Food and Chemical Toxicology 48 (2010) 2633–2637

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Dieckol isolated from *Ecklonia cava* inhibits α-glucosidase and α-amylase in vitro and alleviates postprandial hyperglycemia in streptozotocin-induced diabetic mice

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ARTICLE INFO

Article history:
Received 24 March 2010
Accepted 21 June 2010

Keywords:
Ecklonia cava
Dieckol
α-Glucosidase
α-Amylase
Postprandial hyperglycemia
Diabetic mice

ABSTRACT

This study was designed to investigate whether dieckol may inhibit α-glucosidase and α-amylase activities, and alleviate postprandial hyperglycemia in streptozotocin-induced diabetic mice. Dieckol isolated from *Ecklonia cava*, brown algae, evidenced prominent inhibitory effect against α-glucosidase and α-amylase. The IC_{50} values of dieckol against α-glucosidase and α-amylase were 0.24 and 0.66 mM, respectively, which evidenced the higher activities than that of acarbose. Dieckol did not exert any cytotoxic effect in human umbilical vein endothelial cells (HUVECs) at various concentrations (from 0.33 to 2.69 mM). The increase of postprandial blood glucose levels were significantly suppressed in the dieckol administered group than those in the streptozotocin-induced diabetic or normal mice. Moreover, the area under curve (AUC) was significantly reduced via dieckol administration (259 versus 483 mmol min/l) in the diabetic mice as well as it delays absorption of dietary carbohydrates. Therefore, these result indicated that dieckol might be a potent inhibitor for α-glucosidase and α-amylase.

1. Introduction

Diabetes mellitus is a chronic disease representing one of the world's most serious health concerns, developing increasingly with the increasing obesity and advancing age in the general global population. Diabetes mellitus is a complex disorder characterized by hyperglycemia. The disease is primarily classified into insulin-dependent diabetes mellitus (type I diabetes) and non-insulin-dependent diabetes mellitus (type II diabetes). The prevalence of type II diabetes is increasing globally (Zimmet et al., 2001). Postprandial hyperglycemia plays an important role in the development of type II diabetes, as well as in complications associated with the condition, including micro-vascular and macro-vascular diseases (Baron, 1998). Therefore, the control of postprandial hyperglycemia has been shown to be important in the treatment of diabetes and the prevention of cardiovascular complications.

One of the therapeutic approaches adopted thus far to ameliorate postprandial hyperglycemia involves the retardation of glucose absorption via the inhibition of carbohydrate-hydrolyzing enzymes including α-glucosidase and α-amylase, in the digestive organs (Bhandari et al., 2008). The powerful synthetic α-glucosidase and α-amylase inhibitors, such as acarbose, miglitol, and voglibose, function directly in reducing the sharp increases in glucose levels that occur immediately after food uptake (Saito et al., 1998; Sels et al., 1999; Stand et al., 1999). However, the continuous use of those synthetic agents should be limited because those agents may induce side effects such as flatulence, abdominal cramps, vomiting, and diarrhea (Hanefeld, 1998). Additionally, there have been some reports describing an increased incidence of renal tumors, serious hepatic injury, and acute hepatitis (Diaz-Gutierrez et al., 1998; Charpentier et al., 2000). Therefore, a number of studies have been conducted in the search for naturally derived α-glucosidase and α-amylase inhibitors that induce no deleterious side effects (Matsui et al., 2007; Kim et al., 2008; Heo et al., 2009).

Marine algae are known to generate an abundance of bioactive compounds with great potential in the pharmaceuticals, food, and biomedical industries. In particular, the brown algae harbor a variety of biological compounds, including pigments, fucoidans,

Abbreviations: AUC, area under curve; HUVECs, human umbilical vein endothelial cells; pNPG, p-Nitrophenyl-α-glucopyranoside; pNPM, p-Nitrophenyl-α-maltopenotoglycoside.

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doi:10.1016/j.fct.2010.06.032

phycocolloids, and polyphenolic compounds (Halliwell and Gutteridge, 1999). The brown alga, *Ecklonia cava*, is popular in Korea and Japan as a food ingredient and a medicine. It is rich in the biological polyphenolic compounds referred to as phlorotannins (Heo et al., 2005). The phlorotannin components of *E. cava* include phenolic secondary metabolites such as eckol (a closed-chain phloroglucinol trimer), 6,6′-bieckol (a hexamer), dieckol (a hexamer), phlorofucofuroeckol (a pentamer), and triphlorethol-A, all of which influence biological activities (Kang et al., 2005a, b; Ahn et al., 2007). Among these phlorotannins, dieckol is one of the major and most active compounds.

