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Original Article

Anti-oxidant and Anti-inflammatory Effects of Rutin and Its Metabolites

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Abstract

Rutin is one of the major flavonoids found in buckwheat (*Fagopyrum esculentum* Moench). While rutin is already known to exhibit anti-oxidative, anti-inflammatory, and anti-carcinogenic activities. However, the health beneficial function of rutin metabolites is not well understood. In DPPH radical scavenging assays, the present study found that 3,4-dihydroxyphenyl acetic acid had the highest total anti-oxidant activity, followed by 3,4-dihydroxyphenylacetic acid, rutin, homovanillic acid, and 3-hydroxyphenyl acetic acid. Further, 3,4-dihydroxyphenylacetic acid strongly reduced LPS-induced IL-6 production in RAW 264.7 cells, compared with other metabolites. Therefore, these results suggest that rutin metabolites have potential to be utilized as food ingredients with anti-oxidant and anti-inflammatory activities.

Keywords : Anti-oxidant, Anti-inflammatory, Rutin, Rutin metabolites, Buckwheat

Introduction

Oxidative stress induced by free radicals has been associated with several cellular toxic processes including oxidative damage to proteins and DNA, membrane lipid oxidation, enzyme inactivation, and gene mutation that may lead to inflammation and carcinogenesis (Poulsen et al. 1998). Inflammation is a response of organisms to the presence of pathogens, chemicals and mechanical injury. However, continuous inflammation can lead to various chronic diseases such as cancer, Alzheimer's, and atherosclerosis (Kwon et al. 2010). During the process of inflammation, macrophages actively participate in inflammatory responses by releasing the pro-inflammatory cytokines, tumor necrosis factor (TNF)-α, interleukin (IL)-6, and cyclooxygenase-2 (COX-2), as well as other inflammatory factors. Especially, IL-6 is a cytokine with multiple biological activities including regenerative and anti-inflammatory effects, and it contributes to the host defense against pathogens. Whereas, the accelerated production of IL-6 plays a significant pathological role in cancer (Clavin et al. 2007 Muthusamy et al. 2011; Kim et al. 2012). Therefore, the intake of sufficient amounts of anti-oxidants is necessary to prevent free radical-induced oxidative stress and inflammation.

Rutin (*3-O-rhamnosylglucosyl-quercetin*, Figure 1A) is a major flavonoids found in a variety of plants. Especially, buckwheat (*Fagopyrum esculentum* Moench) plant including the leaves, flowers, stalks, and seeds is known as a major source of rutin (Watanabe et al. 1997; Holasova et al. 2002). First, rutin is hydrolyzed into its aglycone, quercetin, by the intestinal microflora (Gao al. 2006; Jaganath et al. 2009; Pashikanti et al. 2010), and then, quercetin is converted to various metabolites, such as 3,4-dihydroxyphenyl acetic acid (DHPAA), 3-hydroxyphenyl acetic acid (HPAA), 3,4-dihydroxyphenylacetic acid (DHT), and homovanillic acid (HVA) Figures. 1B, C, D, E). Considerable attention has been focused on the health-benefiting biological activities of rutin and quercetin, including their anti-oxidative (Chen et al. 2006), anti-inflammatory (Lin et al. 2009; Pastore et al. 2009; Yu et al. 2009), and anti-carcinogenic (Yang et al. 2000) effects. However, the biological activities of rutin metabolites have received little attention. Accordingly, the present study investigated the anti-oxidant and anti-inflammatory effects of rutin metabolites.

Materials and Methods

Chemicals

Dulbecco's modified Eagle's medium (DMEM), the penicillin-streptomycin, and 0.5% trypsin-EDTA were obtained from GIBCO® Invitrogen (Auckland, NZ). The fFetal bovine serum (FBS), rutin, DHPAA, HPAA, DHT, HVA,2-diphenyl-1-picryl-hydrazyl (DPPH) vitamin C, and lipopolysaccharides (LPS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The eEnzyme-linked immunosorbent assays (ELISA) set against mouse IL-6 was obtained from BD Biosciences (San Jose, CA, USA).

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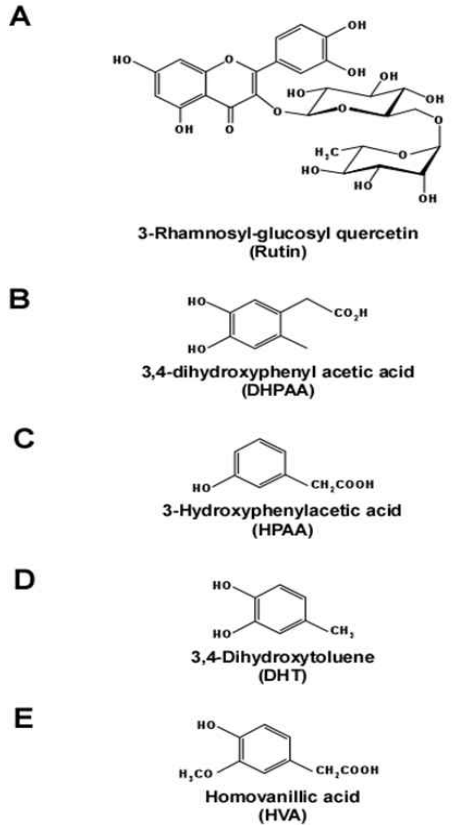

