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Bioactive compounds isolated from apple, tea, and ginger protect against dicarbonyl induced stress in cultured human retinal epithelial cells

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ABSTRACT

Background: Methylglyoxal (MGO) is known to be a major precursor of advanced glycation end products (AGEs) which are linked to diabetes and its related complications. Naturally occurring bioactive compounds could play an important role in countering AGEs thereby minimizing the risk associated with their formation.
Methods: In this study, eight specific bioactive compounds isolated from apple, tea and ginger were evaluated for their AGEs scavenging activity using Human Retinal Pigment Epithelial (H-RPE) cells treated with MGO.
Results: Among the eight specific compounds evaluated, (-)-epigallocatechin 3-gallate (EGCG) from tea, phloretin in apple, and [6]-shogaol and [6]-gingerol from ginger were found to be most effective in preventing MGO-induced cytotoxicity in the epithelial cells. Investigation of possible underlying mechanisms suggests that that these compounds could act by modulating key regulative detoxifying enzymes via modifying nuclear factor-erythroid 2-related factor 2 (Nrf2) function. MGO-induced cytotoxicity led to increased levels of AGEs causing increase in Nε-(Carboxymethyl) lysine (CML) and glutathione (GSH) levels and over expression of receptor for advanced glycation end products (RAGE). Data also showed that translocation of Nrf2 from cytosol to nucleus was inhibited, which decreased the expression of detoxifying enzyme like heme oxygenase-1 (HO-1). The most potent bioactive compounds scavenged dicarbonyl compounds, inhibited AGEs formation and significantly reduced carbonyl stress by Nrf2 related pathway and restoration of HO-1 expression.
Conclusions: These findings demonstrated the protective effect of bioactive compounds derived from food sources against MGO-induced carbonyl stress through activation of the Nrf2 related defense pathway, which is of significant importance for therapeutic interventions in complementary treatment/management of diabetes-related complications.

Introduction

Diabetes is one of the chronic diseases associated with obesity, which is an emerging global epidemic. The International Diabetes Federation reported an alarming increase of obesity in the Middle East and North Africa (MENA) region where Kuwait, Qatar and Saudi Arabia, top the list with prevalence rates in the range 23–24% (IDF 2013). During prolonged incubation and at high concentrations of glucose, it reacts with proteins/lipids non enzymatically generating reactive dicarbonyl intermediates like α-oxoaldehydes such as Methylglyoxal (MGO) and glyoxal (GO) which induce the formation of advanced glycation end products (AGEs) (Hegab et al. 2012). These intermediate compounds, once generated, react quickly with amino acids like lysine, arginine and cysteine on proteins exerting toxicity to cells and tissues (Nagaraj et al. 2002). It has been reported that accumulation of AGEs exacerbates diabetes-related complications affecting the eyes, kidneys, nervous system and blood vessels (Hegab et al. 2012). Hence, trapping of MGO and GO result in limiting the formation of AGEs and could serve as a basis for effective prevention/management of diabetic complications for a long term (Rahbar 2007). The mechanism

Abbreviations: MGO, Methylglyoxal; AGEs, Advanced glycation end products; H-RPE, Human Retinal Pigment Epithelial cells; CML, ε-Carboxymethyl lysine; GSH, Glutathione; RAGE, Receptor for advanced glycation end products; HO-1, Heme oxygenase-1; Nrf2, Nuclear factor-erythroid 2-related factor 2; AG, Aminoguanidine; EGCG, (-)-epigallocatechin 3-gallate; ECG, (-)-epicatechin 3-gallate; EC, (-)-epicatechin; PH, Phloretin.

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reported in the literature suggests that AGEs bind to a receptor for advanced glycation end products (RAGE) which, in turn, impairs the vessel functions inducing a series of inflammatory processes, such as increasing the levels of oxidative stress, up-regulating the expression of adhesion molecules, enhancing intima proliferation and promoting angiogenesis (Goldin et al. 2006). *In vitro* and *in vivo* studies suggest that limiting the expressions of RAGE in vascular cells could modulate the levels of various proinflammatory mediators and prevent the development of vascular dysfunction (Figarola et al. 2007). Nε-(Carboxymethyl) lysine (CML) and Nε-(Carboxyethyl) lysine (CEL) are the best-characterized AGEs generated from GO and MGO, respectively. The levels of CML and CEL in diabetic patients are about 2–3 folds higher than those in healthy people (Teerlink et al. 2004). The generation of CML has been associated with progression of inflammation, atherosclerotic calcification, and diabetes (Hofmann et al. 2002). Earlier studies have shown that Nrf2 phosphorylation activates antioxidant responsive element (ARE), glutamate cysteine ligase (GCL) and HO-1 which converts MGO into lactic acid by glyoxalase-1 (Desai & Wu 2007; Vander Jagt 2008; Keum, Owuor, Kim, Hu, & Kong 2003; Li & Kong 2009).

Naturally occurring compounds, especially polyphenols, are of great interest considered as potential candidates to prevent the formation of AGEs, especially given their proven safety and efficacy (compared to synthetic compounds) in the prevention of cancer, hyperglycemia, heart disease and aging. Phenolic compounds like quercetin have been reported to possess a strong ability to attenuate oxidative damage by activating Nrf2 (Yeh & Yen 2006; Weng, Chen, Yeh & Yen 2011). For instance, higher intakes of quercetin have been found to reduce the risk of type 2 diabetes (Knekt et al. 2002). Apples being a rich source of polyphenols are considered to be beneficial in promoting good health. Qi et al. (2006), reported that consumption of whole apples and cereal bran decreased plasma glucose levels in non-insulin-dependent diabetic patients. It was also shown that women who had consumed an apple or more a day reduced their risk for type 2 diabetes by up to 28% (Song et al. 2005).

