Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Nutritional Biochemistry 16 (2005) 729–735

Journal of Nutritional Biochemistry

Pro-oxidative effect of β-carotene and the interaction with flavonoids on UVA-induced DNA strand breaks in mouse fibroblast C3H10T1/2 cells

Shu-Lan Yeh[a], Wei-Yu Wang[b], Chin-Hsiu Huang[b], Miao-Lin Hu[b,*]

[a]*Institute of Nutritional Science, Chung Shan Medical University, Taichung, Taiwan 402, ROC*
[b]*Department of Food Science, National Chung-Hsing University, Taichung, Taiwan 402, ROC*

Received 23 November 2004; received in revised form 3 March 2005; accepted 24 March 2005

Abstract

It has been suggested that β-carotene itself is unstable under certain conditions and that a combination of antioxidants may prevent the pro-oxidative effects of β-carotene. Thus, the present study aimed to investigate the interaction of β-carotene with three flavonoids—naringin, rutin and quercetin—on DNA damage induced by ultraviolet A (UVA) in C3H10T1/2 cells, a mouse embryo fibroblast. The cells were preincubated with β-carotene and/or flavonoid for 1 h followed by UVA irradiation, and DNA damage was measured using comet assay. We showed that β-carotene at 20 μM enhanced DNA damage (by 35%; $P < .05$) induced by UVA (7.6 kJ/m^2), whereas naringin, rutin and quercetin significantly decreased UVA-induced DNA damage. When each flavonoid was combined with β-carotene during preincubation, UVA-induced cellular DNA damage was significantly suppressed and the effects were in the order of naringin ≥ rutin > quercetin. The flavonoids decreased UVA-induced oxidation of preincorporated β-carotene in the same order. Using electron spin resonance spectroscopy, we showed that the ability of these flavonoids to quench singlet oxygen was consistent with protection against DNA damage and β-carotene oxidation. All three flavonoids had some absorption at the UVA range (320–380 nm), but the effects were opposite to those on DNA damage and β-carotene oxidation. Taken together, this cell culture study demonstrates an interaction between flavonoids and β-carotene in UVA-induced DNA damage, and the results suggest that a combination of β-carotene with naringin, rutin or quercetin may increase the safety of β-carotene.
© 2005 Elsevier Inc. All rights reserved.

Keywords: β-carotene; Flavonoids; UVA; Interaction; DNA damage

1. Introduction

β-Carotene has been extensively investigated as a chemopreventive agent that may protect against skin photodamage [1–6]. Recently, β-carotene has been used as a component of some cosmetics [7,8]. However, inconsistent findings exist in various studies [9–14]. For example, Black [11] found that β-carotene-supplemented semidefined diets, in contrast to commercial closed formulas, not only fail to protect against UV-induced carcinogenesis but also lead to significant exacerbation in mice. Black et al. [12] pointed out that the inconsistency in the photoprotective effect of β-carotene in animal studies [10,11] may have been due to the interaction between β-carotene and other dietary antioxidants including phytochemicals. Similarly, an in vitro study showed that preincubation of skin fibroblasts with either β-carotene or lycopene (0.1–1.0 μM) increases ultraviolet A (UVA)-induced expression of metalloproteinase 1 (MMP-1) [14], a collagenase associated with skin aging [15], while concurrent addition of vitamin E or vitamin C during preincubation suppresses the increase in MMP-1 mRNA. However, limited data exist regarding the interaction of β-carotene with other phytochemicals on UVA-induced oxidative damage.

Flavonoids are a major type of phytochemicals that are ubiquitously present in fruits and vegetables. Growing evidence demonstrate an inverse relation between the dietary intake of flavonoids and the incidence of several chronic diseases and cancers [16,17]. In addition, flavonoids may protect against photodamage [18,19]. These beneficial effects of flavonoids have been attributed to their actions as antioxidants, enzyme inhibitors and cell cycle regulators under various conditions [20–24]. An in vitro study showed that flavonoids, including naringenin, rutin and flavonoid extracts from apple skin, prevent UVB-induced DNA

* Corresponding author. Tel.: +886 4 2281 2363; fax: +886 4 2281 2363.
E-mail address: mlhuhu@dragon.nchu.edu.tw (M.-L. Hu).

