Effect of six Korean plants on glucagon like peptide-1 release

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Abstract Glucagon like peptide-1 (GLP-1) plays a major role in the regulation of energy homeostasis as it causes satiety and suppresses appetite. *Angelica dahurica*, *Coreanomecon hylomeconoides*, *Chrysanthemum morifolium*, *Portulaca oleracea*, *Stellaria alsine*, and *Stellaria media* have traditionally been used as famine relief foods in Korea. The aim of the present study was to determine whether the aqueous ethanolic extracts of the six plants stimulate GLP-1 secretion. The results demonstrated that each extract of the plants stimulated GLP-1 secretion from enteroendocrine NCI-H716 cells, respectively. Among the extracts examined, the extract of *Portulaca oleracea* showed the highest activity on GLP-1 release. The results may suggest that the GLP-1 secretion induced by the six plants is a possible mechanism for the six plants exerting effects on satiety increase and appetite suppression.

Keywords Famine relief foods · *Portulaca oleracea* · Glucagon like peptide-1 · Satiety · Enteroendocrine cells

Introduction

Obesity results from an overflow of energy intake compared to energy expenditure. The control of energy homeostasis is multifactorial, involving the communication between the gastrointestinal (GI) tract and the brain. GI hormones are secreted from enteroendocrine cells (EEC) of the GI tract into circulation or into vagus nerve to act on the central nervous system, particularly the hypothalamus and the brainstem, which regulate energy homeostasis (Lai et al., 2015). Among GI hormones, glucagon like peptide-1 (GLP-1) plays a major role in the regulation of energy homeostasis as it causes satiety and suppresses appetite through signaling the brain to decrease gastric emptying and intestinal motility (Zanchi et al., 2017).

GLP-1 is secreted when stimulated by nutrients such as glucose, amino acids, and fatty acids. Meanwhile, recent studies have showed that non-nutrient edible plant extracts such as green tea extract (Liu et al., 2014), sweet potato leaf extract (Nagamine et al., 2014), and *Spergularia marina* extract (Kim et al., 2014) stimulated GLP-1 secretion. By means of established model such as the human cell line NCI-H716, derived from a caecal adenocarcinoma (Parker et al., 2009), the GLP-1-secreting function of various components can be assessed.

The release of GLP-1 involves interaction between nutrients and G-protein coupled receptors (GPCRs) that are expressed on EECs (Psichas et al., 2015). The GPCRs related to GLP-1 release include sweet taste receptor, umami taste receptors, bitter taste receptors (Reimann et al., 2008), Takeda G-protein-coupled receptor 5 (Behrens and Meyerhof, 2011), free fatty acid receptors 1–4 (Edfalk et al., 2008; Hirasawa et al., 2005; Nohr et al., 2013), and G-protein receptor 119 (Reimann et al., 2008). Activation of the GPCRs increases intracellular Ca^{2+}

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concentration, alters membrane potential, and secretes GLP-1 (Kinnamon, 2012; Reimann et al., 2012). Therefore, the changes of intracellular Ca^{2+} levels coupled to GLP-1 secretion are often measured.

Angelica dahurica (AD), *Coreanomecon hylomeconoides* (CH), *Chrysanthemum morifolium* (CM), *Portulaca oleracea* (PO), *Stellaria alsine* (SA), and *Stellaria media* (SM) have traditionally been used as famine relief foods in Korea (Guh et al., 2015; Kim and Cha, 2013; Lee, 2002). Famine relief foods provide satiety and suppress appetite, possibly via the stimulation of GLP-1 secretion. In this study, the aqueous ethanolic extracts (AEE) of the six plants were tested for their ability to stimulate GLP-1 secretion and intracellular Ca^{2+} changes from NCI-H716 human enteroendocrine L-cells.

