, samples were incubated with the corresponding primary anti-heme oxygenase (HO)-1 (sc-10789) (Santa Cruz, CA, U.S.A.), receptor for AGE (RAGE) (sc-05563) (Santa Cruz, CA, U.S.A.), polyclonal N°-(carboxymethyl)lysine (CML), monoclonal N°-(carboxyethyl)lysine (CEL), monoclonal GA-pyridine (the three antibodies were kindly provided by Dr. R. Nagai (Kumamoto University, Japan)), or β-actin (A5316) (Sigma Chemical Co., St. Louis, MO, U.S.A.) antibody overnight at 4 °C. Thereafter, the membranes were washed at room temperature and incubated with goat anti-rabbit and/or goat anti-mouse IgG horseradish peroxidase conjugated secondary antibody (sc-2004 and sc-2005) (Santa Cruz, CA, U.S.A.) for 90 min at room temperature. After washing, each specific protein was visualized using ECL Western Blotting Detection Reagents (GE Healthcare, Piscataway, NJ, U.S.A.) and detected by chemiluminescence with LAS-1000 plus (Fujifilm, Japan).

Statistical Analysis The effect of GS on each parameter was examined using one-way Analysis of Variance. Individual differences among groups were analyzed by Dunnett’s test. p<0.05 was considered significant. The results are expressed as means±S.E.M.

RESULTS

Body Weight and Serum Glucose Levels As shown in Fig. 2A, normal rats revealed a significantly higher body weight gain than diabetic control rats during the experimental period (p<0.01), but the oral administration of GS did not affect body weight changes in diabetic rats. On the other
hand, the diabetic rats showed significantly higher serum glucose levels than normal rats. The serum glucose level of diabetic control rats was increased during the diabetic period, but it was maintained to nearly the same value as on day 0 by the 20- and 100-mg GS treatments (Fig. 2B).

Renal Function and Serum Constituent  Table 1 shows the results of biochemical analysis. The kidney weight of diabetic control rats was 1.6-fold heavier than that of normal rats, but it was reduced by administrations of GS at both 20 and 100 mg ($p<0.01$). Although the diabetic control group showed a slight increase in the Ccr and serum Cr levels compared to normal rats, the urine volume and amount of urinary protein were significantly higher than in the normal group. However, GS effectively ameliorated the increases in serum Cr and urinary protein levels to nearly normal levels ($p<0.01$). On the other hand, serum albumin and total protein levels were significantly decreased under diabetes, but the albumin level was significantly increased in GS-treated groups ($p<0.01$). In the analysis of serum lipid levels, i.e., total cholesterol and triglycerides, 20-d treatment with GS did not lead to a significant decrease in these elevated lipid levels under diabetes. Similarly, GS treatment did not lead to

Table 1. Biochemical Analysis

<table>
<thead>
<tr>
<th>Item</th>
<th>Normal rats ($n=5$)</th>
<th>Diabetic rats ($n=8$)</th>
<th>Control ($n=8$)</th>
<th>GS 20 mg/kg/d ($n=8$)</th>
<th>GS 100 mg/kg/d ($n=8$)</th>
</tr>
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<tr>
<td>Kidney weight, mg/100 g BW</td>
<td>669±19</td>
<td>1079±14**</td>
<td>998±32**</td>
<td>994±18**</td>
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<tr>
<td>Ccr, ml/kg BW/min</td>
<td>7.66±0.64</td>
<td>8.16±0.24</td>
<td>7.63±0.50</td>
<td>7.39±0.35</td>
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<tr>
<td>Urine volume, ml/d</td>
<td>15±3</td>
<td>127±7**</td>
<td>111±7**</td>
<td>108±4**</td>
<td></td>
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<tr>
<td>Urinary protein, mg/d</td>
<td>20.1±2.0</td>
<td>31.6±2.0**</td>
<td>21.2±2.3</td>
<td>19.8±2.4</td>
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<tr>
<td>Serum Cr, mg/dl</td>
<td>0.348±0.015</td>
<td>0.381±0.008</td>
<td>0.329±0.015</td>
<td>0.293±0.014**</td>
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<tr>
<td>Serum albumin, g/dl</td>
<td>3.50±0.05</td>
<td>2.93±0.03**</td>
<td>3.13±0.06**</td>
<td>3.24±0.08**</td>
<td></td>
</tr>
<tr>
<td>Serum total protein, g/dl</td>
<td>5.25±0.08</td>
<td>4.58±0.11**</td>
<td>4.74±0.07**</td>
<td>4.70±0.08**</td>
<td></td>
</tr>
<tr>
<td>Serum total cholesterol, mg/dl</td>
<td>49±3</td>
<td>121±8**</td>
<td>106±10**</td>
<td>103±9**</td>
<td></td>
</tr>
<tr>
<td>Serum triglyceride, mg/dl</td>
<td>92±9</td>
<td>429±45*</td>
<td>335±50*</td>
<td>272±78</td>
<td></td>
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<tr>
<td>Water intake, ml/d</td>
<td>39±5</td>
<td>159±10**</td>
<td>143±8**</td>
<td>141±6**</td>
<td></td>
</tr>
<tr>
<td>Food intake, g/d</td>
<td>19.1±0.5</td>
<td>32.3±0.5**</td>
<td>31.8±0.6**</td>
<td>30.3±0.8**</td>
<td></td>
</tr>
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</table>

