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Quercetin-3-O-(2''-galloyl)-α-L-rhamnopyranoside prevents TRAIL-induced apoptosis in human keratinocytes by suppressing the caspase-8- and Bid-pathways and the mitochondrial pathway

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ABSTRACT

Quercetin and its derivatives have antioxidant and anti-inflammatory effects. Nevertheless, in human keratinocytes, compared to the reports on other toxic insults, researches on the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis that may be involved in skin diseases are rare. Furthermore, the effect of quercetin-3-O-(2''-galloyl)-α-L-rhamnopyranoside (QGR), a new quercetin derivative, on TRAIL-induced apoptosis in keratinocytes has not been studied. In this respect, we investigated the effect of QGR on TRAIL-induced apoptosis in human keratinocytes. TRAIL triggers apoptosis by inducing a decrease in Bid, Bcl-2, Bcl-xL and survivin protein levels, increase in Bax and VDAC1 levels, loss of the mitochondrial transmembrane potential, release of cytochrome *c*, activation of caspases (−8, −9 and −3), cleavage of PARP-1, and an increase in the tumor suppressor p53 levels. Treatment with QGR prevented TRAIL-induced apoptosis-related protein activation, formation of reactive oxygen species, nuclear damage, and cell death. In contrast, quercetin induces cytotoxicity and had an additive effect on TRAIL-induced apoptosis-related protein activation and cell death. These results suggest that the QGR, unlike quercetin, may reduce TRAIL-induced apoptosis in human keratinocytes by suppressing the activation of the caspase-8- and Bid-pathways and the mitochondria-mediated cell death pathway, which is associated with the formation of reactive oxygen species. These data suggest that QGR could be effective in the prevention of TRAIL-induced apoptosis-mediated skin diseases.

1. Introduction

The tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) has been shown to be involved in inflammatory and immune responses and apoptosis in skin diseases, such as atopic dermatitis [1–3]. Increased expression of TRAIL is detected in T cells and monocytes in the peripheral blood and skin lesions in patients with atopic dermatitis [3,4]. Keratinocytes are considered to play a critical role in the pathogenesis of the inflammatory skin diseases atopic dermatitis and psoriasis [5,6]. Dysregulated apoptosis has been suggested to induce inflammation and cancer in the skin [7]. Dysregulated apoptosis in keratinocytes may reduce the defensive efficiency of the dermis against allergens and infections. Apoptosis of keratinocytes caused by skin-infiltrating T cells may be involved in the formation of eczema in atopic dermatitis [1,8]. TRAIL induces apoptosis via the activation of the cell death receptors and the mitochondria-mediated apoptotic pathway [9,10].

The flavonoid quercetin and its derivatives have anti-oxidant, anti-inflammatory, and anti-tumor effects [11–13]. Quercetin and quercetrin hydrate reduce the oxidative stress-induced cell death in keratinocytes through their anti-oxidant action [12]. Quercetin inhibits the UV irradiation-induced inflammatory cytokine production in primary human keratinocytes by suppressing the NF-κB pathway [13]. The derivative quercetin 3-O-α-L-arabinofuranoside inhibits the lipopolysahharide-stimulated expression of nitric oxide synthase and cyclooxygenase-2 and the production of PGE_2 in HaCaT cells [14]. Quercetin attenuates the H_2O_2-induced mitochondrial and cell damage and apoptosis in the human keratinocyte cell line NCTC 2544 cells and HaCaT cells [12,15]. In contrast, quercetin induces cell death in various cancer cells and enhances the sensitivity of cancer cells to anticancer drugs and TRAIL [16–19]. In addition, it has been shown that polyphenols, including quercetin does not prevent UVC-induced apoptosis in both human HaCaT cells and breast cancer cells MCF 7 [20]. Therefore, quercetin may have a differential effect on cell dysfunction and death depending on the type of cell or the toxic insult.