We demonstrated previously that the dieckol isolated from *E. cava* is a potential therapeutic agent that may ameliorate the damage induced by diabetes-associated hyperglycemia-induced oxidative stress (Lee et al., 2010). Therefore, in this study, we attempted to determine whether or not dieckol may inhibit α-glucosidase and α-amylase activities, and also whether or not dieckol alleviates postprandial hyperglycemia in streptozotocin-induced diabetic mice.

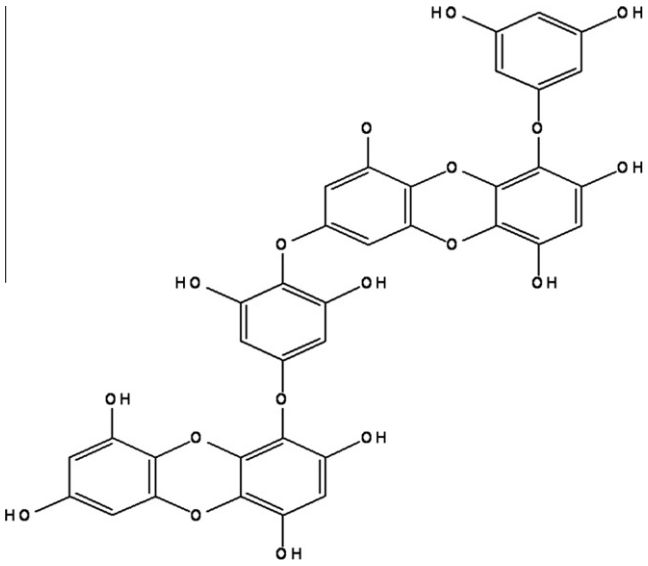

Fig. 1. Chemical structure of dieckol isolated from *E. cava*.

2. Materials and methods

2.1. Materials

The brown alga *E. cava* (Phylum Phaeophyta, Class Phaeophyceae, Order Laminariales, Family Alariaceae) was collected from the coast of Jeju Island, south Korea. Salt, sand and epiphytes were using tap water. Then, the samples were rinsed carefully with fresh water and freeze–dried. Dried alga sample was ground and sifted through a 50-mesh standard testing sieve. All chemicals and reagents used were of analytical and obtained from commercial sources.

The UV and FT-IR spectra were recorded on a Pharmacia Biotech Ultrospec 3000 UV/Visible spectrometer and a SHIMAZU 8400s FT–IR spectrometer, respectively. NMR spectra were recorded on a Varian INOVA 400 MHz spectrometer. CD_3OD were used as a solvent for the NMR experiments, and the solvent signals were used as an internal reference. The HPLC was carried out on a YoungLin Instrument HPLC system equipped with a YoungLin acme 9000 UV/VIS detector and Autochrome software using C_{18} column (Waters Spherisorb® DOS-2 RP-18, 4.6 × 250 mm, 5 μm, Waters Co.).

2.2. Extraction and isolation

The dried *E. cava* powder (500 g) was extracted three times with 80% methanol, and filtered. The filtrate was evaporated at 40 °C to obtain the methanol extract, which was dissolved in water, then partitioned with ethyl acetate. The ethyl acetate fraction (45.65 g) was mixed with Celite. The mixed Celite was dried and packed into a glass column, and eluted in the order of hexane, methylene chloride, diethyl ether, and methanol. The diethyl ether fraction (26.69 g) was subjected to Sephadex LH-20 column chromatography using stepwise gradient chloroform/methanol (2/1 to 1/1 to 0/1) solvents system, and then finally purified by reversed-phase HPLC to give compound dieckol (275.8 mg). The structure of the dieckol (Fig. 1) was identified by comparing the NMR spectral data with those in existing literature.

2.2.1. Dieckol

^{1}H NMR (400 MHz, methanol-d_4) δ 6.15 (1H, s), 6.13 (1H, s), 6.09 (1H, d, 2.9 Hz), 6.06 (1H, d, 2.9 Hz), 6.05 (1H, d, 2.9 Hz), 5.98 (1H, d, 2.8 Hz), 5.95 (1H, d, 2.8 Hz), 5.92 (3H, m); ^{13}C NMR (100 MHz, methanol-d_4) δ 161.8, 160.1, 157.8, 155.9, 154.5, 152.4, 147.3, 147.2, 147.1, 146.9, 144.3, 144.1, 143.4, 143.3, 138.6, 138.5, 126.5, 126.2, 125.6, 125.5, 124.9, 124.6, 124.5, 99.9, 99.7, 99.5, 99.4, 97.6, 96.2, 95.8, 95.7, 95.3.