0955-2863/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2005.03.012

damage [25]. Administration of quercetin significantly reduces oxidative damage produced by UVA radiation in rats [23,26]. Similarly, naringin has been found to suppress radiation-induced genomic instability in mouse bone marrow [27]. However, whether these flavonoids affect the behavior of β-carotene under UVA exposure is unclear. The UVA (320–380 nm) component of sunlight is particularly associated with oxidative damage involved in photoaging [28,29]. We therefore investigated the interaction of β-carotene with naringin, quercetin and rutin on UVA-induced DNA damage in C3H10T1/2 (C3H) cells, a mouse embryo fibroblast that has been used for photodamage studies in vitro [30].

2. Methods and materials

2.1. Reagents

All chemicals used were of reagent or higher grade. β-carotene, rutin, naringin and quercetin were from Sigma Chemical (St. Louis, MO, USA). Tetrahydrofuran (THF) and dimethyl sulfone (DMSO) were from Wako (Japan). 2,2,6,6-Tetramethylpiperidine (TEMP) was from Aldrich (St. Louis, MO, USA). Basal medium of Eagle, fetal bovine serum (FBS), trypsin, penicillin, streptomycin, sodium pyruvate and nonessential amino acids were from GIBCO (MD, USA).

2.2. Cell culture and β-carotene and flavonoids incorporation

C3H cells were cultured in basal medium of Eagle containing 10% (vol/vol) FBS, 0.37% (wt/vol) $NaHCO_3$, penicillin (100 U/ml), streptomycin (100 U/ml), 0.1-mM nonessential amino acid and 1-mM sodium pyruvate at 37°C in a humidified incubator under 5% CO_2 and 95% air. The cells were harvested at approximately 90% confluence. A THF–β-carotene solution (10 mM) was prepared freshly before each experiment. The purity of commercial β-carotene, as calibrated using an extinction coefficient, 1.4×10^{-5} M^{-1}/cm^{-1} [31], was approximately 93%, which compared well with the 95% purity claimed by Sigma. THF or THF–β-carotene (20 μl) was added to C3H cells (approximately 10^6 cells/dish) in 10 ml of medium at 37°C in the dark for 1 h. The cells were then washed three times in phosphate-buffered saline (PBS, pH7.4, containing 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4 and 8.1 mM Na_2HPO_4), and the concentrations of β-carotene were determined by HPLC with a detection wavelength of 450 nm after extraction in ethanol and hexane (1:2, vol/vol) as described previously [32]. Naringin, rutin and quercetin were prepared in DMSO at a stock concentration of 50 mM and incubated with C3H cells at final concentrations of 10 and 23 μM. The final concentration of DMSO was 0.046%. Control cells were incubated with THF and/or DMSO solvent. After extraction in methanol, the concentrations of naringin in cells were determined by HPLC and detected at 280 nm [33].

2.3. UVA irradiation

Cells in 10-cm^2 dishes were washed and then covered with 10 ml of Hanks balanced salt solution (1.3 mM $CaCl_2$, 5.4 mM KCl, 0.4 mM KH_2PO_4, 0.5 mM $MgCl_2.6H_2O$, 0.4 mM $MgSO_4.7H_2O$, 136.7 mM NaCl, 4.2 mM $NaHCO_3$ and 0.3 mM $NaH_2PO_4.H_2O$). Irradiation

