Food Research International 44 (2011) 109–113

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Plant flavonol quercetin and isoflavone biochanin A differentially induce protection against oxidative stress and inflammation in ARPE-19 cells

Niina M.M. Saviranta [a], Laura Veeroos [a], Lars J. Granlund [a], Viivi H. Hassinen [a], Kai Kaarniranta [b], Reijo O. Karjalainen [a,c,*]

[a] University of Eastern Finland, Department of Biosciences, P.O. Box 1627, 70211 Kuopio, Finland
[b] University of Eastern Finland and Kuopio University Hospital, Department of Ophthalmology, P.O. Box 1627, 70211 Kuopio, Finland
[c] AgriFood Research Finland, 31600 Jokioinen, Finland

ARTICLE INFO

Article history:
Received 29 June 2010
Accepted 31 October 2010

Keywords:
Functional ingredients
Oxidative stress
Inflammation
Eye health
Quercetin
Biochanin A

ABSTRACT

In this work, differential ability of plant flavonol quercetin and plant isoflavone biochanin A to modulate oxidative stress and inhibit inflammation-related responses was investigated using human retinal pigment epithelial cells (RPE) at gene expression level. Quercetin protected cells from oxidative stress-induced cell death, whereas biochanin A had no statistically significant protective effects. Quercetin reduced the expression of cytokines IL-6 and IL-1β in cells treated with H_2O_2, and expression levels of Nrf2 and HO-1 were increased by quercetin treatment suggesting protective function against oxidative stress. Our data indicate that quercetin may protect cells by inhibiting the production of pro-inflammatory factors such as IL-6, and by inducing the expression of ROS-catalyzing phase II proteins such as HO-1. Therefore, plant extracts rich in flavonol quercetin may be an interesting resource for functional food products and other foods targeted for reduced risks of age-related macular degeneration.

1. Introduction

Functional type foods containing increased levels of health-promoting compounds hold a great potential to lower the risk or even prevent certain chronic diseases capable of causing huge costs for health care. Food companies are currently focusing great efforts for the development of functional "mixture" drinks which contain significant amounts of distinct types of bioactive compounds from different fruits or berries (González-Molina, Moreno and García-Viguera, 2009). There are some evidence supporting the idea that additive and synergistic effects of phytochemicals may be responsible for the antioxidant properties in a diet rich in fruits and vegetables (Liu, 2003; Park et al., 2008). Thus, development of functional type of foods targeted for chronic diseases such as neurodegenerative diseases (Zafrilla, Morillas, Rubio-Perez and Cantos Villar, 2009) may benefit if bioactive compounds possessing different levels of anti-inflammatory and anti-oxidative properties will be combined in the specific functional food target (Park et al., 2008; Ho et al., 2009).

Age-related macular degeneration (AMD) is the most common cause of irreversible loss of central vision in elderly people in developed countries. According to World Health Organization (WHO), 50 million persons suffer from AMD symptoms and 14 million persons are blind or severely visually impaired because of AMD (Gehrs, Anderson, Johnson and Hageman, 2006). AMD is divided into early and late diseases, as well as the atrophic and exudative degeneration categories. Both forms of AMD involve the appearance of local inflammation but the neovascularisation through Bruch's membrane and the RPE layer is a diagnostic marker for exudative AMD (Kaarniranta et al., 2010). Primarily AMD is characterized by degeneration of the macular retinal pigment epithelial (RPE) cells. RPE cells are exposed to chronic oxidative stress; they are constantly absorbing light energy and undergoing phagocytization of the lipid rich shed tips of photoreceptor outer segments involved in physiological visual cycle. Oxidative stress refers to progressive cellular damage and chronic inflammation that contributes to protein misfolding and functional abnormalities in the RPE cells during cellular senescence (Kaarniranta & Salminen, 2009; Kaarniranta et al., 2010). Degeneration and cell death of RPE cells cause secondary adverse effects on neural retina leading to visual loss. In the absence of effective preventive treatment for AMD, the number of patients severely disabled by AMD is expected to triplicate during the next decades. Interestingly, nutritional factors might prevent AMD development that is demonstrated in the Age-Related Eye Disease Study

Abbreviations: AMD, age-related macular degeneration; ARE, antioxidant response element; COX-2, cyclooxygenase-2; GST, glutathione S-transferase; HO-1, heme oxygenase-1; IL-1β, interleukin-1β; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; NF-κB, nuclear factor-κB; Nrf2, nuclear factor-erythroid 2-related factor-2; qRT-PCR, quantitative real-time polymerase chain reaction; ROS, reactive oxygen species; TNF-α, tumor necrosis factor α.