Materials and methods

Plant materials and extraction

AD, CH, CM, PO, SA, and SM were obtained from a local market (Jeonnam, Korea). The information about family, local name, voucher number, and part used are listed in Table 1. The plants were freeze dried and the drying yields were 15.2%, 14.9%, 24.7%, 10.6%, 17.7%, and 17.8% for AD, CH, CM, PO, SA, and SM, respectively. The freeze dried plants were extracted with 50% ethanol (1:40 w/v) for 72 h and filtered. The extraction was done in triplicate. The filtered extracts were combined, evaporated under reduced pressure at 40 °C, and freeze dried. The extraction yields for AD, CH, CM, PO, SA, and SM were 35.11%,

22.90%, 34.01%, 24.06%, 18.30%, and 31.91%, respectively (w/w; based on the dry weight).

High-performance liquid chromatography (HPLC) analysis

The extracts were analyzed by HPLC (Jasco, Tokyo, Japan) consisted of PU-8980 pump, CO-976 column oven, AS-2057 plus intelligent sampler, and MD-2010 plus multi-wavelength detector. The column was a C18 reversed phased Waters XTerra RP18 (4.6 mm × 150 mm, 5 µm; Milford, MA, USA), and the mobile phases were 0.05% trifluoroacetic acid (A) and acetonitrile (B). The gradient was: 0–5 min, 90% A; 5–10 min, 90–80% A; 10–20 min, 80–50% A; 20–25 min, 50–38% A; 25–28 min, 38–25% A; 28–35 min, 25–0% A; 35–45 min, 0–25% A; 45–50 min, 25–90% A. The equilibration time was 6 min, the flow rate was 1.0 mL/min, and the injection volume was 20 µL. The standards (chlorogenic acid, trans-caffeic acid, sinapic acid, rutin, quercitrin, myricetin, luteolin, naringenin, kaempferol, and apigenin; 0.1 mg/mL each) and the samples (10 mg/mL) were prepared in methanol. Detection was performed at 327 nm. The peaks were identified by comparing retention time and UV spectra.

Cell culture and GLP-1 secretion studies

NCI-H716 cells (the American Type Culture Collection, Manassas, VA, USA) were cultured and differentiated as reported (Reimer et al., 2001). GLP-1 secretion from the cells was measured using the method previously described (Kim et al., 2014). In brief, the cells were plated at 5×10^4 cells per well in 96-well microplates coated with Matrigel

Table 1 Plants used and EC_{50} values for GLP-1 secretion and Ca^{2+} response

Abb. ^a	Scientific name	Family	Local name	Part used	Voucher number	EC_{50} (µg/mL) ^b	
						GLP-1 secretion	Ca^{2+} response
AD	<i>Angelica dahurica</i> (Fisch. Ex Hoffm.) Benth. & Hook. F. Ex Franch. & Sav.	Umbelliferae	Guritdae	Aerial part of whole plant	101	737.97	260.74
CH	<i>Coreanomecon hylomeconoides</i> Nakai	Papaveraceae	Maemikkot	Aerial part of whole plant	98	848.47	208.97
CM	<i>Chrysanthemum morifolium</i>	Compositae	Gukhwa	Flowers	92	543.31	290.28
PO	<i>Portulaca oleracea</i> L.	Portulacaceae	Soebileum	Aerial part of whole plant	107	297.31	201.59
SA	<i>Stellaria alsine</i> var. <i>undulata</i> (Thunb.) Ohwi	Caryophyllaceae	Byeoluknamul	Aerial part of whole plant	100	859.32	80.17
SM	<i>Stellaria media</i> (L.) Vill.	Caryophyllaceae	Byeolkkot	Aerial part of whole plant	99	772.75	236.76

^aAbbreviation

^b EC_{50} was calculated when the response to 250 mM glucose was considered to be the maximum

and incubated for 48 h. The cells were incubated with the extracts in Krebs–Ringer bicarbonate buffer (128.8 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4 , 1.2 mM MgSO_4 , 2.5 mM CaCl_2 , 5 mM NaHCO_3 , 10 mM HEPES, and 0.2% bovine serum albumin, pH 7.4) for 1 h. GLP-1 in the supernatants were measured by a GLP-1 active ELISA kit (Millipore, Billerica, MA, USA) and expressed as folds of the untreated control.