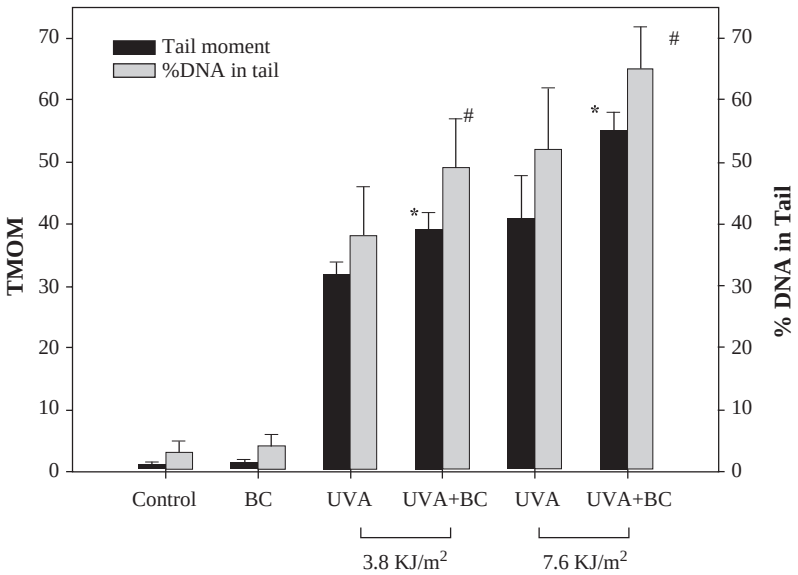

Fig 1. Effect of β-carotene (BC) on DNA strand breaks in C3H cells irradiated with 3.8 or 7.6 kJ UVA/m^2. Cells were preincubated with 20 μM of BC in the dark at 37°C for 1 h and then washed before UVA irradiation. DNA strand breaks were expressed as TMOM and %DNA in tail. Values (means±S.D. of triplicate assays) are compared statistically within each UVA dose between groups with and those without BC by Student's *t* test. $^*P<.05$ for TMOM; $^{\#}P<.05$ for %DNA in tail.

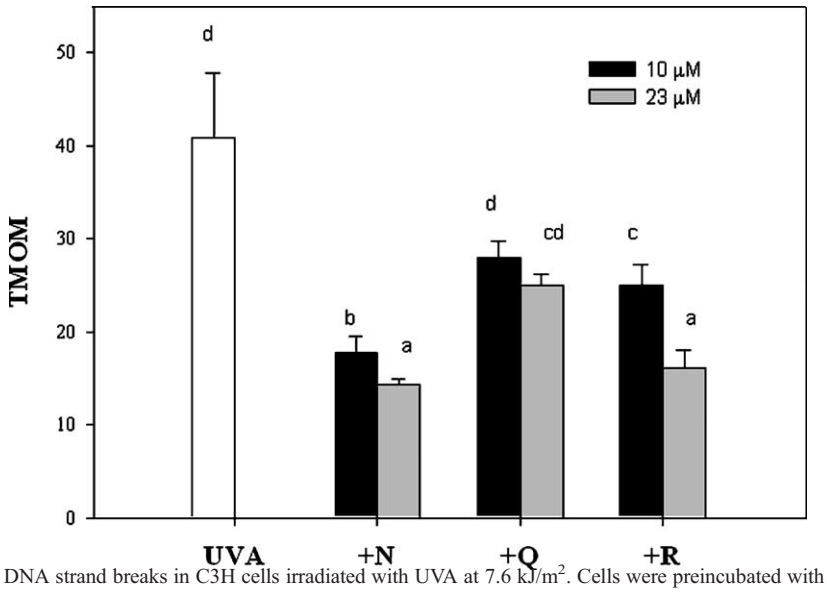

Fig 2. Effects of flavonoids on DNA strand breaks in C3H cells irradiated with UVA at 7.6 kJ/m^2. Cells were preincubated with 10 or 23 μM of naringin (N), quercetin (Q) or rutin (R) for 1 h in the dark at 37°C and then washed before UVA irradiation. Values (means±S.D., $n=3$) not sharing a common letter are significantly different ($P<.05$).

was carried out in a UVA irradiation chamber (XL-1000 UV cross-linker) as described before [30] with an accumulated dose of 7.6 kJ/m^2. The UVA light source emits radiation at a range of 320–380 nm with main output at 365 nm. Sham-irradiated cells were treated in the same manner except that they were not irradiated.