Data are expressed as the mean±S.E.M. *$p<0.05$, **$p<0.01$ compared with normal rats; *$p<0.01$ compared with diabetic control rats.
significant differences in elevated water and food intakes of diabetic control compared to normal rats.

**TBARS and HO-1 Protein Expression** The effects of GS on TBARS levels were evaluated in serum and renal mitochondria. As shown in Fig. 2C, the serum TBARS level of diabetic control rats was significantly elevated from that of normal (2.2-times) and the oral administration of GS at 20 and 100 mg significantly inhibited this increase compared to diabetic control group ($p<0.01$). The renal mitochondrial TBARS level was also significantly increased in diabetic control rats ($1.98 \pm 0.07 \text{ nmol/mg protein}$) compared with normal rats ($1.56 \pm 0.06 \text{ nmol/mg protein}$), but levels in groups treated with GS were significantly reduced to the normal level ($1.53 \pm 0.09$ and $1.48 \pm 0.07 \text{ nmol/mg protein}$, respectively, $p<0.01$) (Fig. 2D).

HO-1 protein expression in the renal cortex on day 20 was considerably higher in untreated diabetic rats than normal rats, but both 20 and 100 mg GS-treated rats showed markedly reduced expressions than those of control rats, respectively (Fig. 2E).

**Renal Glucose, Intracellular Glycation, and AGE Levels** At the end of the experiment, the renal glucose level in diabetic control rats ($6.47 \text{ mg/g wet tissue}$) was significantly increased from normal rats ($0.68 \text{ mg/g wet tissue}$). However, 20 and 100 mg of GS treatment led to significant decrease by 4.99 and 4.54 mg/g wet tissue, respectively (Fig. 3A), which effect was similar to serum glucose level (Fig. 2B). Additionally, diabetic control rats showed an increase in the fluorescent AGE level of about 1.5 times compared with that of normal rats ($p<0.01$). However, in rats treated with GS for 20 d, it was slightly decreased in the 20 mg-treated group and significantly decreased in the 100 mg-treated group ($p<0.01$) (Fig. 2B).

We also measured renal MG and GA levels using enzymatic principles. As shown in Fig. 3C, the renal glucose level in diabetic control rats showed 1.4-times higher renal MG level than normal rats ($p<0.05$), but there was no marked increase in the renal GA level (Fig. 3D). However, significant decreases in the renal MG level were observed in both 20 and 100 mg-treated groups ($p<0.05$, respectively), and the GA level was also decreased to the normal value in the 100 mg-treated group.

To elucidate the expression levels of AGE-related proteins, we performed immunoblotting analyses in the renal cortex, and the results are presented in Fig. 3E. Diabetic rats showed up-regulated protein expression of RAGE and increased accumulations of CML, CEL, and GA-pyridine, with only GA-pyridine not reaching significance. In contrast, oral administration of GS successfully reduced the CML level in the 100 mg-group. The levels of RAGE, CEL, and GA-pyridine likewise were down-regulated as the dose increased.