Figure 1. Chemical structures of rutin (A), 3,4-dihydroxyphenyl acetic acid (DHPAA) (B), 3-hydroxyphenyl acetic acid (HPAA) (C), 3,4-dihydroxyphenylacetic acid (DHT) (D) and homovanillic acid (HVA) (E).

Cell culture

The RAW 264.7 mouse macrophage cell line was obtained from the Korean Cell Line Bank (Seoul, Korea). The RAW 264.7 cells were cultured in DMEM and incubated at 37℃ in a 5% CO_2 incubator. The cells were then incubated in DMEM containing 10% FBS, 2 mM L-glutamine, and 100 μg penicillin/ 100 μg/mL streptomycin.

DPPH radical scavenging activity

The method of Brand-Williams et al. was used with slight modifications. The DPPH radicals were dissolved in 80% aqueous methanol, and various concentrations of 0.1 mL of the sample solution were added to 2.9 mL of the DPPH radical solution. The mixture was then shaken vigorously and allowed to stand at 23℃ in the dark for 20 min, at which time the decrease in absorbance at 517nm was measured using a microplate reader (Sunrise-Basic Tecan, Tecan Austria GmbH 5082 Groding, Austria). The control solution consisted of 0.1 mL of 50% aqueous methanol and 2.9 mL of the DPPH radical solution. The radical stock solution was freshly prepared each day.

Sandwich ELISA

For the sandwich ELISA, the RAW 264.7 cells were seeded at a density of 5 $\times 10^5$ per well in a 96-well plate. After 24 h incubation, the cells were stimulated with 4 mg/mL LPS in the presence or absence of rutin, DHPAA, HPAA, DHT, and HVA for the indicated periods. Thereafter, the media were collected to measure the IL-6 level. The sandwich ELISA was performed according to the manufacturer's protocol. The 96-well plates were read at an absorbance of 450 nm.

Statistical analysis

When applicable, the data are expressed as mean and standard deviation (SD) values, and Student's t test was used for single statistical comparisons. A probability value of $p<0.05$ was used as the criterion for statistical significance.

Results and Discussion

Free radical scavenging activities of rutin and rutin metabolites

Epidemiological studies indicate that a high intake of flavonoid may prevent inflammatory diseases by suppressing oxidative stress (Garcia et al. 2005; Weseler et al. 2011; Gillette-Guyonnet et al. 2013; Tanaka 2013). Rutin is a well-known flavonoid found in various, fruits, vegetables, and grains, including buckwheat (Kreft et al. 1999). It has been shown that rutin is not absorbed to any degree in the small intestine and that sizable amounts pass into the colon (Jaganath et al. 2009). Rutin is hydrolyzed into quercetin and sugar moiety by enzymes such as β-rhamnosidases and β-glucosidases produced by colonic bacteria. The cleavage of B-ring in quercetin results in the production of phenolic acids such as DHPAA, HPAA, DHT and HVA. In the presence of glucose, there was 60–97% conversion of quercetin to DHPAA in the fecal samples (Jaganath et al. 2009). These metabolized compounds are considered as actual active components of various foods. However, the anti-oxidant and anti-inflammatory effects of rutin metabolites have not been well investigated. Therefore, the anti-oxidant activity of rutin and its metabolites was initially investigated by measuring the DPPH free radical scavenging capacities. Vitamin C, a well-known anti-oxidant, was used as the positive control. In the DPPH radical scavenging assays, DHPAA showed the highest total anti-oxidant activity (6.8 μ

g/mL vitamin C equiv/50 μM DHPAA), followed by DHT (6.4 μg/mL vitamin C equiv/50 μMDHT), rutin (8.3 μg/mL vitamin C equiv/50 μMrutin), HVA (5.6 μg/mL vitamin C equiv/50 μM HVA), and HPAA(1.7 μg/mL vitamin C equiv/50 μM HPAA) (Figure 2), indicating that DHPAA and DHT could be applied as anti-oxidant agents, whereas HPAA had no affect on the DPPH free radical scavenging capacities.

