Food Chemistry 133 (2012) 445–450

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Effects of quercetin metabolites on the enhancing effect of β-carotene on DNA damage and cytochrome P1A1/2 expression in benzo[a]pyrene-exposed A549 cells

Yan-Zin Chang [a], Hsiao-Chun Lin [b], Shu-Ting Chan [b], Shu-Lan Yeh [b,c,*]

[a] Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan, ROC
[b] Department of Nutritional Science, Chung Shan Medical University, Taichung, Taiwan, ROC
[c] Department of Nutrition, Chung Shan Medical University Hospital, Taichung, Taiwan, ROC

ARTICLE INFO

Article history:
Received 29 October 2011
Received in revised form 6 January 2012
Accepted 19 January 2012
Available online 27 January 2012

Keywords:
Quercetin-3-glucuronide
Quercetin-3′-sulphate
Isorhamnetin
β-Carotene
Cytochrome P450
Benzo[a]pyrene

ABSTRACT

A549 cells were pre-incubated with β-carotene (BC) alone or in combination with quercetin or three major quercetin metabolites in human plasma, quercetin 3-glucuronide (Q3G), quercetin 3′-sulphate (Q3′S) and isorhamnetin, followed by incubation with benzo[a]pyrene (BaP), to investigate the effects of these compounds on the BaP-induced harmful effects of BC. All the quercetin metabolites at 10 μM inhibited BaP + BC-induced cell death. Q3′S, Q3G and isorhamnetin also significantly decreased BaP ± BC-induced DNA damage by 64%, 60% and 24%, respectively. In a similar order, these compounds suppressed BaP + BC-induced cytochrome P450 (CYP)1A1/1A2 expression by 10–50%. Q3G and Q3′S significantly decreased the intracellular reactive oxygen species formation induced by BaP + BC; however, Q3G had the best effect on decreasing the loss of BC induced by Fe/NTA. The combined effects of quercetin metabolites were additive. This study indicates that quercetin metabolites decrease the BaP-induced harmful effect of β-carotene in A549 cells by downregulating the expression of CYP1A1/1A2, at least in part.

1. Introduction

Quercetin is a phytochemical which is widely present in foods of plant origin, such as apples and onions. It has attracted much attention in relation to prevention of degenerative diseases such as atherosclerosis and cancers (Gupta, Kim, Prasad, & Aggarwal, 2010; Terao, Kawai, & Murota, 2008). Quercetin has a wide range of possible biological properties, including regulation of inflammatory cytokines and antioxidant activities (Valério et al., 2009). However, conjugated quercetin metabolites, such as quercetin glucuronides and quercetin sulphates, rather than quercetin aglycone, are present in human plasma due to its efficient phase II metabolism (Day et al., 2001). The biochemical and biophysical properties among quercetin and quercetin metabolites may be different because of structure modification (Loke et al., 2008; Suri et al., 2008). For example, quercetin 3-glucuronide (Q3G) rather than quercetin 3′-sulphate (G3′S) or quercetin aglycone significantly reduces the stimulant-induced calcium influx in human neutrophils (Suri et al., 2008). In contrast, compared to quercetin, Q3′S retains considerable lipoxygenase inhibitory activity while Q3G possesses antioxidant activity rather than lipoxygenase inhibitory activity at physiological concentrations (Loke et al., 2008). Thus, to understand the possible mechanisms by which quercetin exerts its health benefits *in vivo*, it is important to investigate the biological activities of quercetin metabolites.

