SUPPLEMENTARY MATERIAL

Inhibition of resveratrol glucosides (REs) on advanced glycation endproducts (AGEs) formation: inhibitory mechanism and structure-activity relationship

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Abstract

The study on inhibitory effects of resveratrol glucosides (REs) on advanced glycation endproducts (AGEs) formation is still unmet. Herein, for the first time, the antiglycation activities of five REs in the fetal bovine serum proteins (FBS)/fructose system were evaluated, and its structure-activity relationship and antiglycation mechanism were further explored. These REs showed remarkable inhibition toward AGEs formation. Among them, Piceatannol-3'-O-glucoside (PG) exhibited highest antiglycation activity as reflected in approximately 80% inhibition of fluorescent AGEs at the concentration of 1.0 mM. The structure-activity relationship analysis indicated that glucoside attached to the B ring of resveratrol displays a superior antiglycation activity. Moreover, the results of antiglycation mechanism showed that the antiglycation activity of REs was proportional to their antioxidant capacity and methylglyoxal (MGO) trapping capacity. Therefore, the REs are promising candidates worthy of further exploration for preventing AGEs accumulation in vivo, thereby treating AGEs-associated diseases.

Keywords Resveratrol glucosides (REs); Advanced glycation endproducts (AGEs); Structure-activity relationship; Antiglycation mechanism
Experimental

Apparatus and reagents

The fluorescence spectra were recorded on a HITACHI F-7000 fluorometer (Tokyo, Japan). UV-vis spectra were recorded on UV-2450 UV-vis spectrophotometer (Shimadzu, Kyoto, Japan). Seven stilbenoids were studied: Resveratrol (>98.0%), Isorhapontigenin (>98.0%), (E)-Polydatin (>99.0%), Resveratroloside (>98.0%), Piceatannol-3’-O-glucoside (>98.0%), 2,3,5,4’-Tetrahydroxystilbene-2-O-β-D-glucoside (THSG, >98.0%), Astringin (>97.0%) were all purchased from Yuanye Bio-Technology Co., Ltd (Shanghai, China). Structures of seven stilbenoids tested in this study are shown in Figure S4.

Fetal bovine serum (FBS) was obtained from Sigma. The working solution of FBS (10 μM) was prepared by directly diluting the above FBS with 100 mM phosphate buffer solution before use. The working solutions of stilbenoids (1.0 mM) were prepared by dissolving each stilbenoid with methanol.

1, 1-diphenyl-2-picrylhydrazyl (Aladin Co. Ltd, Shanghai, China) was employed to determine the antioxidant activity of stilbenoids. Buffer solution used all over the experiments was the 100 mM phosphate buffer solution with pH at 7.4. Ultrapure water was purified by a Milli-Q system (Millipore, Bedford, MA, USA). All other reagents and solvents were of analytical grade and purchased from Chemical Reagent Factory of Hunan Normal University (Changsha, Hunan, China).

Measurement of AGE-inhibitory activity

The fructose-derived AGEs were prepared according to the previous method with some modifications (Sompong et al., 2015; Takeuchi et al., 2010). The FBS (10 μM) solutions was incubated with 50 mM fructose in 100 mM phosphate buffer saline (PBS), pH 7.4 at 37 °C for 10 days. In addition, the resveratrol and its derivatives were added to the glycation model in the concentration range of 0-10 mM, and aminoguanidine (AG) was used as the positive control. After incubation, the fluorescent intensity was measured to assess fructose-derived AGE formation via using the fluorometer at the excitation wavelength of 330 nm and emission wavelength of 410 nm, respectively. The percentage of inhibition of fructose-derived
AGEs formation was calculated by the following formula:

\[
\text{Inhibition rate (\%)} = \left[ \frac{(F_C - F_{CB}) - (F_S - F_{CB})}{F_C - F_{CB}} \right] \times 100\% \\
\]

where \(F_C\) and \(F_{CB}\) are the fluorescent intensity of control with and without fructose, \(F_S\) is the fluorescent intensity of the sample with fructose.

**Measurement of antioxidant capacity**

Antioxidant activities of these REs were measured on the basis of the scavenging 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical and 2, 20-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) ABTS free radical.

**DPPH free radical scavenging activity**

The DPPH free radical scavenging activity of the REs was measured according to literatures with slight modifications (Cao et al., 2015; Ren et al., 2013). The samples with different volumes were added to 500 \(\mu\)L of DPPH solution (1.0 mM in 50% ethanol) and diluted with 50% methanol to 1.0 mL. Following incubation in the dark for 30 min, the absorbance at 517 nm was measured. The REs were replaced by methanol in the control group. The blank group consisted of 750 \(\mu\)L methanol and 250 \(\mu\)L of Millipore water. The DPPH free radical scavenging potential was calculated using the following formula:

\[
\text{DPPH free radical scavenging activity (\%)} = \left(1 - \frac{A_1}{A_0} \right) \times 100\% \\
\]

where \(A_0\) is the absorbance of the control, and \(A_1\) is the absorbance of samples. Each sample was tested three times (\(n=3\)). The absorbance was found to be reproducible within experimental errors.

