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3,4-Dihydroxyphenylacetic acid is a predominant biologically-active catabolite of quercetin glycosides

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ABSTRACT

Since dietary flavonoid glycosides, including quercetin 4′-glucoside from onion, are poorly absorbed from the gastrointestinal tract, they are converted into smaller phenolic acids, which can be absorbed into the circulation. The purpose of this study was to compare the effects of the major phenolic acid catabolites of quercetin 4′-glucoside, including 3,4-dihydroxyphenylacetic acid (DOPAC), 3-hydroxyphenylacetic acid, 3,4-dihydroxybenzoic acid (protocatechuic acid) and hippuric acid, on the antioxidant activity and phase II cytoprotective enzyme induction *in vitro*. Both DOPAC and protocatechuic acid, having a catechol moiety, exhibited both DPPH radical scavenging and superoxide dismutase-like activities, whereas 3-hydroxyphenyl acetic acid and hippuric acid did not. DOPAC also more potently enhanced the gene expression of several phase II drug-metabolizing enzymes than the other phenolic acid catabolites. DOPAC significantly inhibited the hydrogen peroxide-induced cytotoxicity in hepatocytes with the enhancement of the total glutathione S-transferase activity. In conclusion, DOPAC may play a key role in the antioxidative potential of the colonic lumen after the ingestion of the quercetin glycoside-rich onion.

1. Introduction

Flavonoids, the biggest group of dietary plant-originated polyphenols, may decrease the risk of chronic diseases such as heart disease and cancers (Duthie, Duthie, & Kyle, 2000). Most of the flavonoids have antioxidative activities and show biological responses including the inhibition of cell growth and modulation of signal transduction (Yang, Landau, Huang, & Newmark, 2001). Although flavonoid glycosides, a major form of flavonoids in nature, can be metabolized and partly absorbed in the small intestine, considerable amounts of them can pass through the small intestine (Murota & Terao, 2003). In the colon, almost all of the conjugated glycosides, glucuronides, and sulfates are removed from the flavonoid molecules by bacterial enzymes. The formed aglycones can be further catabolized into ring fission products such as phenolic acids (Rechner et al., 2002). It is thus suggested that the phenolic acid catabolites can be absorbed and thus may contribute to the health-promoting effects of flavonoid consumption.

Quercetin is one of the typical flavonol-type flavonoids in a wide variety of fruits and vegetables, usually occurring as glycosides (Yang et al., 2001). Quercetin glycosides containing a sugar group at the 3-position, such as quercetin 3-*O*-β-glucoside (Q3G, isoquercetrin), are commonly distributed in various fruits and vegetables, whereas quercetin 4′-*O*-β-glucoside (Q4′G) is one of the major glycosides in onion (Tsushida & Suzuki, 1995), which is the main source of flavonoids in the European diet (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993). Q4′G in comparison to Q3G or quercetin aglycone showed very little inhibition of either the azo radical- or ferrous ion-induced lipid peroxidation of human plasma LDL *in vitro* (Yamamoto, Moon, Tsushida, Nagao, & Terao, 1999). On the other hand, Murota et al. demonstrated that Q4′G is superior to Q3G in the protection of the rat intestinal mucosa from iron ion-induced lipid peroxidation, despite its lower free radical and iron ion-chelating activity (Murota et al., 2004). These data imply that vegetables rich in Q4′G, such as onion, may be

Abbreviations: Q3G, quercetin 3-*O*-β-glucoside; Q4′G, quercetin 4′-*O*-β-glucoside; OPAC, 3-hydroxyphenylacetic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; PCA, protocatechuic acid (3,4-dihydroxybenzoic acid); HPA, hippuric acid; DPPH, 1,1-diphenyl-2-picrylhydrazyl; SOD, superoxide dismutase; XA, xanthine; XOD, xanthine oxidase; NBT, nitroblue tetrazolium; FOX, ferrous ion oxidation-xylenol orange; PBS, phosphate buffered saline; GST, glutathione *S*-transferase; CDNB, 1-chloro-2,4-dinitrobenzene; GSH, glutathione; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); NQO1, NAD(P)H:quinone oxidoreductase 1; GCLC, glutamate-cysteine ligase, catalytic subunit; xCT, cystine/glutamate exchanger; HO-1, hemeoxygenase 1; CYP1A1, cytochrome P450 1A1; BHT, 2,6-di-*tert*-butyl-4-methylphenol; AhR, aryl hydrocarbon receptor; ARE, antioxidant response element; XRE, xenobiotic responsive element.

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beneficial in preventing oxidative damage to the intestinal tract. Also, the antioxidative potential of Q4′G might be corroborated by the effective bacterial metabolism in the intestinal mucosa (Murota et al., 2004). Moreover, a previous study using radiolabeled Q4′G ([2-^{14}C]Q4′G) revealed that Q4′G passes through the gastrointestinal tract of rats and almost all of Q4′G is converted to phenolic acids (Mullen et al., 2008). This study also showed that substantial amounts of 3-hydroxyphenylacetic acid (OPAC) and 3,4-dihydroxyphenylacetic acid (DOPAC) and smaller quantities of 3,4-dihydroxybenzoic acid (PCA) were detected in the colorectum, whereas no quercetin conjugates were found. The urine and feces mainly contained radiolabeled hippuric acid (HPA) and OPAC with only trace amounts of the quercetin metabolites. The products from the catabolism of Q4′G are presented in Fig. 1.

