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Proanthocyanidins are the major anti-diabetic components of cinnamon water extract

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ABSTRACT

Cinnamon consumption has been found to associate with the attenuation of diabetes mellitus. The misfolding of human islet amyloid polypeptide (hIAPP) is regarded as a causative factor of type 2 diabetes mellitus (T2DM). Here, we investigated whether cinnamon has any beneficial effect on the toxic aggregation of hIAPP. We found that cinnamon water extract (CWE) inhibited the amyloid formation of hIAPP in a dose-dependent manner, and identified proanthocyanidins as the major anti-amyloidogenic compounds of CWE. Proanthocyanidins affected the secondary structures of hIAPP and delayed the structural transition from unstructured coils to β-sheet-rich structures. Further studies showed that proanthocyanidins not only inhibited the formation of hIAPP oligomers, but also significantly attenuated the membrane damaging and cytotoxic effects caused by the hIAPP aggregation. Together, these results suggest a possible way by which cinnamon shows beneficial effects on T2DM, and indicate a potential pharmacological usage of proanthocyanidins as an anti-diabetic drug candidate.

1. Introduction

Cinnamon has been used in several cultures for centuries as a spice and as a traditional herbal medicine (Gruenwald et al., 2010). A clinic trial in multi-ethnic type 2 diabetes patients in the United Kingdom has shown that a daily intake of 2 g cinnamon for 12 weeks can reduce the levels of HbA1c, systolic blood pressure and diastolic blood pressure (Akilen et al., 2010). Cinnamon consumption also reduces postprandial intestinal glucose absorption, inhibits gluconeogenesis and stimulates glucose metabolism, glycogen synthesis and insulin release *in vitro*; in diabetic animal models, cinnamon shows multiple beneficial effects including attenuation of diabetes-associated weight loss, reduction of fasting blood glucose and HbA1c, and upregulation of circulating insulin levels (Ranasinghe et al., 2012).

Diabetes is a common metabolic disease and the number of adult patients is expected to reach 439 million by 2030 (Shaw et al., 2010). Type 2 diabetes mellitus (T2DM) accounts for over 90% of the diagnosed diabetics (Clark et al., 1987) and is characterized by insulin resistance, progressive loss of pancreatic β-cell function, decrease in β-cell mass and accumulation of human islet amyloid peptide (hIAPP) deposits. hIAPP is a 37 residue peptide hormone (Fig. 1A) co-secreted with insulin by pancreatic β-cells (Scherbaum, 1998) and plays a role in regulating glucose metabolism (Westermark et al., 2011). In the disease state, hIAPP aggregates and causes dysfunction of pancreatic β-cells (Hebda and Miranker, 2009; Ritzel and Butler, 2003). During aggregation, hIAPP monomers first form β-sheet-rich oligomers which further assemble into fibrillar amyloid (Lopes et al., 2007). And the oligomeric species are thought to be cytotoxic in disrupting the cellular membrane permeabilization and causing cell dysfunction and death (Glabe, 2006; Haataja et al., 2008). Previous structural studies on the nontoxic rat IAPP and hIAPP in membrane-mimicking detergent micelles have shown that the N-terminus that deeply buried within the micelles causes most of the membrane damage and the conformal changes in the C-terminus, and may thus be the modulator of the amyloid formation (Nanga et al., 2011,

Abbreviations: CD, circular dichroism; CWE, cinnamon water extract; EGCG, (−)-epigallocatechin 3-gallate; HFIP, hexafluoroisopropanol; hIAPP, human islet amyloid polypeptide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PA, proanthocyanidins; PBS, phosphate buffered saline; PICUP, photo-induced cross-linking of unmodified proteins; POPG, 2-oleoyl-1-palmitoyl-sn-glycero-3-phosphorac-(1-glycerol) sodium salt; RP-HPLC, reverse phase high performance liquid chromatography; Ru(bpy), Tris(2,2′-bipyridyl)dichlororuthenium(II); TEM, transmission electron microscopy; ThT, thioflavin-T; T2DM, type 2 diabetes mellitus.

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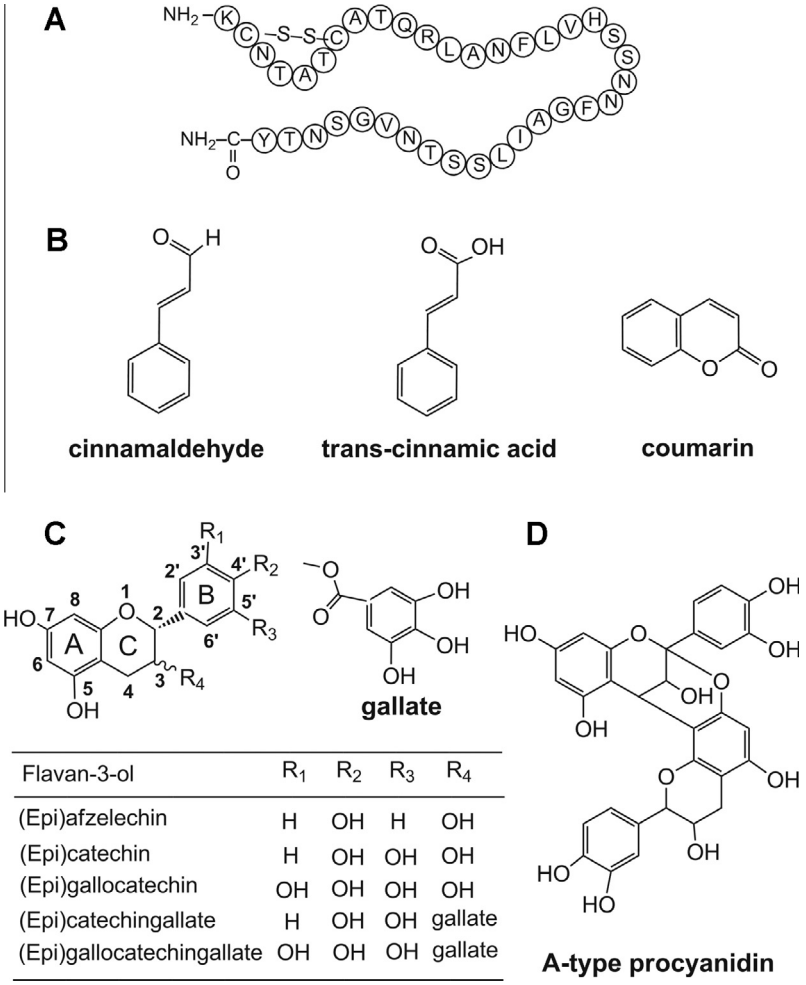