2.4. Comet assay

Comet assay was adapted from the method of Singh et al. [34]. Cells were suspended in low melting point agarose in PBS at 37°C and placed onto a frosted glass microscope slide precoated with a layer of 1% normal melting point agarose. After 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% dimethyl sulfoxide) for 1 h at 4°C. The slides were then placed in an electrophoresis tank, allowing the DNA to unwind for 15 min in the alkaline solution. Electrophoresis was performed using the method of Collins et al. [35]. The image was analyzed by computer using the Image Pro Plus software (Media Cybernetics, USA) as tail moment (TMOM) by the formula TMOM = %DNA in tail×tail length.

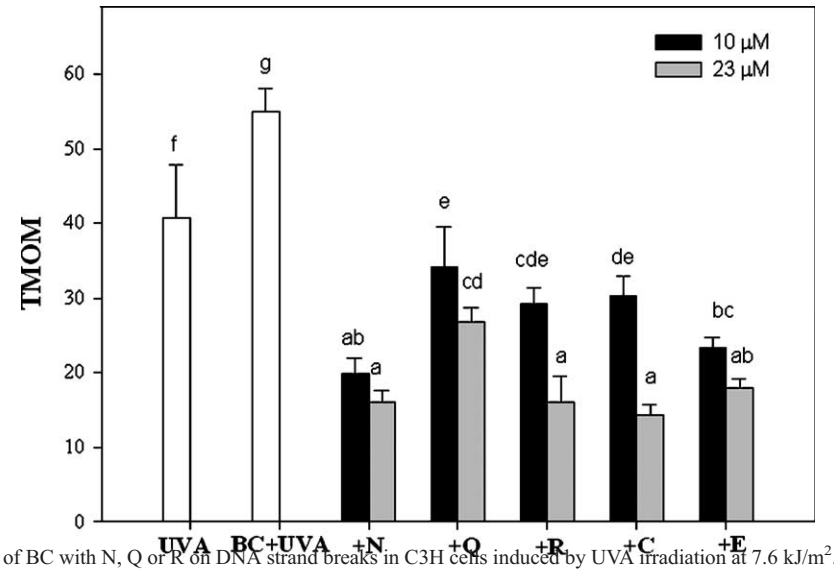

Fig 3. Effects of coincubation of BC with N, Q or R on DNA strand breaks in C3H cells induced by UVA irradiation at 7.6 kJ/m^2. Cells were preincubated with 20 μM of BC with and without 10 or 23 μM of N, Q, R, vitamin C (C) or vitamin E (E) for 1 h followed by washing before UVA irradiation. Values (means±S.D., $n=3$) not sharing a common letter are significantly different ($P<.05$).

Table 1
Effects of naringin (N), quercetin (Q) and rutin (R) on the loss of incorporated β-carotene (BC) in C3H cells irradiated with 3.8 kJ UVA/m^2

Treatments	BC concentration (nmol/10^{6}cells)	%
BC before UVA irradiation	3.50±0.12^e	100±3
BC/UVA	0.84±0.11^a	24±3
N+BC/UVA	2.73±0.07^d	78±2
Q+BC/UVA	1.12±0.07^b	32±2
R+BC/UVA	2.21±0.18^c	63±5

The cells were preincubated for 1 h with 20 μM of BC with or without 23 μM of a flavonoid followed by washing before UVA irradiation. Values (means±S.D., $n=3$) sharing a common letter are not significantly different ($P>.05$).

2.5. Detection of singlet oxygen ($^1O_2$)

The formation of singlet oxygen by UVA irradiation was determined by electron spin resonance (ESR) spectroscopy using 2,2,6,6-TEMP as the spin trap. The reaction mixture contained 23 μM of a flavonoid in 25-mM phosphate buffer pH7.4 and 32.5-mM TEMP as a trap molecule, as modified from previous studies [36,37]. The mixture was irradiated with UVA and recorded immediately on a Bruker EMX-10 spectrometer with 100-kHz field modulation and the following settings: microwave powder, 20 mW; center field, 3483 G; sweep width, 100 G; sweep time, 200 s.

2.6. Statistical analysis

Values are expressed as means±S.D. of three replicates and analyzed by using Student's t test for two-group comparison or using one-way analysis of variance followed by Duncan's multiple range test for comparisons of group means. A P value less than .05 was considered statistically significant.