Measurement of Ca^{2+} response in NCI-H716 cells

Ca^{2+} response in the cells was measured as described previously (Kim et al., 2014). The cells (5×10^4 cells per well) were differentiated in Matrigel coated 96-well clear-bottom plates for 48 h at 37 °C. The medium was replaced to a calcium indicator dye from the FLIPR Calcium 5 Assay Kit (Molecular Devices, Sunnyvale, CA, USA) and incubated for 1 h at 37 °C. Ca^{2+} response was measured using a FlexStation 3 (Molecular Devices) according to the manufacturer's instruction. The response was expressed as ΔRFU (relative fluorescent units), calculated as (maximum fluorescent value)—(minimum fluorescent value).

Statistical analysis

The data were analyzed by SPSS (SPSS 20 software, SPSS inc., Armonk, NY, USA) with a paired *t* test to determine significance compared to untreated control. The half-maximal effective concentrations (EC_{50}) of the extracts for GLP-1 secretion and Ca^{2+} response were calculated when the response to 250 mM glucose was considered to be the maximum using SigmaPlot® version 12.0 (Systat Software, San Jose, CA, USA). The correlation analysis was performed between the EC_{50} value of GLP-1 secretion and EC_{50} of Ca^{2+} response using Xlstat version 2015 (Addinsoft, Paris, France).

Results and discussion

HPLC chromatogram of the plant extracts

The HPLC chromatograms of the extracts and standards at 327 nm are shown in Fig. 1. Among the tested flavonoids, chlorogenic acid, luteolin, and apigenin were detected in AEE-CM and quantified as 1.19, 1.22, and 0.36 $\mu\text{g}/\text{mg}$ dried weight of AEE-CM, respectively. The presence of these flavonoids has been reported in the flower of CM (Lin and Harnly, 2010). The presence of some of the flavonoids analyzed has been reported in PO (Siriamornpun and Sut-tajit, 2010) and SM (Miksatkova et al., 2014). However, none of the flavonoids analyzed were detected in AEE-PO and AEE-SM as well as in the other extracts. The

differences in flavonoid compositions may due to differences in cultivated area and extraction method.

Effects of plant extracts on GLP-1 secretion and Ca^{2+} response

The stimulation of GLP-1 secretion by the extracts was evaluated and compared to that of glucose (Fig. 2A). The half-maximal effective concentrations (EC_{50}) of the extracts for GLP-1 secretion were calculated using glucose at 250 mM as maximum and are shown in Table 1. Among the extracts, AEE-CM, AEE-PO, and AEE-SA significantly induced GLP-1 secretion at all the concentrations tested ($p < 0.05$) while AEE-CH and AEE-SM showed significant GLP-1 secretion stimulatory effects only at the highest concentration tested (500 $\mu\text{g}/\text{mL}$; $p < 0.05$). AEE-PO showed the lowest EC_{50} value followed by AEE-CM, AEE-AD, AEE-SM, AEE-CH, and AEE-SA (Table 1). Also, the cells treated with 500 $\mu\text{g}/\text{mL}$ of AEE-PO showed the highest increase in GLP-1 secretion of about threefold compared to untreated control.

The extract of AD root and the extract of PO seed have been reported to stimulate GLP-1 secretion (Heidarzadeh et al., 2013; Park et al., 2016). In this study, the extract of the aerial portions of AD and PO stimulated GLP-1 secretion in NCI-H716 cells. AEE-SM has been reported to have anti-obesity effect (Rani et al., 2012) and its anti-obesity effect might be, in part, due to GLP-1 secretory activities of AEE-SM.

