Caffeoylated Phenylpropanoid Glycosides from Brandisia hancei Inhibit Advanced Glycation End Product Formation and Aldose Reductase in Vitro and Vessel Dilation in Larval Zebrafish in Vivo

Song Yi Yu1*, Ik-Soo Lee1*, Seung-Hyun Jung1, Yun Mi Lee1, Yu-Ri Lee1, Joo-Hwan Kim2, Hang Sun3, Jin Sook Kim1

Abstract

In our continuing efforts to identify effective naturally sourced agents for diabetic complications, five caffeoylated phenylpropanoid glycosides, acteoside (1), isoacteoside (2), poliumoside (3), brandioside (4), and pheliposide (5) were isolated from the 80% EtOH extract of Brandisia hancei stems and leaves. These isolates (1–5) were subjected to an in vitro bioassay evaluating their inhibitory activity on advanced glycation end product formation and rat lens aldose reductase activity. All tested compounds exhibited significant inhibition of advanced glycation end product formation with IC₅₀ values of 4.6–25.7 µM, compared with those of aminoguanidine (IC₅₀ = 1056 µM) and quercetin (IC₅₀ = 28.4 µM) as positive controls. In the rat lens aldose reductase assay, acteoside, isoacteoside, and poliumoside exhibited greater inhibitory effects on rat lens aldose reductase with IC₅₀ values of 0.83, 0.83, and 0.85 µM, respectively, than those of the positive controls, 3,3-tetramethyleneglutaric acid (IC₅₀ = 4.03 µM) and quercetin (IC₅₀ = 7.2 µM). In addition, the effect of acteoside on the dilation of hyaloid-retinal vessels induced by high glucose in larval zebrafish was investigated. Acteoside reduced the diameters of high glucose-induced hyaloid-retinal vessels by 69% at 10 µM and 81% at 20 µM, compared to the high glucose-treated control group. These results suggest that B. hancei and its active components might be beneficial in the treatment and prevention of diabetic vascular complications.

Abbreviations

AGE: advanced glycation end product
AG: aminoguanidine
ANOVA: one-way analysis of variance
AR: aldose reductase
EtOH: ethanol
HG: high glucose
SEM: standard error of the mean
TMG: 3,3-tetramethyleneglutaric acid
VEGFR: vascular endothelial growth factor receptor

Introduction

Diabetic vascular complications are the leading cause of end-stage renal failure, acquired blindness, various neuropathies, and accelerated atherosclerosis. Chronic hyperglycemia plays a major role in the initiation of diabetic vascular complications through various hyperglycemia-induced metabolic and hemodynamic derangements, including increased AGE formation, increased polyol pathway metabolism, activation of protein kinase C isomers, and increased hexosamine biosynthesis [1–4].

Accumulation of AGE, heterogeneous molecules derived from non-enzymatic glycation between amino acid residues and oxidative derivatives of glucose or pentose, leads to the development of diabetic vascular complications, including diabetic retinopathy, neuropathy, and nephropathy [5]. Increasing evidence suggests that AGE accumulation is irreversible, causing structural and functional changes in proteins, such as collagen, elastin, and albumin. AR (aldose reductase; aldotol:: - NADP⁺ 1-oxidoreductase, E.C.1.1.1.21) is the key enzyme in the polyol pathway that catalyzes NADH-dependent reduction of glucose to sorbitol. During hyperglycemic events, elevated glucose levels enhance AR activity by increasing glucose flux through the polyol pathway, inducing
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Results and Discussion

The 80% EtOH extract of *B. hancei* stems and leaves that significantly inhibited AGE formation (IC50 = 3.19 µg/mL) and RLAR (IC50 = 3.27 µg/mL) was subjected to a series of chromatographic techniques. This led to the isolation of five caffeoylated phenylpropanoid glycosides (1–5) (Fig. 1). By comparing their physicochemical and spectral data with those in the literature, these isolates were identified as acteoside (1), isoacteoside (2), poliurosido (3), brandioside (4), and pheliposide (5) [11, 15, 16]. The inhibitory effects of the isolated compounds (1–5) on AGE formation and RLAR in vitro were examined as described previously [17] and results are presented in Table 1. All tested compounds markedly inhibited AGE formation, with IC50 values of 4.6–25.7 µM, compared with those of the positive controls AG (IC50 = 1056 µM), the first AGE inhibitor for the treatment of diabetic nephropathy [18], and quercetin (IC50 = 28.4 µM), a commercially available natural compound. Of the tested compounds, acteoside and pheliposide exhibited the strongest AGE-inhibitory activity, with IC50 values of 5.1 and 4.6 µM, respectively. In the RLAR assay, acteoside, brandioside, and pheliposide, exhibited greater inhibitory effects on RLAR with IC50 values of 0.83, 0.83, and 0.85 µM, respectively, than those of the positive controls, TMG (IC50 = 4.03 µM) and quercetin (IC50 = 7.2 µM), while isoacteoside and poliurosido exhibited less potent RLAR inhibitory effects. Acteoside has previously been isolated from the aerial parts of *Monochasma savatieri* with potent AR inhibitory activity [19]. However, this is the first report of other caffeoylated phenylpropanoid glycosides possessing inhibitory activity on AGE formation and RLAR.