3. Results

3.1. DNA damage

Comet images of DNA damage in C3H were determined after UVA irradiation at 3.8 and 7.6 kJ/m^2. As shown in Fig. 1, UVA irradiation significantly led to comet formation (expressed as TMOM and %DNA in tail) in cellular DNA in a dose-dependent manner and preincubation of cells with 20 μM of β-carotene significantly increased DNA break by 26–35% over that of UVA irradiation alone. Because the two parameters of comet assay were highly correlated ($r=.99$; $P<.01$) in full range of DNA damage, we used only TMOM in other data presented here. Preincubation of naringin, rutin or quercetin (10 and 23 μM) with C3H cells significantly decreased DNA damage induced by UVA at 7.6 kJ/m^2 (Fig. 2), and the effects of naringin and rutin were dose dependent. These flavonoids inhibited DNA damage in the order of naringin≥rutin>quercetin, with the highest inhibition (65%) achieved by 23 μM of naringin. Similar results were observed when 3.8 kJ UVA/m^2 was used (data not shown).

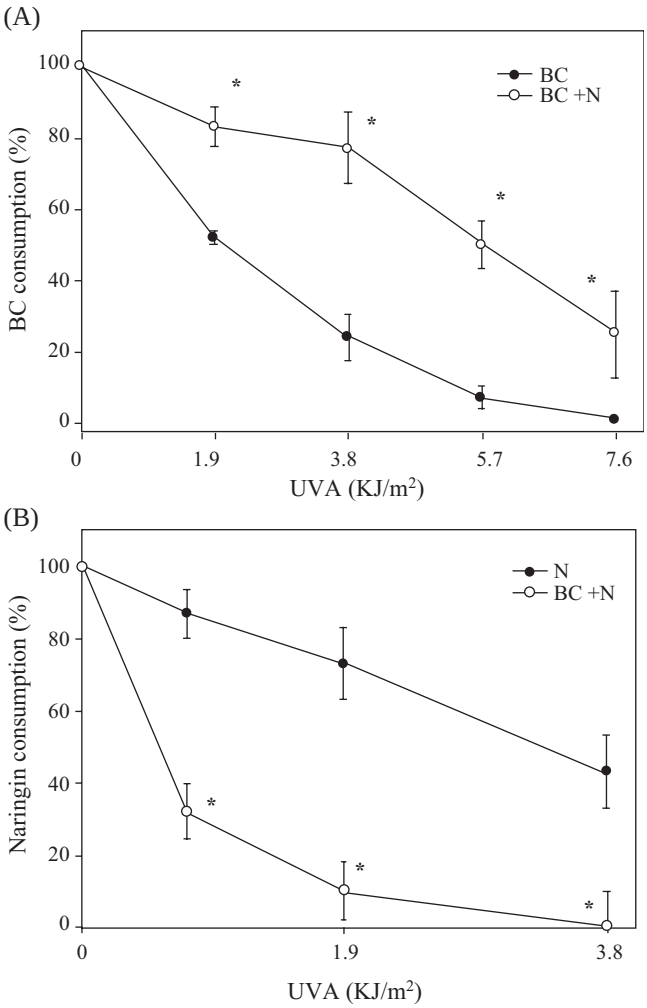

Fig 4. The consumption of intracellular BC (A) and N (B) induced by UVA irradiation. C3H cells were preincubated with 20 μM of BC plus 23 μM of N or individually for 1 h followed by washing before UVA irradiation. Values (means±S.D., $n=3$) in the BC+N group with an asterisk are significantly different from BC or N alone at the same UVA dose ($P<.05$).