Several human and animal supplementary studies (Chan et al., 2011; Liu, Russell, & Wang, 2003; The Alpha-tocopherol, Beta Carotene Cancer Prevention Study Group, 1997) have demonstrated the harmful effects of the supplementation of high dose β-carotene (BC) on lungs exposed to cigarette smoking. These findings are contradictory to the epidemiologic studies, which show an inverse correlation between the risk of lung cancer and the intake of foods rich in BC in smokers (Peto, Doll, Buckley, & Sporn, 1981). The absence of other beneficial compounds, which are also commonly present in plant foods, in BC-supplemented studies may contribute to the contradictory findings (Russell, 2004). An *in vitro* study demonstrated that ascorbic acid and α-tocopherol decrease the production of oxidative products of BC induced by lung postnuclear fractions from ferrets exposed to cigarette smoke through antioxidative activity, at least in part (Liu, Russell, & Wang, 2004). The oxidative products of BC, in turn, lead to a cascade of harmful effects in the lung of cigarette smoke-exposed animals or smokers (Russell, 2004). Except for α-tocopherol and ascorbic acid, our previous studies show that quercetin suppresses the stimulant-induced harmful effects of BC by several mechanisms

Abbreviations: BaP, benzo[a]pyrene; BC, β-carotene; CYP, cytochrome P450; Q3G, quercetin-3-glucuronide; Q3′S, quercetin 3′-sulphate.

* Corresponding author at: Department of Nutritional Science, Chung Shan Medical University, No. 110, Sec. 1, Jianguo N. Rd., Taichung, Taiwan, ROC. Tel.: +886 4 24730022x12201.
E-mail address: suzyyeh@csmu.edu.tw (S.-L. Yeh).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2012.01.060

(Chan et al., 2011; Yeh & Wu, 2006). For example, quercetin decreases the enhancing effect of BC on benzo[a]pyrene (BaP)-induced DNA damage by inhibiting CYP 1A1 expression in A549 cells (Yeh & Wu, 2006). However, it is unclear whether quercetin metabolites also exert such effects. BaP is a well-known environmental and cigarette smoke-associated carcinogen in multiple organs, including lungs, and requires metabolic activation by CYP450; it is commonly used in cell- and animal studies as a carcinogen (Yeh & Wu, 2006). CYP1A1 and CYP1A2 are the major isoforms of CYP in human lung cells (Alexandrov, Rojas, & Satarug, 2010).

A549 cells, a human lung cell line, have characteristic features of type II cells of the pulmonary epithelium, including metabolic and transport capacities (Foster, Oster, Mayer, Avery, & Audus, 1998). Therefore, in the present study, we preincubated A549 cells with three major quercetin metabolites present in plasma of humans, Q3G, Q3′S and isorhamnetin (a methylated quercetin), alone or mixed (concentration: Q3′S/Q3G/isorhamnetin = 2/2/1), in combination with BC, followed by BaP exposure. We wanted to investigate (1) the influence of individual or mixed quercetin metabolites on the harmful effects of BC induced by BaP and (2) whether quercetin metabolites exert their effects through the modulation of CYP1A1/2 expression. Physical concentrations of quercetin metabolites (mainly at 10 μM) were used in the present study (Day et al., 2001). We also compared the effects of quercetin metabolites with that of quercetin itself.

2. Materials and methods

2.1. Reagents

All chemicals used were of reagent grade or higher. β-Carotene and quercetin were from Sigma Chemical Co. (St. Louis, MO). Basal Eagle medium, foetal bovine serum, trypsin, penicillin, streptomycin, sodium pyruvate, and nonessential amino acids were purchased from Gibco/BRL (Rockville, MD). Isorhamnetin was purchased from Extrasynthèse (Genay, France). Q3G was synthesised and purified according to the methods described by Day et al. (2001), and Chen, Chen, Li, and Zeng (2005). Q3′S was synthesised by the method described by Day, Bao, Morgan, and Williamson (2000). According the method described by Day et al. (2001), we confirmed the synthesised conjugated metabolites of quercetin by the retention times of HPLC and mass spectrometry (Day, Bao, & Morgan, 2000). Mass spectrometric analysis was performed with electrospray ionisation in the negative-ion mode and in the range of *m/z* 100–700. The purity of Q3G and Q3′S were checked by HPLC and were found to be about 95% and ⩾99%, respectively. The level of free quercetin in each of the synthetic compounds was undetectable (the limit of detection ⩽6 pmole/injection).