The regulation of disrupted apoptosis may confer a benefit in the treatment of inflammatory skin diseases [7]. In human

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keratinocytes, compared to the reports on other toxic insults, the quercetin researches on the TRAIL-induced apoptosis that may be involved in skin diseases are rare. Furthermore, the effect of quercetin-3-O-(2''-galloyl)-α-L-rhamnopyranoside (QGR), a new quercetin derivative, on TRAIL-induced apoptosis in keratinocytes has not been studied. To assess the preventive effect of QGR on apoptosis-mediated skin diseases, we investigated effect of QGR on TRAIL-induced apoptosis in human keratinocytes in relation to apoptotic process.

2. Materials and methods

2.1. Materials

The TiterTACS™ colorimetric apoptosis detection kit was purchased from Trevigen, Inc. (Gaithersburg, MD, USA). The assay kits for human cytochrome *c* (Quantikine® M) and caspases (−8, −9 and −3) were purchased from R&D systems (Minneapolis, MN, USA). The antibodies (Bid, Bax, Bcl-2, Bcl-xL, survivin, cytochrome *c*, voltage-dependent anion channel (VDAC1), PARP-1, p53, and β-actin) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). TRAIL (Apo2L; human recombinant), horseradish peroxidase-conjugated anti-mouse IgG, z-Asp-(OMe)-Gln-Met-Asp(OMe) fluoromethyl ketone (z-DQMD.fmk), and z-Ile-Glu-(O-ME)-Thr-Asp(O-Me) fluoromethyl ketone (z-IETD.fmk) were all purchased from EMD-Calbiochem (La Jolla, CA, USA). The SuperSignal® West Pico chemiluminescence substrate for the cytochrome *c* detection in Western blot analysis was purchased from PIERCE Biotechnology Inc. (Rockford, IL, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), z-Leu-Glu-(O-ME)-His-Asp(O-Me) fluoromethyl ketone (z-LEHD.fmk), 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6(3)$), and other chemicals were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA).

2.2. Extraction, isolation and structural identification of quercetin-3-O-(2''-galloyl)-α-L-rhamnopyranoside

Quercetin-3-O-(2''-galloyl)-α-L-rhamnopyranoside (QGR) was isolated from the leaves of *Acer ginnala* Maxim collected from Korea National Arboretum (Pocheon, Korea, Fig. 1). The leaves (1 kg) of *Acer ginnala* were extracted several times with 80 % methanol at room temperature. After the removal of methanol under vacuum, aqueous solution was filtered through filter paper (Tokyo Roshi Kaisha Ltd., Japan). The solution filtered was concentrated (227.5 g), applied to the column filled with Sephadex LH-20 (25–100 μm, 2 kg, 10 × 80 cm, GE Healthcare Bio-Science AB, Uppsala, Sweden) and the solution then was eluted with H_2O contained methanol (30–100% gradient), which afforded 3 sub-fractions. The fraction 3 (43 g) was applied to the columns filled with MCI-gel CHP20P (75–150 μm, 600 g, 5 × 60, Mitsubishi Chemical, Tokyo, Japan) with a gradient elution system of water–methanol. From these procedures, quercetin-3-O-(2''-galloyl)-α-L-rhamnopyranoside (QGR, 850 mg) was yielded. Purity of QGR was analyzed by high performance liquid chromatography (Waters 600 system, Milford, MA, USA). The yield had approximately 98% purity.

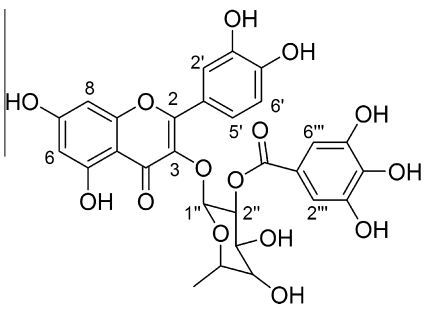

Fig. 1. The chemical structure of QGR.

The structural identity of QGR was characterized by spectral analyses. The 1D nuclear magnetic resonance (NMR) findings such as, ^{1}H- (600 MHz) and ^{13}C- (150 MHz) NMR were recorded with Gemini 2000 (Varian, Palo Alto, CA, USA). Low-resolution fast atom bombardment mass spectrum (LRFAB-MS) was recorded with JMS-600W (JEOL, Tokyo, Japan).