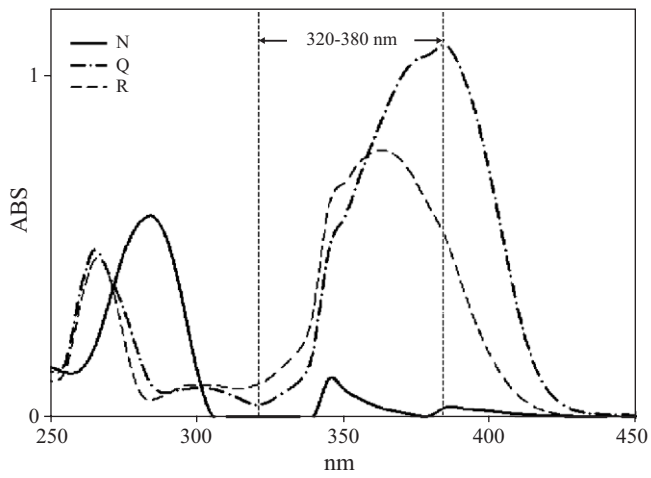

Fig 5. Absorption spectra of N, Q and R in DMSO. The concentration of each flavonoid was 23 μM.

When each of the three flavonoids was combined with β-carotene during preincubation, the enhanced DNA damage by β-carotene in cells exposed to UVA at 7.6 kJ/m^2 was significantly inhibited as compared with the extent of UVA-induced DNA damage in cells preincubated with β-carotene alone (Fig. 3). The protection by naringin at 10 μM was more efficient than that by the other two flavonoids, and the effect was comparable with that of vitamin E and better than that of vitamin C at the same concentration. The protective effects of naringin and rutin at 23 μM were similar and significantly better than those of quercetin.

3.2. Consumption of cellular β-carotene and absorption spectra of flavonoids in UVA

UVA exposure led to rapid consumption of β-carotene in the cells. Approximately 24% of incorporated β-carotene remained in cells irradiated with 3.8 kJ UVA/m^2 (Table 1), whereas 85% of β-carotene remained in cells without subsequent UVA irradiation (data not shown). Coincubation of a flavonoid with β-carotene decreased UVA-induced oxidation of β-carotene, with 78%, 63% and 32% of β-carotene remaining in cells preincubated with naringin, rutin and quercetin, respectively, after subsequent UVA irradiation at 3.8 kJ/m^2.

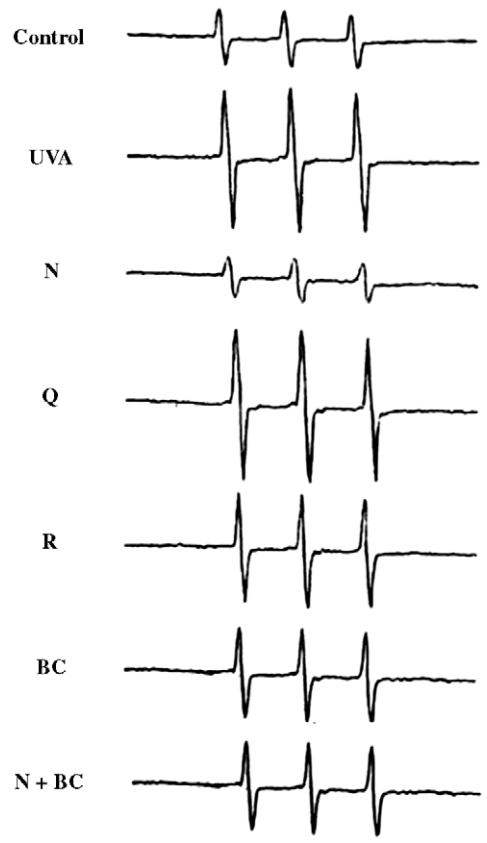

Fig 6. Effects of N, Q, R, BC or the combination of N with BC on the formation of TEMP-singlet oxygen adducts in a cell-free system induced by UVA irradiation at 7.6 kJ/m^2. The concentration of each flavonoid was 23 μM, and the concentration of BC was 20 μM.

To further characterize the protective effects of naringin, we then used 23 μM of naringin to study the consumption kinetics of preincorporated β-carotene as a function of UVA irradiation. Fig. 4A shows a rapid and dose-dependent loss of β-carotene by UVA, whereas loss of β-carotene in cells was significantly inhibited by naringin preincubation. In contrast, the loss of naringin in cells was significantly enhanced by β-carotene Fig. 4B. Fig. 5 shows the absorption of UVA (320–380 nm) by flavonoids, in which quercetin had the strongest absorption, followed by rutin then by naringin, whose absorption was substantially lower than that of the other two flavonoids.