2.2. Cell culture

A549 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and were cultured in BME containing 10% (v/v) foetal bovine serum, 0.37% (w/v) $NaHCO_3$, penicillin (100 U/mL), and streptomycin (100 U/mL) at 37 °C in a humidified incubator under 5% CO_2 and 95% air. The cells were harvested at approximately 90% confluence (approximately 10^6 cells/10-cm dish) for various experiments. Both the concentrations and the method of incorporation of tested compounds into A549 cells were adapted from a previous study (Yeh & Wu, 2006). Stock solutions of THF-β-carotene (10 mM) were freshly prepared before each experiment. Quercetin and its metabolites were prepared in methanol at a stock concentration of 20 mM. To prevent autoxidation, the test compounds were then added to the culture medium to give the final concentrations indicated, and the cells were incubated at 37 °C in the dark for 4 h. In our preliminary study, we found that after 4 h incubation the protective effects of quercetin metabolites (or quercetin) on BaP or BaP + BC-induced damage in A549 cells reached their maxima (data not shown). The cells were then washed three times in phosphate-buffered saline (PBS; pH 7.4, containing 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, and 8.1 mM Na_2HPO_4), followed by incubation with fresh BME with or without 20 μM BaP at 37 °C for 24 h. Control cells were incubated with THF and/or methanol alone. In all experiments, the total level of solvents was not greater than 0.2% (v/v).

2.3. DNA damage assay (comet assay)

The comet assay, adapted from the method of Singh, McCoy, Tice, and Schneider (1988), was used to measure DNA strand breaks in cells. Cells were suspended in low-melting-point agarose in PBS at 37 °C and were then placed on a frosted glass microscope slide precoated with a layer of 1% normal-melting-point agarose. After the application of a third layer of 1% normal-melting-point agarose, the slides were immersed in cold-lysing solution (10 mM Tris, 2.5 M NaCl, 100 mM Na_2EDTA, 1% sodium *N*-laurylsarcosine, 1% Triton X-100, and 10% dimethylsulphoxide) for 1 h at 4 °C. The slides were then placed in an electrophoresis tank, and the DNA was allowed to unwind for 15 min in the alkaline solution. Electrophoresis was performed using the method described by Collins, Ma, and Duthie (1995). The images were analysed by computer with the Interactive Image Analysis Comet Assay III (Perceptive Instruments, Haverhill, UK), and DNA strand breaks were expressed as a percentage of DNA in the tail (% DNA in tail).

2.4. Western blots of CYP1A1/2

Western blot analysis was performed to detect the expression of CYP1A1/2 in treated cells (Liu et al., 2003). The cells were harvested and lysed with 20% sodium dodecyl sulphate containing 1 mM phenylmethylsulphonyl fluoride. The lysate was sonicated for 30 s on ice and was then centrifuged for 30 min at 4 °C. The protein (40 μg) from the supernatant fluid was resolved by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. After blocking with TBS buffer (20 mM Tris–HCl, 150 mM NaCl, pH 7.4) containing 5% nonfat milk, the membrane was incubated with CYP1A1/2 polyclonal antibodies (Affinity BioReagents, Rockford, IL) and then with horseradish peroxidase-conjugated antirabbit IgG, followed by visualisation with an enhanced chemiluminescence detection kit (Western Lightning Chemiluminescence Reagent Plus; PerkinElmer Life Sciences, Inc., Waltham. MA). The relative density of the immunoreactive bands was quantified by using a luminescent image analyzer (LSA-100, Fujifilm, Japan).

2.5. Intracellular level of reactive oxygen species (ROS)

According to the method described by Amer, Goldfarb, and Fibach (2003), we used dichlorofluorescin as a fluorescent probe to determine the intracellular level of ROS. Briefly, A549 cells were first incubated with quercetin or its metabolites for 4 h. After washing twice with PBS, the cells were loaded with DCFH-DA at 0.4 mM (final concentration) for 15 min, followed by washing with PBS and incubation with BaP for 30 min. We then measured the intracellular fluorescence by using a flow cytometer (FACSCalibur, BD Biosciences, Franklin Lakes, NJ).