Yellow amorphous powder, negative FAB-MS: *m/z* 599 $[M-H]^-$. ^{1}H-NMR (600 MHz, CD_3OD) : δ 1.03 (3H, d, *J* = 5.4 Hz, H-6″), 3.46–3.48 (^{2}H, m, H-4″,5″), 4.02 (^{1}H, dd, *J* = 3.6, 9.0 Hz, H-3″), 5.49 (^{1}H, d, *J* = 1.5 Hz, H-1″), 5.62 (^{1}H, dd, *J* = 1.5, 3.6 Hz, H-2″), 6.19 (^{1}H, d, *J* = 1.8 Hz, H-6), 6.37 (^{1}H, d, *J* = 1.8 Hz, H-8), 6.94 (^{1}H, d, *J* = 8.4 Hz, H-5′), 7.07 (^{2}H, s, H-2‴, 6‴), 7.34 (^{1}H, dd, *J* = 2.4, 8.4 Hz, H-6'), 7.37 (^{1}H, d, *J* = 2.4 Hz, H-2′). ^{13}C-NMR (125 MHz, CD_3OD) : δ 16.4 (C-6″), 69.3 (C-3″), 70.8 (C-5″), 72.1 (C-2″), 72.4 (C-4″), 93.3 (C-8), 98.5 (C-6), 99.1 (C-1″), 104.4 (C-10), 108.9 (C-2‴, 6‴), 115.1 (C-5′), 115.5 (C-2′), 119.8 (C-1‴), 121.4 (C-6′), 121.5 (C-1′), 134.2 (C-3), 138.5 (C-4‴, 145.0 (C-3‴, 5‴), 148.4 (C-4′), 157.1 (C-9), 157.9 (C-2), 161.7 (C-5), 164.4 (C-7), 166.1 (C-7‴), 178.0 (C-4).

2.3. Keratinocyte culture

Human keratinocytes (HEK001, tissue: skin; morphology: epithelial; cell type: human papillomavirus 16 E6/E7 transformed) were purchased from American Type Culture Collection (Manassas, VA, USA) and cultured in keratinocyte-SFM supplemented with bovine pituitary extract, recombinant epidermal growth factor, 100 U/ml penicillin, and 100 μg/ml streptomycin (GIBCO®, Invitrogen Co., Grand Island, NY, USA).

2.4. Cell viability assay with MTT reduction

Cell viability was measured using the MTT assay, which is based on the conversion of MTT to formazan crystals by mitochondrial dehydrogenases dehydrogenases, and frequently used to measure cellular growth and survival [21]. Keratinocytes (3×10^4 cells/200 μl) were treated with TRAIL for 24 h at 37 °C. The medium (200 μl) was then incubated with 10 μl of 10 mg/ml MTT solution for 2 h at 37 °C. After centrifugation at 412*g* for 10 min, the culture medium was removed, and 100 μl of dimethyl sulfoxide was added to each well to dissolve the formazan. The absorbance was measured at 570 nm using a microplate reader (Magellan, TECAN, Salzburg, Austria). Cell viability was expressed as a percentage of the absorbance value of control cultures.

2.5. Cell viability assay with neutral red uptake

Cell viability was determined using the neutral red uptake assay, which is based on the observation that neutral red is accumulated in the lysosomes of live cells [22]. Keratinocytes (3×10^4) were treated with TRAIL for 24 h at 37 °C. The cell suspension (200 μl) was then incubated with 10 μl of 1 mg/ml neutral red solution for 3 h at 37 °C. After centrifugation at 412*g* for 10 min, the culture medium was removed, and the dye was extracted with 100 μl of a 1% acetic acid and 50% ethanol solution for 20 min. The absorbance was measured at 540 nm using a microplate reader.

2.6. Measurement of oligonucleosomal DNA fragmentation

DNA fragmentation due to the activation of endonucleases was assessed by gel electrophoresis. Keratinocytes (4×10^6 cells/ml) were treated with TRAIL for 16 h at 37 °C and then washed with PBS. DNA was isolated with the DNA purification kit, according