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Original Article

Identification of biosynthetic intermediates of teaghrelins and teaghrelin-like compounds in oolong teas, and their molecular docking to the ghrelin receptor

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ABSTRACT

Teaghrelins are unique acylated flavonoid tetraglycosides found in Chin-shin oolong tea, and have been demonstrated to be promising oral ghrelin analogues. The biosynthetic pathway of teaghrelins from quercetin-3-O-rutinoside (rutin) or kaempferol-3-O-rutinoside (nicotiflorin) was proposed to comprise three enzymatic steps according to the identification of putative intermediates in Chin-shin oolong tea. In addition to the two known teaghrelins in Chin-shin oolong tea, four teaghrelin-like compounds with different attachments of glycosides were identified in various oolong teas. Molecular modeling and docking were used to evaluate theoretically whether the putative biosynthetic intermediates of teaghrelins and the four teaghrelin-like compounds could be potential candidates of ghrelin analogues. The results showed that the attachment of a coumaroyl group was crucial for these tea compounds to bind to the ghrelin receptor. However, the additional attachment of a rhamnosyl glycoside to the flavonoid backbone of teaghrelin-like compounds at C-7 significantly reduced their binding affinity with the ghrelin receptor.

1. Introduction

Ghrelin is a peptide hormone consisting of 28 amino acids, in which the third serine residue is acylated with an *n*-octanoyl group essential for its biological functions [1–3]. It is colloquially called the "hunger hormone", and its target receptor is a G protein-coupled receptor also known as the growth hormone secretagogue-1a receptor [1,4]. The remarkable physiological functions of ghrelin via activation of the ghrelin receptor are promotion of appetite by stimulation of

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hypothalamic arcuate nucleus and induction of growth hormone release from the anterior pituitary gland [5,6]. In addition, several other biological effects of ghrelin have been reported, including influence on the reproductive system, the gastrointestinal system, glucose metabolism, and cardiovascular functions [7–9]. On the basis of the multiple biological activities of ghrelin, both peptidyl and nonpeptidyl ghrelin analogues were synthesized and aimed to develop potential therapeutic applications of several diseases, e.g., gastrointestinal deficiency and anorexia [10–13]. Unfortunately, no synthetic oral ghrelin analogues were satisfactory and approved for clinical application thus far.

Tea is one of the most widely consumed beverages around the world [14], and its major ingredients, flavonols and polyphenols, have been demonstrated to provide a variety of health benefits [15–17]. According to the degree of fermentation in preparation, teas are mainly classified as green tea (unfermented), oolong tea (semifermented), and black tea (fully fermented), where the term fermentation refers to natural browning reactions induced by oxidative enzymes in the cells of tea leaves [18]. Over the past few decades, oolong tea has been the most favorite choice of Taiwanese because of its special taste and flavor [19,20].

Teaghrelins are unique acylated flavonoid tetraglycosides found in Chin-shin oolong tea, and have been demonstrated to be responsible for the hunger induction just as the endogenous hunger hormone, ghrelin [21,22]. Similar to ghrelin, teaghrelins are able to induce hunger sensation of rats as well as stimulate growth hormone secretion of rat primary anterior pituitary cells [21]. According to the observation in the aforementioned animal study and cell line assay, teaghrelins were proposed to be promising oral ghrelin analogues, provided they undergo necessary clinical trials.

In addition to teaghrelins, some flavonoid derivatives were also found in Chin-shin oolong tea [23]. Presumably, these flavonoid derivatives might serve as intermediates in the biosynthetic pathway of teaghrelins. Moreover, several teaghrelin-like compounds, acylated flavonoid tetraglycosides with different attachments of glycosides, were detected in various oolong teas [24]. In this study, we aimed to search for more potential oral ghrelin analogues by screening flavonoid derivatives in Taiwan oolong teas. We first identified the putative biosynthetic intermediates of teaghrelins in Chin-shin oolong tea and teaghrelin-like compounds in various oolong teas. To evaluate the possibility if the putative biosynthetic intermediates of teaghrelins and the teaghrelin-like compounds could be potential candidates of ghrelin analogues, molecular modeling of these compounds docking to the ghrelin receptor was exhibited.

2. Methods

2.1. Chemicals and materials

All chemicals were purchased from E. Merck Co. (Merck KGaA, Darmstadt, Germany) unless stated otherwise. High-performance liquid chromatography (HPLC) grade acetonitrile was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Acetic acid (99.7%) was obtained from J.T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA). Rutin was purchased from Sigma Co. (Sigma-Aldrich, St. Louis, MO, USA). Purified water was afforded by a Millipore clear water purification system (Direct-Q, Millipore, Billerica, MA, USA). Various oolong teas prepared from tea plants (*Camellia sinensis* L.), including Alishan Chin-shin, Dongding Chin-shin, Wuyi, Baihao, and Tieguanyin, were gifts or purchased from local tea producers. Teaghrelins were purified from Chin-shin oolong teas according to the protocol as described previously [22].

2.2. Preparation and HPLC analysis of tea infusions

Tea infusions were prepared by adding 20 mL of boiling water to 1 g of various oolong teas for 10 minutes. After cooling to room temperature, the brew was filtered through a 0.22 μm polyvinylidene difluoride (PVDF) membrane filter (Pall Corporation, Glen Cove, NY, USA) for the following analysis. Chemical constituents in oolong tea infusions were analyzed by HPLC system coupled to a 600E photodiode array detector (Waters Corporation, Milford, MA, USA), and separation was performed on the Syncronis C18 column (4.6 × 250 mm inner diameter, 5 μm, Thermo Scientific, Waltham, MA, USA). The separated condition of HPLC analysis was prepared according to Lo et al [21]. The mobile phase consisted of (A) water containing 0.5% acetic acid and (B) acetonitrile. The gradient was as follows: 0–60 minutes, linearly gradient from 10% to 30% B; 61–70 minutes 30% B; and 71–100 min, linearly gradient from 30% to 10% B. The column was maintained at room temperature and the injection volume was 10 μL at a flow rate of 1 mL/min. The ultraviolet (UV) absorbance detection wavelength was set at 280 nm.

2.3. Mass spectrometric analysis

Mass spectrometric analysis was performed on a LTQ (linear trap quadrupole) tandem mass spectrometer (Thermo Electron, San Jose, CA, USA) equipped with an electrospray ionization (ESI) interface and connected to a Surveyor LC system (Thermo Electron, San Jose, CA, USA) with a 5 μL sample loop. The analytes were separated with the same condition of HPLC analysis. The flow rate was 1 mL/min. The mass spectra were obtained with negative ESI mode. The heated capillary temperature was 300°C, and the spray voltage was 4.5 kV. Flow rates of sheath gas, auxiliary gas, and sweep gas were 50, 13, and 3 arbitrary units, respectively. Data-dependent acquisition (DDA) was used to perform under automatic gain control conditions. The first scan was operated in full-scan mode with m/z values ranging from 150 to 1500. The other scans were set as the data-dependent MS^n scan using the high-purity helium (>99.99%) as the collision gas and the relative collision energy of 33–35%. The flavonoid derivatives shown in the HPLC profiles were identified according to the same procedure as described previously [23].

2.4. Homology modeling of three-dimensional structure of human ghrelin receptor

The protein sequence of a human ghrelin receptor (growth hormone secretagogue receptor, Genbank accession number AAI13548) was uploaded to the SWISS-MODEL website (http://