The interest in fermented products formed by the colonic microbiota in the large intestine, including the metabolites of polyphenols as well as the short-chain fatty acids, and their role in bowel diseases has started to rapidly grow. Because they more precisely reflect the physiological conditions than intact polyphenols *in vitro*, studies with the bacterial metabolites of polyphenols are needed to explain the actual biological properties *in vivo*. In the present study, we compared the effects of the major phenolic acid catabolites of Q4′G, including DOPAC, OPAC, PCA and HPA on DPPH radical, superoxide and detoxification enzyme level *in vitro*. We also demonstrated that DOPAC is a predominant antioxidative catabolite of Q4′G formed by the colonic microbiota in the large intestine.

2. Materials and methods

2.1. Materials

Quercetin and the major phenolic acid metabolites of Q4′G (DOPAC, OPAC, PCA and HPA) were purchased from Sigma (Poole, United Kingdom). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Tokyo Chemical Industry (Tokyo, Japan). All other chemicals were obtained from Wako Pure Chemicals Industries (Osaka, Japan).

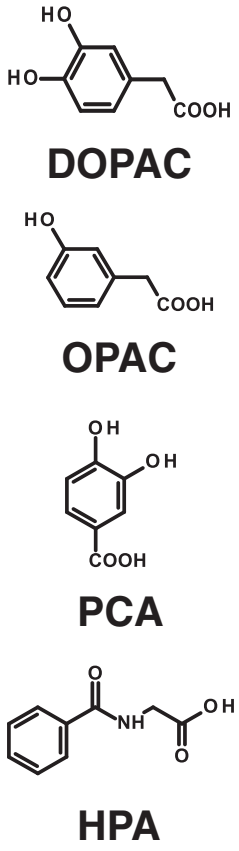

Fig. 1. Chemical structures of phenolic acids from the catabolism of Q4′G.

2.2. DPPH radical scavenging assay

The DPPH radical scavenging activity was evaluated as reported previously (Nakamura, Watanabe, Miyake, Kohno, & Osawa, 2003). A test compound (ethanol solution, 2 ml) mixed with a 100 mM Tris-HCl buffer (pH 7.4, 2 ml) was added to 0.5 mM DPPH in ethanol (1 ml), and the mixture was shaken vigorously and kept for 20 min at room temperature in the dark. The DPPH radical scavenging activity is expressed as the ratio of the relative decrease in the absorbance of the test sample mixture at 517 nm to that of the 1 mM Trolox solution. The experiment was done in triplicate. DPPH radical scavenging activity (%) = {(ethanol alone) − (test compound)} / {(ethanol alone) − (Trolox)} × 100.

2.3. Superoxide scavenging assay in the xanthine/xanthine oxidase system

Superoxide dismutase (SOD)-like activity was measured by a xanthine (XA)/XA oxidase (XOD) system using a SOD Test Wako kit (Nakamura, Ohto, Murakami, & Ohigashi, 1998) accordingly to the manufacturer's instruction. In this system, SOD-like superoxide scavenging activity was estimated by measuring the nitroblue tetrazolium (NBT) reduction.

2.4. Determination of hydrogen peroxide formation

Hydrogen peroxide amount was determined by the ferrous ion oxidation-xylenol orange (FOX) method (Qi et al., 2011). The FOX reagent-A contained 25 mM H_2SO_4, 100 mM sorbitol, 250 μM $Fe(NH_4)_2(SO_4)_2$, and 125 μM xylenol orange. The FOX-B contained 25 mM H_2SO_4, 100 mM sorbitol, and 250 μM $Fe(NH_4)_2(SO_4)_2$. This assay method was applicable to determine hydrogen peroxide concentrations as low as 0.2 μM. Briefly, the collected sample (45 μl) was mixed with 5 μl of methanol and incubated at room temperature for 30 min. The FOX-A reagent (0.45 ml) or FOX-B reagent for reference was added and incubated for 30 min. The solutions were then centrifuged at 15,000g for 10 min at room temperature and the absorbance at 560 nm was measured. The concentration of hydrogen peroxide was calculated from standard curve.

2.5. Cell cultures

The mouse hepatoma cell line Hepa1c1c7, obtained from the American Type Culture Collection, was grown and maintained at 37 °C in α-minimum essential medium (α-MEM, Nissui Pharmaceutical, Tokyo, Japan) containing 10% fetal bovine serum, 4 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. For experiments, cells were seeded in complete medium and treated with each reagent or DMSO vehicle (final 0.1%, v/v). RL34 cells were obtained from the Health Science Research Resources Bank, Osaka, Japan (Yamada, Okigaki, & Awai, 1987). The cells were grown as monolayer cultures in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (0.3 mg/ml), pyruvic acid (0.11 mg/ml), and 0.37% $NaHCO_3$ at 37 °C in an atmosphere of 95% air and 5% CO_2. The post-confluency RL34 cells were exposed to the test compounds in a medium containing 10% fetal bovine serum.

2.6. Reverse transcription-polymerase chain reaction (RT-PCR)

Cells were washed with ice-cold phosphate buffered saline (PBS) (−). Total cellular RNA was isolated using Trizol reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's recommendations. RNA was quantified by measuring absorbance at 260 nm. Total

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