* Corresponding author. University of Eastern Finland, Department of Biosciences, P.O. Box 1627, 70211 Kuopio, Finland. Tel.: +358 40 3553834; fax: +358 17 2811510.
E-mail address: reijo.karjalainen@uef.fi (R.O. Karjalainen).

0963-9969/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2010.10.056

(AREDS, 2001). This study indicates that people at high risk of developing advanced stages of AMD lowered their risk by about 25% when treated with a high-dose combination of different antioxidants.

Inability to protect cells from oxidative stress often leads to the accumulation of reactive oxygen species (ROS) which induce the activation of nuclear factor-κB (NF-κB) resulting in the coordinated expression of inflammatory and innate immunity genes and secretion of pro-inflammatory cytokines (Surh & Na, 2008). Accumulated data strongly suggest that continuous up-regulation of pro-inflammatory mediators (e.g., tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), IL-6, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS)) is induced during the aging process due to an age-related redox imbalance that activates many pro-inflammatory signaling pathways (Chung et al., 2009). One indication of that is the higher level of the systemic IL-6 that is independently associated with progression of AMD. Activation of antioxidant defense and phase II enzymes is a key system to protect cells from oxidative damages. Nuclear factor-erythroid 2-related factor-2 (Nrf2) is one of the most essential transcription factors which plays a key role in antioxidant response element (ARE)-mediated activation of phase II and antioxidant enzymes (Nguyen, Nioi and Pickett, 2009). In transgenic Nrf2 deficient mice, increased disease susceptibility was associated with decreased expression of antioxidant/phase II detoxifying enzymes in parallel with up-regulation of pro-inflammatory cytokine markers (Li et al., 2008) suggesting that Nrf2-mediated protection against oxidative stress may be based both on activation of cellular antioxidant systems and suppression of pro-inflammatory signaling pathways. These findings suggest that Nrf2 bind to *cis*-acting elements in the promoters of the antioxidant target genes such as coding for glutathione S-transferase (GST) and heme oxygenase-1 (HO-1), but the well-known detoxifying enzymes, catalases and superoxide dismutase, may also be the molecular target of Nrf2 (Braun et al., 2002).

Polyphenolic compounds can also significantly inhibit the formation of ROS and thereby limit the oxidative and inflammation-related harmful impacts on target cells. Experimental data indicate (Kimura et al., 2009) that quercetin protected cells from UVA oxidative damages by elevating intracellular antioxidant activity by enhancing the activation of transcription factor Nrf2. Further, Johnson, Maher and Hanneken (2009) found in RPE cells that citrus flavonoid (eriodictyol) provided long-term protection against oxidative stress by the activation of Nrf2, and the induction of phase II enzymes. Consequently, recent data suggest that targeting Nrf2/ARE signaling pathway represents a promising avenue to identify phytochemicals for the construction of specific functional products to reduce the risks of developing age-related macular degeneration.

In this work, we will concentrate on two phenolic compounds of potentially different protective activity, quercetin and biochanin A (Fig. 1). Flavonol quercetin occurs abundantly in apples, onions and many berries. Quercetin is well known for its high antioxidant ability and epidemiological evidence suggests that high intake of quercetin is positively associated with the reduced occurrence of cardiovascular diseases (Knekt et al., 2002). Plant isoflavone, biochanin A occurs abundantly in red clover leaves and blossoms (Saviranta, Anttonen, von Wright and Karjalainen, 2008). It was recently found (Park et al., 2008) that combined treatments with low concentrations of several natural phenolic compounds such as phytoestrogenic genistein, resveratrol and flavonol quercetin may be useful in the treatment of obesity through the suppression of adipogenesis and enhanced adipocyte apoptosis. In this work, we provide new information about the differential ability of quercetin and biochanin to modulate oxidative stress and the ability of quercetin to inhibit inflammation-related responses in human retinal pigment epithelial (RPE) cells.