The accumulation of AGE in retinal blood vessels plays an important role in the onset and development of diabetic retinopathy [20]. Experimental studies have shown that specific inhibitors of AGE formation are effective in reducing retinal microvascular lesions in diabetic animal models [21, 22]. Recently, it was reported

<table>
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<tr>
<th>Compounds</th>
<th>Inhibitory effect (IC50, µM)*</th>
<th>RLAR</th>
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<tr>
<td>Acteoside (1)</td>
<td>5.11 ± 0.17</td>
<td>0.83 ± 0.02</td>
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<tr>
<td>Isoacteoside (2)</td>
<td>25.69 ± 1.43</td>
<td>26.23 ± 1.41</td>
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<tr>
<td>Poliurosido (3)</td>
<td>19.69 ± 0.18</td>
<td>8.47 ± 0.05</td>
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<tr>
<td>Brandioside (4)</td>
<td>12.04 ± 1.05</td>
<td>0.83 ± 0.04</td>
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<tr>
<td>Pheliposide (5)</td>
<td>4.63 ± 0.21</td>
<td>0.85 ± 0.02</td>
</tr>
<tr>
<td>Quercetin*</td>
<td>28.41 ± 0.21</td>
<td>7.21 ± 0.16</td>
</tr>
<tr>
<td>AG*</td>
<td>1056.47 ± 57.25</td>
<td>–</td>
</tr>
<tr>
<td>TMG*</td>
<td>–</td>
<td>4.03 ± 0.02</td>
</tr>
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*Results are expressed as means ± SD (n = 3); IC50 indicates the concentration (µM) at which the inhibition percentage of the AGE formation or RLAR was 50%, and the values were determined by regression analysis; † After incubating for 14 days, the fluorescent reaction products were assayed on a spectrofluorometric detector; ‡ Quercetin, aminoguanidine (AG), and 3,3-tetramethyleneglutaric acid (TMG) were used as positive controls.
that hyperglycemia-induced adult zebrafish have an increased vessel diameter in the retina [23]. Thus, acteoside, which exhibited a potent AGE formation inhibitory activity and was isolated in a large amount, was chosen for further investigation to examine the effect on retinopathy in vivo in a diabetic zebrafish model. The change in hyaloid-retinal vessel dilation was assessed with flk:EGFP transgenic zebrafish embryos under HG (30 mM) conditions. The dilation of hyaloid-retinal vessels in larval zebrafish induced by HG was reduced by treatment with acteoside. HG-induced hyaloid-retinal vessels treated with acteoside and VEGFR inhibitor were visibly thinner than those of the HG-treated control group (Fig. 2B–E), and no cytotoxicity was observed at the concentrations used. Quantitative analysis showed that acteoside reduced the diameter of HG-induced hyaloid-retinal vessels by 69% at 10 µM and 81% at 20 µM compared to the HG-treated control group, whereas the positive control, VEGFR inhibitor, exhibited 77% inhibition at a concentration of 1 µM (Fig. 2F–G). This suggests that acteoside inhibits the development of experimental retinopathy in the early larval stages. Development and investigation of AGE formation and AR inhibitors, particularly naturally sourced agents with few side effects, might provide a novel therapeutic approach for delaying and preventing diabetes-related complications. Of the many naturally occurring compounds previously found to inhibit AGE formation or AR, the most significant are flavonoids, such as quercetin, quercitrin, isoquercitrin, and luteolin [24, 25]. To date, more than 100 flavonoids have been reported to exhibit inhibitory activity on AGE formation or AR. The other major classes of compounds found in natural products are polyphenolics, anthraquinones, alkaloids, and monoterpenoid glycosides [26–29]. Phenylpropanoids and their derivatives, a large conglomerate of naturally occurring phenolic compounds, are widespread throughout the plant kingdom, and various plants used in traditional medicine contain significant amounts of these compounds. They have low toxicity and a variety of pharmacological activities including antitumor, antibacterial, antiviral, analgesic, neuroprotective, hepatoprotective, anti-inflammatory, and antioxidant effects [30–32], which makes them promising novel candidate drugs of natural origin. The current study of effective agents for diabetic complications from the stems and leaves of B. hancei resulted in the isolation of five caffeoylated phenylpropanoid glycosides (1–5): acteoside (1), isoacetoside (2), poliumoside (3), brandioside (4), and pheliposide (5). These isolates (1–5) displayed considerable inhibition of AGE formation; acteoside and pheliposide exhibited the strongest AGE inhibition, while in the RLAR assay, acteoside, isoacetoside, and poliumoside exhibited greater inhibitory effects. Acteoside also reduced the dilation of HG-induced hyaloid-retinal vessels in a diabetic zebrafish model. These results suggest that B. hancei and its active components might be beneficial in the treatment and prevention of diabetic vascular complications and other related diseases.