3.3. Involvement of $^1O_2$

To determine the ability of flavonoids in scavenging $^1O_2$ induced by UVA, we used the ESR spectroscopic technique with TEMP as a spin trap in an aqueous system, which upon attack by $^1O_2$ forms a nitroxyl radical TEMPO. As shown by ESR spectroscopy (Fig. 6), naringin was more effective than rutin in quenching $^1O_2$ induced by UVA, while quercetin had essentially no $^1O_2$-quenching ability in this system. β-carotene itself moderately decreased the TEMP–$^1O_2$ adducts, but the combination of naringin and β-carotene did not produce an additive effect.

4. Discussion

β-Carotene is a pro-oxidant under certain conditions such as UVA irradiation [11–14], and it has been suggested that a combination of antioxidants may prevent the pro-oxidative effects of β-carotene. In this study, we examined the interaction on cellular DNA damage between β-carotene and some common flavonoids—naringin, rutin and quercetin. Using C3H cells, we showed that β-carotene (20 μM) was pro-oxidative because it significantly enhanced DNA damage in cells irradiated with 3.8 and 7.6 kJ UVA/m^2. In contrast, naringin, rutin and quercetin protected against UVA-induced DNA damage. When combined with β-carotene, each of these flavonoids suppressed the pro-oxidative effect of β-carotene in C3H cells induced by UVA irradiation. The doses of UVA used here, which significantly led to comet formation in cellular DNA, are much lower than the average dose (500 kJ/m^2) capable of inducing minimal erythema in human skin [38]. Because β-carotene readily autoxidizes during incubation, we adopted a short preincubation time (≤1 h) with a relatively high concentration of β-carotene (20 μM) [39–41] as compared with concentrations (0.1–5 μM) in other UVA studies [13,14]. The concentrations of flavonoids (10 and 23 μM) used here were based on those used by Lin et al. [42] (i.e., 1, 10 and 25 μM of quercetin) and were comparable with the β-carotene concentration (20 μM) used in the present study. We used 23-μM flavonoids because cytotoxicity was somewhat evident at 25 μM or higher levels (data not shown).

A possible mechanism underlying the interaction of the flavonoids with β-carotene in UVA-induced DNA damage

is the antioxidant activity of the flavonoids, which is closely related to their ability of donating hydrogen atoms [21,43–45]. Several structural characteristics are essential for the antioxidant activities of flavonoids: orthohydroxylation on the B ring, the number of free hydroxyl groups, a C2–C3 double bond in the C ring and the presence of a 3-hydroxyl group [43,44]. Flavonol 3-*O*-glycosides with a blocked C3 hydroxyl group, such as rutin, may result in lower antioxidant ability than its aglycon, quercetin [21]. However, our finding that naringin and rutin were much stronger than quercetin in preventing UVA-induced DNA damage is inconsistent with the notion of hydrogen donation by flavonoids. Since much of the damaging effect of UVA radiation can be attributed to the generation of $^1O_2$ [28,36], we determined the ability of $^1O_2$ quenching by the three flavonoids. We found that naringin was more effective than rutin in quenching $^1O_2$ while quercetin had no effect. Thus, the ability of quenching $^1O_2$ may explain, at least in part, the order of protective effects of the three flavonoids. Our observation that the combination of naringin and β-carotene did not produce an additive effect on $^1O_2$ quenching may be explained by the fact that β-carotene enhances the consumption of naringin induced by UVA. Taken together, our results indicate that the ability of the flavonoids to quench $^1O_2$ is more important than their ability to donate hydrogen atoms in inhibiting UVA-induced DNA damage. However, it cannot be excluded that these flavonoids may also react with other reactive species including β-carotene radical or its oxidative products to afford protection against DNA damage.