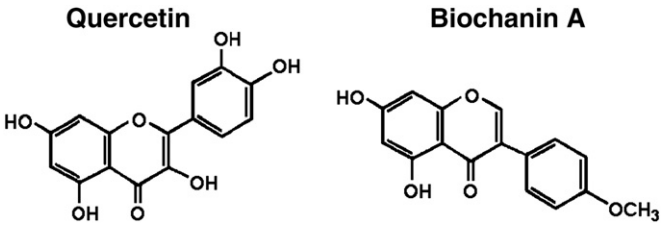

Fig. 1. Chemical structures of quercetin and biochanin A.

2. Materials and methods

2.1. Cell culture

ARPE-19 human retinal pigment epithelial (RPE) cell line was obtained from American type culture collection (ATCC, Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle Medium nutrient mixture F12-Ham's (Gibco, Paisley, UK) supplemented with 10% fetal bovine serum (FBS) (ThermoFisher Scientific, Erembodegem, Belgium), 2 mM L-glutamine (Lonza, Walkersville, MD, USA), 100 U/ml penicillin (Lonza) and 100 U/ml streptomycin (Lonza), at 37 °C, in 10% CO_2. Cells were harvested with trypsin (1×TRP/EDTA, 25200-072, Gibco), and seeded in 12-well plates.

ARPE-19 cells were exposed to different concentrations of quercetin or biochanin A (Sigma Chemical Co., St. Louis, MO, USA) in fresh culture medium. Compounds were diluted in DMSO and the final DMSO concentration of culture medium was 0.5% for cytotoxicity tests and 0.25% for oxidative stress tests. The cytotoxicity of compounds was tested by incubating cells with 0–200 μM quercetin or 0–300 μM biochanin A for 24 h. In order to determine the protective effects of quercetin and biochanin A against oxidative stress-mediated cell death, cells were incubated with 0–150 μM quercetin or 0–50 μM biochanin A for 2 h before addition of hydrogen peroxide (J. T. Baker, Deventer, The Neatherlands), target set being 50–60% cell mortality, and further incubated for 24 h. Expression levels of selected genes were measured from cells treated with 100 μM quercetin for 2 h before addition of H_2O_2 (final concentration 1.1 μM), and further incubated for 24 h.

2.2. MTT assays

Cellular viability was assessed via the MTT assay. In the MTT assay, the cells ability to metabolize 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazolium bromide is measured spectrophotometrically. The assay was performed as previously described (Hansen, Nielsen and Berg, 1989).

2.3. RNA extraction and quantitative real-time PCR of IL-6, IL-1β, Nrf2, GSTP1 and HO-1

Cells were lyzed and RNA for quantitative real-time PCR (qRT-PCR) was extracted using illustra RNAspin Mini isolation Kit (GE Healthcare, Buckinghamshire, England) with DNase I on-column digestion. Samples were stored at −70 °C after being lyzed and also after RNA isolation. The cDNA was synthesized from 1 μg of total RNA using DyNAmo cDNA Synthesis Kit (Finnzymes, Espoo, Finland). Quantitative real-time PCR reactions were carried out using Dynamo HS SYBR Green kit (Finnzymes), the reaction volume being 20 μl. The concentration of gene-specific primers was 1 μM and the amount of diluted cDNA 2 μl, deriving from 2.5 or 25 ng of total RNA as a template. The qRT-PCR primers, ordered from Oligomer (Helsinki, Finland) for human IL-6 and IL-1β were from Dussault and Pouliot (2006), and Nrf2, GSTP1 and HO-1 from Kokot et al. (2009). Acid riboprotein P0 (RPLP0) (Malinen et al., 2008) was used as a reference gene.

The reactions were performed as triplicates in iCycler iQ Real-Time PCR device (Bio-Rad). The PCR reaction conditions were: 95 °C for