Flavan-3-ol	R_1	R_2	R_3	R_4
(Epi)afzelechin	H	OH	H	OH
(Epi)catechin	H	OH	OH	OH
(Epi)gallocatechin	OH	OH	OH	OH
(Epi)catechingallate	H	OH	OH	gallate
(Epi)gallocatechingallate	OH	OH	OH	gallate

Fig. 1. Primary sequence of hIAPP and chemical structures of the major compounds from CWE. (A) hIAPP has an amidated C terminus and a disulfide bridge between Cys-2 and Cys-7; (B) chemical structures of cinnamaldehyde, cinnamic acid and coumarin; (C) chemical structures of the flavan-3-ol units in PA; (D) major constituents of cinnamon PA.

2009, 2008). Therefore, inhibiting the formation of toxic hIAPP amyloid provides a plausible therapeutic approach for the prevention and treatment of T2DM (Scrocchi et al., 2002).

Although the beneficial effects of cinnamon consumption on diabetes have been known for a long time (Khan et al., 1990), the effect of cinnamon on the toxic aggregation of hIAPP has not been studied. We hypothesize that cinnamon may exert its beneficial function on diabetes through affecting the amyloidogenecity of hIAPP. To test this idea, a series of assays were applied to measure the effect of cinnamon water extract (CWE) on the amyloidogenecity of hIAPP and to identify the active anti-amyloid components of CWE.

2. Materials and methods

2.1. Materials

Synthetic hIAPP (Fig. 1A) was obtained from Genscript (Piscataway, NJ, USA). Proanthocyanidins (PA), cinnamic acid, cinnamaldehyde and coumarin were purchased from Aladdin-reagent INC. (Shanghai, China). Hexafluoroisopropanol (HFIP), thioflavin-T (ThT), carboxyfluorescein, Tris(2,2′-bipyridyl)dichlororuthenium(II) (Ru(bpy)) and 2-oleoyl-1-palmitoyl-sn-glycerol-3-phospho-rac (1-glycerol) sodium salt (POPG) were obtained from Sigma–Aldrich (St. Louis, USA). Fresh blood was drawn from healthy volunteers using heparin as anticoagulant. INS-1 cells were obtained from the China Center for Type Culture Collection (CCTCC). All other chemicals were of the highest grade available.

2.2. Preparation of the cinnamon water extract (CWE)

Cinnamon powder (5 g) was soaked in 50 mL water for 5 h at 40 °C. The extract was centrifuged at 7000*g* for 10 min, and the supernatant (cinnamon water extract (CWE)) was lyophilized and stored at −20 °C until use.

2.3. RP-HPLC analysis

RP-HPLC was used to identify the components of CWE. Proanthocyanidins, cinnamic acid, cinnamaldehyde and coumarin were used as standards. RP-HPLC was performed on a Hitachi L-2000 HPLC system (Hitachi, Tokyo, Japan) with an Apollo C18 column (Grace, USA) thermostatted at 40 °C and detected with a UV detector set at 280 nm. The mobile phase consists of 60% acetonitrile, and the flow rate was 1 mL/min.

2.4. Amyloid formation and Thioflavin-T (ThT) fluorescence assay

For amyloid formation, hIAPP (13 μM) was incubated at 25 °C in 25 mM phosphate buffered saline (PBS; pH 7.4, 50 mM NaCl) containing 1% (v/v) HFIP in the presence or in the absence of CWE or CWE-derived compounds. hIAPP was first dissolved in HFIP and sonicated for 2 min to homogenize. Concentrated hIAPP in HFIP was diluted to the solution containing different concentrations of CWE or compounds to start the aggregation. 10 μL of the reaction mixture was removed at designated time for thioflavin-T (ThT) fluorescence assay on a Hitachi FL-2700 fluorometer (Hitachi, Tokyo, Japan). The assay solution contains 25 mM PBS (pH 7.4, 50 mM NaCl) and 26 μM ThT. The excitation and emission wavelengths were set at 450 nm and 482 nm, respectively. All experiments were repeated at least three times. The kinetic curves were calculated as we previously described (Zhang et al., 2011).