**DISCUSSION**

Polyphenols are the most abundant dietary antioxidants, being common constituents of fruits, vegetables, cereals, nuts, seeds, chocolate, and beverages, such as tea, coffee, or wine. They have been postulated to lead to many health benefits, such as the prevention of cancer, neurodegenerative diseases, cardiovascular diseases, and diabetes as well as exhibit anti aging effects, because oxidative stress is be-
lieved to influence many acute and chronic diseases. In our previous study, we demonstrated that (−)-epigallocatechin 3-O-gallate (EGCG), a major green tea polyphenol, could alleviate renal damage caused by abnormal glucose metabolism-associated oxidative stress involved in renal lesions of diabetic nephropathy. To add to these findings, we screened one low-molecular-weight polyphenol, GS, as a constituent of Cornus officinalis, and showed its beneficial effect on the early stage of the diabetic kidney; however, our is the sole research on its biological activities to date.

Intensive hyperglycemic control in patients with type 1 diabetes, which was performed by The Diabetes Control and Complications Trial Research Group, has been emphasized to delay the onset and slow the progression of diabetic complications such as retinopathy, neuropathy, and nephropathy. Importantly, in our experimental protocol, 20-d GS treatment of STZ-induced diabetic rats led to a significant amelioration of the hyperglycemic state (p < 0.01). Additionally, serum and renal mitochondrial TBARS levels were markedly reduced to normal values, although there were no significant reductions in serum lipid levels. Moreover, overexpression of HO-1 in the diabetic renal cortex was significantly decreased following treatment. In fact, many researchers have accumulated extensive evidence concerning diabetic oxidative stress. In particular, reliable data were obtained by Nishikawa et al., showing that the TCA cycle was the source of increased ROS-generating substrates induced by hyperglycemia, and that normalizing levels of mitochondrial reactive oxygen species could prevent diabetic pathological changes. On the other hand, research has been focused on the beneficial role of HO-1, a rate-limiting enzyme in heme catabolism and heat shock protein 32, involved in protecting a variety of tissues from oxidative stress and inflammatory injury in experimental diabetes. Particularly, it was reported that renal glomerular oxidative stress accelerated in the early stage of diabetes was closely related to mRNA and protein overexpression of HO-1, though there was no significant increase in antioxidant enzymes such as catalase and Cu/Zn-superoxide dismutase, and the overexpression of HO-1 was almost completely normalized by treatment with insulin, vitamin E, or probucol. In this way, the results of the present study successfully demonstrate the beneficial effects of the antioxidant property of GS in the diabetic kidney, represented by the results of renal mitochondrial TBARS and renal HO-1 expression levels.

Increased oxidative stress is also involved in renal structural changes due to oxidative substances affecting glomerular endothelial cells directly, infiltrating into the mesangial area, tubulointerstitial area, and other parts of renal tissue, because of the abundant blood flow in the diabetic kidney. There is no doubt that microalbuminuria is an important indicator of the early stage of diabetic nephropathy. That is, glomerular damage, i.e., changes in the size and charge barrier, or hyperfiltration increases the albumin filtration rate, but proximal tubular reabsorption of this increased albumin is decreased via a decline in its endocytosis due to a loss of megalin expression. This megalin, known as gp330, is part of the LDL receptor family, and participates in the endocytosis of albumin in the proximal convoluted tubule. The results of our experiment presented here demonstrate that diabetic rats showed a significant increase in the urinary protein excretion rate, as well as declines in serum albumin and total protein levels, although there was no change in the Ccr level as a parameter of the glomerular filtration rate. Thus, this could have led to the absence of an increase in the albumin filtration rate; however, protein leakage caused by glomerular damage in diabetic rats was noted in our study, suggesting that this experiment was performed in the early stage of diabetic nephropathy. However, the oral administration of GS for 20 d successfully normalized the urinary protein excretion rate and significantly ameliorated the decreased serum albumin level, indicating that GS has a protective effect against renal injury in the early stage of diabetes.