Effects of the extracts of the six plants on Ca^{2+} response are shown in Fig. 2B. The EC_{50} values of the extracts for Ca^{2+} response were calculated using glucose at 250 mM as maximum (Table 1). All the tested extracts showed significant stimulatory effects at the highest concentration tested (500 $\mu\text{g}/\text{mL}$; $p < 0.05$). Cells treated with AEE-CH and AEE-SA showed higher Ca^{2+} response at 500 $\mu\text{g}/\text{mL}$, and only AEE-SA showed significant increase in Ca^{2+} response at all the concentrations tested ($p < 0.05$). AEE-SA showed the lowest EC_{50} value followed by AEE-PO, AEE-CH, AEE-SM, AEE-AD, and AEE-CM.

As the extracts of six plants increased GLP-1 secretion and Ca^{2+} response, it was suggested that these extracts may induce GLP-1 secretion by increasing intracellular Ca^{2+} .

In order to investigate the relationship between the GLP-1-secreting activity and Ca^{2+} -releasing activity of the extracts, the Pearson's correlation coefficient for the EC_{50} values of GLP-1 secretion and EC_{50} of Ca^{2+} response were calculated from the statistical program. The correlation result demonstrated weak negative relationship ($r = -0.324$). This difference result may due to differences in reaction time and method for measuring GLP-1 and Ca^{2+} (Kim et al., 2014). Moreover, many different

Fig. 1 HPLC chromatograms of the aqueous ethanolic extracts (AEE) of AD, CH, CM, PO, SA, SM, and standards at 327 nm. Full names of the plants are listed in Table 1. (A) chlorogenic acid,

(B) trans-caffeic acid, (C) sinapic acid, (D) rutin, (E) quercitrin, (F) myricetin, (G) luteolin, (H) naringenin, (I) apigenin and (J) kaempferol

receptors are involved in Ca^{2+} response and GLP-1 secretion. As the extracts are mixture of many compounds, they may have activated more than one receptor, resulting in inconsistencies between GLP-1 secretion and Ca^{2+} response. Further studies on the involvement of specific

receptors would be needed to further elucidate mechanisms of GLP-1 secretory activities of the extracts.

GLP-1 secreted from EEC acts on targeted tissues through circulation or vagus nerve. Circulating GLP-1 has a very short half-life due to inactivation by the enzyme dipeptidylpeptidase-4 (DPP-4) in the body (Kieffer et al.,

Fig. 2 Effect of the aqueous ethanolic extracts (AEE) of AD, CH, CM, PO, SA, and SM on GLP-1 secretion (A) and Ca^{2+} response (B) in NCI-H716 cells. Full names of the plants are listed in Table 1. Glucose was used as a positive control. Values are expressed as mean $\pm$ SEM ($n = 15$ per group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. an untreated control

1995). DPP-4 is a membrane-bound X-propyl dipeptidyl peptidase, which involves the degradation of GLP-1 (Lambeir et al., 2003). Therefore, it is thought that the extracts might also act as a DPP-4 inhibitor in NCI-H716 cells. Further study is required to explore whether the extracts can act as a DPP-4 inhibitor.

Phytochemicals found in the plant such as resveratrol (Dao et al., 2011), curcumin (Takikawa et al., 2013), cinnamtannin A2 (Yamashita et al., 2013), isoflavonoids (Kwon et al., 2011), phellopterin (Park et al., 2016), glyceollins (Park et al., 2010), and berberine (Yu et al., 2015) showed GLP-1 secretory activities. Therefore, the flavonoids detected in AEE-CM, chlorogenic acid, luteolin,

and apigenin, may be responsible for the GLP-1 secretory activities. GLP-1 secretory activities of the three flavonoids were not examined in this study and have not been reported. Further research on the elucidation of active components in the extracts is needed.

This study provided preliminary information on the GLP-1 secretory activities of six plants used in famine relief foods traditionally used in Korea. The findings may suggest that the GLP-1 secretion induced by the six plants is a possible mechanism for the six plants exerting effects on satiety increase and appetite suppression.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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