Tea consumption regularly has been reported to reduce cancer, heart disease, obesity and diabetes in humans, animal models, and cell lines (Grove & Lambert 2010; Naito & Yoshikawa 2009). For example, the consumption of Oolong tea has been reported to decrease plasma glucose and fructosamaine levels in diabetic patients (Hosoda et al. 2003) while green tea consumption promoted glucose metabolism in healthy human volunteers and reduced blood glucose levels both in diabetic db^+/db^+ mice and streptozotocin-induced diabetic mice (Tsuneki et al. 2004). A study by Sabu et al. (2002), found that catechins from green tea interacts with glucose metabolism by decreasing serum glucose levels in alloxan-induced diabetic rats. Green, Oolong, and black tea intakes were all reported to reduce plasma and liver triglyceride levels and plasma cholesterol levels in type 2 diabetes in Zucker rats and Sprague-Dawley rats fed with high sucrose diet (Hasegawa, Yamda, & Mori 2003; Yang, Wang, & Chen 2001). It has also been reported that tea catechins exhibited antithrombogenic activity in streptozotocin-diabetic rats by normalizing the thromboxane A2 (TXA2): prostacyclin I2 (PGI2) ratio and thereby improving kidney function (Yang, Choi, & Rhee 1999; Choi, Chang, & Rhee 2002; Rhee, Kim, & Kwag 2002). In another *in vivo* study, green tea extract was found to prevent AGEs formation and collagen crosslinking delaying collagen aging in C57BL/6 mice (Rutter et al. 2003).

Ginger (*Zingiber officinale Rosco*) is derived from Zingiberaceae and has been used worldwide as spice, dietary supplement, and traditional medicine for centuries (Butt and Sultan 2011). Several *in vivo* studies have found that ginger extract possess antidiabetic properties (Bordia, Verma, & Srivastava 1997; Akhani, Vishwakarma, & Goyal 2005; Bhandari, Kanojia, & Pillai 2005; Kadnur & Goyal 2005; Al-Amin, Thomson, Al-Qattan, Peltonen-Shalaby, & Ali 2006; Ojewole 2006; Kato et al. 2006; Islam & Choi 2008; Nammi, Sreemantula, & Roufogalis 2009; Madkor, Mansour, & Ramadan 2011; Ramudu et al. 2011; Shanmugam, Mallikarjuna, Kesireddy, & Sathyavelu Reddy 2011). Saraswat et al. (2010) found that ginger was effective against the development of diabetic cataract in rats mainly through its antiglycation activity. However, the active components in ginger responsible for the observed antidiabetic effects are still unknown.

This study was designed to investigate the cytoprotective properties of eight major bioactive compounds isolated from apple, tea and ginger against MGO-induced carbonyl stress and postulate a possible mode of action for the observed cytoprotective effects.

Materials and methods

Materials

Methylglyoxal, quercetin, chlorogenic acid, phloretin, (-)-epicatechin 3-gallate (ECG), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 4-vinylpyridine and Aminoguanidine (AG) were purchased from Sigma Chemicals (St. Louis, MO, USA). Anti-HO-1 (P 249) antibody was obtained from Cell Signaling Technology (Danvers, MA, USA). Anti-Nrf2 (SC 20) and anti-RAGE (SC 5563) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-GAPDH was purchased from Abcam (Cambridge, MA, USA). Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12), Fetal Bovine Serum (FBS), L- glutamine, Non-Essential Amino Acids (NEAA), Streptomycin/Penicillin at 10,000 μg/ml/10,000 U/ml) and phosphate- buffered saline (PBS) were procured from Hyclone (Belgium). Bioactive compounds (-)-epigallocatechin 3-gallate (EGCG), and (-)-epicatechin (EC) were purchased from Jiang Su Dehe Bio-Tech Co., LTD (Jiangsu, China). Compounds from Ginger ([6]-Gingerol and [6]-Shogaol), were isolated and characterized by members of the research team at North Carolina A&T State University, USA (Sang et al. 2009). All compounds were > 95% pure.

Cell culture and treatments

Human retinal pigmented epithelial (HRPE) cells were obtained from Lonza (Basel, Switzerland). Retinal pigment epithelial (RPE) cells form the outer blood retina barrier and play a key role in the pathological process of neovascularization. HRPE cells are well characterized cell lines expressing growth factors and receptors, which are used in the detoxification of AGEs (Spilsbury et al. 2000; Dunn et al. 1996; Tanihara et al. 1997). Cells were cultured in DMEM/F-12 medium (supplemented with 10% FBS, 3 mM glutamine, NEAA, and antibiotics (streptomycin/penicillin) and incubated at 37 °C in a humidified atmosphere of 5% CO_2.

Viability assay

MGO-induced cell toxicity was assessed using MTT colorimetric assay. For this assay, HRPE cells were seeded in 96-well plates (5×10^4 cells/well). The bioactive compounds (phloretin, ECG, EC, quercetin, [6]-shogaol, chlorogenic acid and [6]-gingerol) were dissolved in DMSO, whereas EGCG was dissolved in water and subsequent dilutions were made using cell growth media. Cells at 70–80% confluence were placed in DMEM/F-12 media free of FBS. The cells in the media were treated with MGO at different concentrations, ranging from 0–100 mM, and incubated for 24–96 h. In a different set of experiment, cells were treated with compounds at different concentrations, ranging from 10–100 μM, and incubated for