Since the administration of GS had a marked affect on glucose and glucose-associated oxidative stress rather than on lipids, as shown in the serum and renal constituents, we subsequently focused on intracellular glycation and AGE formation in kidney tissue. AGE formation, one of the metabolic disorders caused by hyperglycemia, has been focused on as a marker of long-term glycemic control in body tissues. These products have also been strongly implicated in the pathogenesis of diabetic micro- and macrovascular diseases, because AGE-modified proteins can stimulate a variety of cellular responses via a specific cell surface receptor such as RAGE, resulting in the expression and activation of pathogenic mediators, e.g., extracellular matrix, oxidative stress, cytokines, and growth factors. Up to now, several glycation products have been chemically and structurally defined, and, from these, we aimed at elucidating the effect of GS on diabetic glycation products, and these biochemical relationships are summarized in Chart 1.

During diabetes, there is an increased intracellular accumulation of dicarbonyl compounds such as MG, which is well-known to be a highly reactive α-oxoaldehyde formed from triose phosphate during glycolysis. In addition, evidence suggests that MG generated from the Maillard reaction plays an important role in CEL formation. That is, CEL formation during the Maillard reaction was inhibited by aminoguanidine but enhanced by an increasing concentration of phosphate, an enhancer of MG production during the Maillard reaction, while glucosone and 3-deoxyglucosone-modified BSA did not contribute to the formation of CEL. On the other hand, aldehydes such as GA, which is also a Maillard reaction intermediate, can be formed by the hypochlorous acid (HOCl)-serine system via myeloperoxidase (MPO). It was confirmed that GA reacts with proteins to form GA-pyridine and CML in human atherosclerotic lesions and these accumulations were also present in diseased kidneys, showing a positive correlation with the
severity of mesangial matrix expansion and focal glomerulosclerosis.\(^\text{39}\) Furthermore, mitochondrial proteins are prone to posttranslational modifications resulting from glycation and oxidation; especially, MG-induced modifications are targeted to key components of major mitochondrial cycles, such as oxidative phosphorylation and \(\beta\)-oxidation.\(^\text{40}\) In fact, CML is also regarded as a general biomarker of oxidative stress due to it undergoing metal-catalyzed oxidation of polyunsaturated fatty acids in the presence of protein during formation.\(^\text{41}\)

In this study, diabetic rats showed significant increases in glucose, fluorescent AGEs, CML, MG, CEL, and RAGE, which is the best-characterized receptor for AGEs composed of multiple members of the immunoglobulin superfamily,\(^\text{42}\) while they showed only slight increases in GA and GA-pyridine. On the other hand, as administration decreased these increased parameters except for CEL, showing only a tendency to decrease the level, as described in Fig. 3. According to a previous report, Hsu \textit{et al.}\(^\text{43}\) demonstrated that gallic acid could induce apoptotic cell death in 3T3-L1 preadipocytes in a similar way to EGCg,\(^\text{44}\) which results in cell population growth being correlated with antioxidant activity. Additionally, Rosso \textit{et al.}\(^\text{45}\) reported that gallic acid \(n\)-alquil esters have a protective effect on targets of HOCl, a powerful oxidant, through the inhibition of its productive enzyme, MPO activity, and HOCl scavenging. We have previously examined oxidative stress in the STZ treated rats using high and low blood glucose rats. The reduced glucose levels had closely correlated with lipid peroxidation levels in serum and kidney.\(^\text{46}\) Taking these results into consideration, we suggested that GS has effects on glucose reduction and glucose-associated metabolic abnormalities as the target points, especially Maillard reaction-induced CML, \textit{via} the marked inhibition of mitochondrial lipid peroxidation. Hence, the role of GS in maintaining mitochondrial function must be elucidated.

In conclusion, our present study is the first report that GS, only detected from \textit{Cornus officinalis}, has beneficial effects on renal metabolic abnormalities, including renal glucose, AGE formation, and oxidative stress, which are considered to play important roles in the development of diabetic kidney disease. Moreover, these effects were similarly observed at both 20 and 100 mg doses, although GS did not show any toxicity. Therefore, further clarification of the biological activities of GS is expected to provide a novel therapeutic strategy against the development of diabetic nephropathy.

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