**ABTS assay**

The ABTS assay described by Re et al (1999) was used with minor modifications. The ABTS assay assesses the total radical scavenging capacity based on the ability of an antioxidant to scavenge the stable ABTS radical cation (ABTS\(^{+}\)), which was produced by mixing 4.0 mL ABTS stock solution (7.0 mM) with 4.0 mL potassium persulphate (2.45 mM) and allowing to stand in the dark at room temperature for 12-16 h before use. The ABTS\(^{+}\) stock solution was melted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm. The samples with different volumes were
added to 500 μL of ABTS⁺ diluted solution and diluted with 50% methanol to 1.0 mL. Following kept in the dark at room temperature for 5 min, the absorbance was spectrophotometrically determined at 734 nm. The REs were replaced by methanol in the control group. The blank group consisted of 750 μL methanol and 250 μL of Millipore water. The ABTS⁺ scavenging activity was calculated using the following formula:

\[
\text{ABTS radical scavenging activity (\%)} = (1 - \frac{A_1}{A_0}) \times 100\%
\]

where \(A_0\) is the absorbance of the control, and \(A_1\) is the absorbance of sample. Each sample was tested three times (\(n = 3\)). The absorbance was found to be reproducible within experimental errors.

**Evaluation of methylglyoxal (MGO) trapping ability**

The ability of trapping MGO was tested according to the approach described by Peng et al. (2008). MGO (0.25 mL, 1.0 mM) was incubated with 0.25 mL PBS (blank), 1 mM aminoguanidine (AG) in PBS (positive control) and 1.0 mM REs in PBS at 37 °C for 3 h respectively. \(O\)-phenylenediamine (OPD) was used as derivatizing agent, and 5-methylquinoxaline (5-MQ) as the internal standard. After derivatization, MGO was converted to 2-methylquinoxaline (2-MQ) at a UV absorbance of 315 nm. The UPLC analysis was carried out on a reversed-phase Syncronis C18 column (100 mm × 2.1 mm i.d, 1.7 μm, Thermo Fisher Scientific, USA) and a Dionex Ultimate 3000 UPLC instrument. The mobile phase was composed of (A) 0.5% (v/v) acetic acid in water and (B) methanol using an isocratic elution of 60% B. The flow rate was 0.3 mL/min, and the column temperature was maintained at 25 °C. The sample injection volume was 10 μL. The residual MGO after capture reaction was quantified by the ratio of peak area of 2-MQ over 5-MQ and percentage reduction of MGO was calculated as 100 * (quantity of MGO in blank - quantity of MGO in samples with REs/ quantity of MGO in blank - quantity of MGO in AG).

**References**

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Figure S1. Antioxidant capacity of resveratrol and its derivatives. (A: DPPH, the concentration of test samples is 20 μM; B: ABTS, the concentration of test samples is 4.0 μM). Data are represented as means ± SD (n= 3). All differences found are significant at P < 0.05.

Figure S2. MGO Trapping capacity of aminoguanidine (AG), resveratrol and its derivatives. The concentration of test samples is 1.0 mM, and AG was used as positive control in this assay. Data are represented as means ± SD (n= 3). All differences found are significant at P < 0.05.
**Figure S3.** Inhibitory activity of aminoguanidine (AG), resveratrol and its derivatives on total fluorescent AGEs formation. FBS (10 µM) was incubated with fructose (50 mM) in 100 mM phosphate buffer saline (pH 7.4) at 37 °C for 10 days in the absence (control) and presence of each sample (1.0 mM). Aminoguanidine (1.0 mM) was used as a positive control. Fluorescence of samples was measured at excitation 330 nm and emission 410 nm. Data are represented as means ± SD (n= 3). All differences found are significant at P < 0.05.
Figure S4. Structures of resveratrol and its derivatives tested in this study.
**Table S1.** Antioxidant capacity of resveratrol and its derivatives

| Compounds          | IC<sub>50</sub> (μM)<sup>a</sup> | DPPH       | ABTS       |
|--------------------|---------------------------------|------------|------------|
| Resveratrol<sup>b</sup> | 14.45±0.40                      | 3.82±0.02  |
| Isorhapontigenin   | 40.67±0.18                      | 7.49±0.03  |
| Polydatin          | 28.69±0.38                      | 4.41±0.01  |
| Resveratroloside   | 34.61±0.27                      | 5.65±0.03  |
| PG                 | 10.63±0.14                      | 2.18±0.02  |
| THSG               | 16.84±0.15                      | 2.85±0.01  |
| Astringin          | 21.07±0.38                      | 3.09±0.03  |

<sup>a</sup> The IC<sub>50</sub> was defined as the concentration of the 50% inhibition. Data are represented as means ± SD (n= 3). All differences found are significant at P < 0.05.

<sup>b</sup> Positive control
Table S2. Inhibitory activity of aminoguanidine (AG), resveratrol and its derivatives on total fluorescent AGEs formation

| Compounds            | IC$_{50}$ ($\mu$M)$^a$ | FBS-Fructose assay |
|----------------------|-------------------------|--------------------|
| AG$^b$               | 1021.41±4.42            |                    |
| Polydatin            | 1746.68±4.81            |                    |
| Resveratrol          | 521.44±2.83             |                    |
| THSG                 | 280.51±1.62             |                    |
| Resveratroloside     | 762.34±3.08             |                    |
| PG                   | 128.32±1.64             |                    |
| Astringin            | 1241.45±4.92            |                    |
| Isorhapontigenin     | 6321.43±5.76            |                    |

$^a$ The IC$_{50}$ was defined as the concentration of the 50% inhibition. Data are represented as means ± SD (n= 3). All differences found are significant at P < 0.05.

$^b$ Positive control