2.3. Inhibitory effect of dieckol on α-glucosidase and α-amylase in vitro

The α-glucosidase inhibitory assay was done by the chromogenic method described by Watanabe et al. (1997) using a readily available yeast enzyme. Briefly, yeast α-glucosidase (0.7 U, Sigma) was dissolved in 100 mM phosphate buffer (pH 7.0) containing 2 g/l bovine serum albumin and 0.2 g/l NaN_3 and used as an enzyme solution. 5 mM p-Nitrophenyl-α-D-glucopyranoside in the same buffer (pH 7.0) was used as a substrate solution. The 50 μl of enzyme solution and 10 μl of sample dissolved in dimethylsulfoxide were mixed in a microtiter plate and measured absorbance at 405 nm at zero time. After incubation for 5 min, substrate solution (50 μl) was added and incubated for another 5 min at room temperature. The increase in the absorbance from zero time was measured. The α-amylase inhibitory activity was assayed in the same way as described for α-glucosidase inhibitory assay except the using of porcine pancreatic amylase (100 U, Sigma) and blocked p-Nitrophenyl-α-D-maltopentoglycoside (Sigma, St Louis, MO, USA) as enzyme and substrate, respectively. Percent inhibitory activity was expressed as 100 minus relative absorbance difference (%) of test compounds to absorbance change of the control where test solution was replaced by carrier solvent.

2.4. Measurement of cytotoxicity

Cell viability was assessed by measuring the supravital dye neutral red uptake (Fautz et al., 1991). Human umbilical vein endothelial cells (HUVECs) were seeded at the concentration of 2×10^4 cells/ml in 96-well plate and pre-incubated in humidified atmosphere containing 5% CO_2 at 37 °C for 24 h. After that, the cells were treated with various concentrations (0.33, 0.67, 1.34, and 2.69 mM) of dieckol, and further incubated for 24 h. Thereafter, the medium was carefully removed from each well, and replaced with 0.5 ml of fresh medium containing 1.14 mM neutral red. After 3 h of incubation, the medium was removed and the cells were washed twice with phosphate buffered saline (PBS, pH 7.4). The incorporated neutral red was released from the cells by incubation in the presence of 1 ml of the cell lysis buffer [50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 5 mM dithiothreitol (DTT), and Triton X-100 (1%, v/v)] containing acetic acid (1%, v/v) and ethanol (50%, v/v) at room temperature for 15 min. The cell lysis products were centrifuged to measure the dye taken up and absorbance of supernatant was measured spectrophotometrically at 540 nm.

2.5. Experimental animals

Male ICR mice (4 weeks of age; purchased from Joong Ang Lab Animal Co., Korea) were used. All animals were housed individually in a light- (12 h on/12 h off) and temperature-controlled room with food and water available ad libitum. The animals were maintained with pelleted food, while tap water was available ad libitum. After an adjustment period of approximately 2 weeks, diabetes was induced by intraperitoneal injection of streptozotocin (60 mg/kg i.p.) dissolved in a freshly prepared citrate buffer (0.1 M, pH 4.5). After seven days, tail bleeds were performed and animals with a blood glucose concentration above 13.89 mM were considered to be diabetic.

2.6. Measurement of blood glucose level

Normal mice and streptozotocin-induced diabetic mice fasted overnight were randomly divided into two groups. Fasted animals were deprived of food for at least 12 h but allowed free access to water. After overnight fasting, the mice were administrated orally soluble starch (2 g/kg body weight) alone or with dieckol (100 mg/kg body weight). Blood samples were taken from the tail vein at 0, 30, 60, 120 min (Kim, 2004). Blood glucose was measured using a glucometer (Roche Diagnostics Gmbh, Germany). Areas under the curve (AUC) were calculated using the trapezoidal rule.

2.7. Data and statistical analysis

The data are represented as mean ± SD. The statistical analysis was performed using SAS software. The values were evaluated by one-way analysis of variance (ANOVA) followed by post hoc Duncan's multiple range tests.