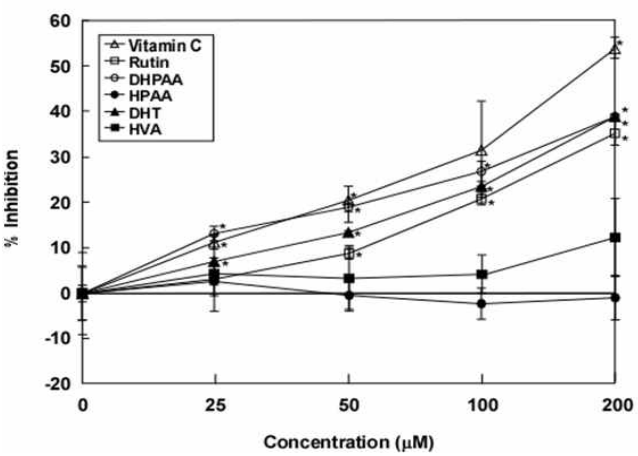

Figure 2. Anti-oxidant activity of rutin or rutin metabolites.

Anti-oxidant activities were measured using the DPPH radical scavenging assays as described in Materials and Methods. Results are expressed as cell viability relative to untreated control. Data are represented as the means ± SD as determined from three independent experiments. The asterisk (*) indicates a significant difference ($p<0.05$) compared with untreated control.

Effects of rutin and rutin metabolites on LPS-induced IL-6 production in Raw 264.7 cells

In a previous studies, it has been suggested that pro-inflammatory cytokines (e.g., TNF-a, IL-6) are highly expressed and secreted by macrophages in response to various pathogens. In particular, IL-6 is one of the most important mediators of the immune and inflammatory responses (Clavin et al. 2007; Muthusamy et al. 2011; Kim et al. 2012). Therefore, the effects of rutin and its metabolites on LPS-induced IL-6 production in RAW 264.7 cells were investigated using a sandwich ELISA. The results showed that DHT strongly suppressed the LPS-induced IL-6 production by 59, 7.2, and 0.6% at the indicated concentrations (10, 20, or 40 μM) (Figure 3). Interestingly, treatment of Raw 264.7 cells with rutin, DHPAA, HPAA, and HVA at the concentration of 40 μM reduced IL-6 production to the level comparable to un-treated control group. These results suggested that rutin metabolites may contribute to the anti-inflammatory activity of diverse foods. However, the obvious linear relationship was not detected between anti-oxidant and anti-inflammatory activities of the rutin and its metabolites.

In some cases, polyphenols which possess similar levels of free radical scavenging activities, can have various effects on inhibition of inflammatory signaling pathways (Kang et al. 2011). In previous studies, quercetin and gallic acid were shown to have a different effect on hydrogen peroxide-induced inhibition of gap junction intercellular communication (GJIC), which is closely related to tumor promotion, despite having similar free radical scavenging activities (Kim et al. 2008; Lee et al. 2010). Furthermore, well-known anti-oxidants, such as gallic acid and butylated hydroxyanisole, did not have any effect (Kim et al. 2009). Therefore, anti-oxidant activity cannot explain all the anti-inflammatory effects of polyphenols, and it is necessary to understand the role of rutin and rutin metabolites against oxidative stress-mediated signaling pathways involved in inflammation.

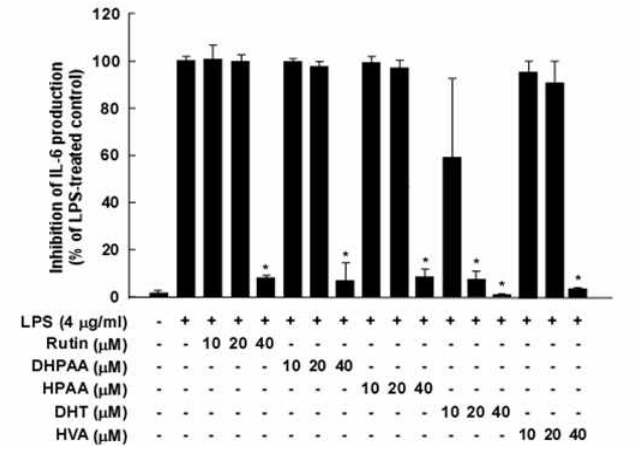

Figure 3. Effects of rutin and its metabolites on LPS-induced IL-6 production in Raw 264.7 cells.

Cells were treated with samples at indicated concentrations (10, 20, 40 μM) for 12 h. The level of IL-6 was measured by sandwich ELISA. Data are represented as the means±SD as determined from three independent experiments. The asterisk (*) indicates a significant.

Conclusion

According to the present study, the free radical scavenging activities of rutin and its metabolites were in the following decreasing order: DHPAA > DHT >rutin> HVA > HPAA. In contrast to expectations, the sandwich ELISA results revealed that DHT showed the strongest suppression of LPS-induced IL-6 production when compared with rutin and the other metabolites. However, no obvious linear relationship was detected between the anti-oxidant activities and anti-inflammatory activities of the rutin metabolites. Thus, further studies are needed to understand the role of rutin and rutin metabolites against oxidative stress-mediated inflammatory

signaling pathways.

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