Materials and Methods

General experimental procedures
Optical rotations were measured on a JASCO P-2000 digital polarimeter. IR spectra were recorded on a JASCO 100 IR spectrometer. 1H (300 MHz) and 13C NMR (75 MHz) spectra were obtained using a Bruker DRX-300 spectrometer with TMS as an internal standard. 2D-NMR experiments (COSY, HMOC, and HMBOC) were run on a Bruker Avance 500 NMR spectrometer. ESI-MS were recorded on a Shimadzu LCMS-IT-TOF spectrometer. Column chromatography was performed using silica gel (70–230 mesh and 230–400 mesh; Merck), YMC-gel ODS-A (5–75 µm; YMC), and Sephadex LH-20 (Amersham Pharmacia Biotech). Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F254 (0.25 mm; Merck) and RP-18 F254a plates (0.25 mm; Merck). Spots were detected by UV light (254 nm) and spraying with 10% H2SO4 followed by heating. The HPLC analysis was performed on an Agilent 1200 HPLC system with a binary pump (G1312A), a vacuum degasser (G1322A), a thermostatted column compartment (G1365B; MWD), and an autosampler (G1329A), using a YMC-pack Pro C18 (5–5 µm, 150 × 4.6 mm i.d.) column.

Plant material
The stems and leaves of Brandisia hancei were collected in Songying, Yunnan, China, in October 2008, and identified by Prof. J.-H. Kim, Gachon University, Republic of Korea. A voucher specimen (DiAB-2008–085) has been deposited in the Herbarium of the Diabetic Complications Research Team, Korea Institute of Oriental Medicine, Republic of Korea.
Extraction and isolation

The air-dried stems and leaves of B. hancei (3.0 kg) were extracted with 80% aqueous EtOH (each 301, three times) at room temperature for 7 days, filtered, and concentrated to give an 80% EtOH extract (332 g). The extract (90 g) was subjected to silica gel column chromatography (70–230 mesh, 100 × 9.5 cm) eluted with a gradient solvent system consisting of CHCl₃/MeOH (10:1); 1:1; 1:2; 1:3; 1:4; 1:5; 1:6; 1:7; 2:1; 3:1; 4:1; 5:1; 6:1; 7:1; 8:1, each 2 L) to yield six fractions [A (2 L, 0.2 g); B (2 L, 0.3 g); C (2 L, 0.5 g); D (2 L, 0.7 g); E (2 L, 1.0 g); F (4 L, 1.4 g)]. Fraction D (0.8 g) was further separated on a Sephadex LH-20 column (60 × 4.5 cm) eluted with an EtOH/H₂O gradient (1:2, 1:1, 2:1, 1:0, each 2 L) to obtain compound 1 (430 mg). Chromatography of fraction D (1.0 g) on a silica gel column (70–230 mesh, 60 × 5.5 cm) eluted with a stepwise gradient of EtOAc/H₂O (1:1, 2:1, 1:0, each 2 L) yielded five fractions [E1 (2 L, 1.0 g); E2 (2 L, 1.5 g); E3 (2 L, 1.8 g); E4 (2 L, 2.8 g); E5 (4 L, 6.0 g)]. Fraction E2 (2.5 g) was further separated on a Sephadex LH-20 column (60 × 3.5 cm) eluted with MeOH (2 L) to yield six fractions [E2.1 (0.5 L, 0.8 g); E2.2 (1 L, 0.7 g); E2.3 (0.5 L, 0.8 g)]. Subfraction E2.2 (0.7 g) was further separated on a Sephadex LH-20 column (35 × 2.0 cm) eluted with an MeOH/H₂O gradient (1:1, 2:1, 1:0, each 0.5 L) to obtain compound 2 (9.3 mg). Chromatography of fraction E4 (2.8 g) on a Sephadex LH-20 column (60 × 3.5 cm) eluted with an MeOH/H₂O gradient (1:2, 1:1, 2:1, 1:0, each 0.5 L) yielded four subfractions [E4.1 (0.5 L, 0.5 g); E4.2 (0.5 L, 0.7 g); E4.3 (0.5 L, 0.6 g); E4.4 (0.5 L, 0.7 g)]. Subfraction E4.2 (0.7 g) was further purified by MPLC [Combiflash RF 200 system; RediSep RF (No. 7008541) column; MeOH/H₂O (1:1); UV detection, 254 nm; flow rate, 50 mL/min] to yield compounds 3 (260 mg) and 4 (21 mg). Compound 5 (307 mg) was isolated from fraction E4.3 (0.6 g) using a YM-MC 18 column (46 × 1.8 cm) eluted with an MeOH/H₂O gradient (1:2, 1:1, 2:1, 1:0, each 0.5 L). The purity of the isolated compounds ranged from 97.0% to 99.5% as assessed by analytical HPLC [Agilent 1200 HPLC system; YMC-pack Pro C18 (5-5 µm, 150 × 4.6 mm i.d.) column; MeOH/H₂O (35:45); UV detection, 254, 290 nm; flow rate, 1.0 mL/min].

AGE formation inhibitory assay

According to an established method, the reaction mixture [bovine serum albumin (10 mg/mL; Sigma; 700 µL) in 50 mM phosphate buffer (pH 7.4) with 0.02% sodium azide] was added to 0.2 M fructose and glucose (100 µL). In screw cap tubes (1.5 mL), the reaction mixture was then mixed with 200 µL of serially diluted compounds or aminoguanidine (99% purity; Sigma) or quercetin (99.5% purity; Sigma). After incubating at 37°C for 7 days, the fluorescent reaction products (200 µL) were transferred to 96-well plates and assayed on a spectrofluorometric detector (Bio-Tek, Synergy HT; excitation wavelength, 350 nm; emission wavelength, 450 nm). The AGE assay was performed in triplicate. Vascular endothelial growth factor receptor inhibitor (99.4% purity; Calbiochem) was used as a positive control.

RLAR inhibitory assay

All experimental protocols for animal care and use were approved by the Korea Institute of Oriental Medicine Institutional Animal Care and Use Committee (approval date and number; March 19, 2012 and 12–020, respectively), and animal husbandry and procedures were performed according to institutional guidelines. Adult zebrafish were maintained under standard conditions at 28.5°C with a 14-h light/10-h dark cycle. Embryos were obtained from crosses between flk:EGFP Tg (transgenic) fish and or quercetin (99.5% purity; Sigma) treated with compound 1 (0.2 mM) days 1–6 post-fertilization (dpf). At 6 dpf, HG-induced embryos were fixed with 4% paraformaldehyde, and the lenses containing hyaloid-retinal vessels were isolated. Fluorescence was visualized using an Olympus SZX16 stereomicroscope, and diameters were analyzed using ImageJ software. Experiments were performed in triplicate. Vascular endothelial growth factor receptor inhibitor (99.4% purity; Calbiochem) was used as a positive control.

Measurement of vessel dilation in larval zebrafish

All experimental protocols for animal care and use were approved by the Korea Institute of Oriental Medicine Institutional Animal Care and Use Committee (approval date and number; March 19, 2012 and 12–020, respectively), and animal husbandry and procedures were performed according to institutional guidelines. Adult zebrafish were maintained under standard conditions at 28.5°C with a 14-h light/10-h dark cycle. Embryos were obtained from crosses between flk:EGFP Tg (transgenic) fish and or quercetin (99.5% purity; Sigma) treated with compound 1 (0.2 mM) days 1–6 post-fertilization (dpf). At 6 dpf, HG-induced embryos were fixed with 4% paraformaldehyde, and the lenses containing hyaloid-retinal vessels were isolated. Fluorescence was visualized using an Olympus SZX16 stereomicroscope, and diameters were analyzed using ImageJ software. Experiments were performed in triplicate. Vascular endothelial growth factor receptor inhibitor (99.4% purity; Calbiochem) was used as a positive control.

Statistical analysis

Hyaloid vessel diameter was used to calculate the percentage inhibition of compound 1 in HG-treated embryos according to the formula:

Percentage inhibition (%)) = 100 – [(T – C)/(HG – C)] × 100,

where C = hyaloid-vessel diameter (AU) of the control embryos; HG = hyaloid-vessel diameter (AU) of the HG-treated embryos; T = hyaloid-vessel diameter (AU) of the HG-treated embryos treated with compound 1. The results are expressed as means ± SEM from multiple experiments. Statistical significance was assessed using ANOVA and Dunnett’s multiple comparison tests with the GraphPad 5.0 Prism software (GraphPad).
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Conflict of Interest

No conflict of interest exists.

References