These flavonoids may also inhibit the pro-oxidative effect of β-carotene on DNA damage by preventing β-carotene oxidation. In this context, Trombino et al. [46] have reported a negative synergistic inhibition of oxidant-induced malondialdehyde production by the combination of β-carotene and ferulic acid, a ubiquitous phenolic compound in nature. They suggested that such effects may be explained by the fact that β-carotene enhances the consumption of ferulic acid whereas ferulic acid decreases the loss of β-carotene and the formation of oxidative products of β-carotene [46]. In our study, the flavonoids significantly decreased the loss of β-carotene in an order that was similar to their protection against UVA-induced DNA damage in cells preincubated with β-carotene (i.e., naringin ≥ rutin > quercetin). In addition, we found that β-carotene enhanced the consumption of naringin. These results suggest that these flavonoids may protect against the pro-oxidative effect of β-carotene on DNA damage through inhibition of β-carotene oxidation. Although it is unclear how these flavonoids inhibit β-carotene oxidation, it is evident that their sunscreen effects should not be a factor because the UVA absorptivity of the three flavonoids (quercetin > rutin > naringin) was opposite to the order of their protection against β-carotene oxidation.

In summary, using C3H cells preincubated with β-carotene and subsequently irradiated with UVA, we have investigated the interaction on cellular DNA damage between flavonoids and β-carotene. We demonstrate that coincubation of naringin, rutin or quercetin with β-carotene protects against the DNA damage and that such actions of the flavonoids are attributed to the ability of quenching $^1O_2$ and, to some extent, of decreasing β-carotene oxidation. Our results suggest that a combination of β-carotene with naringin, rutin or quercetin may increase the safety of β-carotene in various systems and in commercial products including cosmetics.

Acknowledgments

This research was supported by grants (NSC-91-2320-B005-001 and NSC-91-2314-B040-028) from the National Science Council (Republic of China).

References

[1] Mathews-Roth MM. Carotenoid functions in photoprotection and cancer prevention. J Environ Pathol Toxicol Oncol 1990;10:181–92.
[2] Fuller CJ, Faulkner H, Bendich A, Parker RS, Roe DA. Effect of beta-carotene supplementation on photosuppression of delayed-type hypersensitivity in normal young men. Am J Clin Nutr 1992;56:684–90.
[3] Greenberg ER, Baron JA, Stukel TA, Stevens MM, Mandel JS, Spencer SK, et al. A clinical trial of beta carotene to prevent basal-cell and squamous-cell cancers of the skin. The Skin Cancer Prevention Study Group. N Engl J Med 1990;323:789–95.
[4] Stahl W, Sies H. Carotenoids and protection against solar UV radiation. Skin Pharmacol Appl Skin Physiol 2002;15:291–6.
[5] Trekli MC, Riss G, Goralczyk R, Tyrrel RM. Beta-carotene suppresses UVA-induced HO-1 gene expression in cultured FEK4. Free Radic Biol Med 2003;34:456–64.
[6] Biesalski HK, Obermueller-Jevic UC. UV light, beta-carotene and human skin beneficial and potentially harmful effects. Arch Biochem Biophys 2001;389:1–6.
[7] Harang B. Composition having tanning and photoprotective activity, and its cosmetic applications. Official Gazette of the United States Patent and Trademark Office Patents 2000;1237(5).
[8] Runge F, Holm-Hansen J, Michelsen B. Carotenoid formulations, comprising a mixture of beta-carotene, lycopene and lutein. Official Gazette of the United States Patent and Trademark Office Patents 2003;1266(3).
[9] Garmyn M, Ribaya-Mercado JD, Russell RM, Bhawan J, Gilchrest BA. Effect of beta-carotene supplementation on the human sunburn reaction. Exp Dermatol 1995;4:104–11.
[10] Black HS, Mathews-Roth MM. Protective role of butylated hydroxytoluene and certain carotenoids in photocarcinogenesis. Photochem Photobiol 1991;53:707–16.
[11] Black HS. Radical interception by carotenoids and effects on UV carcinogenesis. Nutr Cancer 1998;31:212–7.
[12] Black HS, Okotie-Eboh G, Gerguis J. Diet potentiates the UV-carcinogenic response to beta-carotene. Nutr Cancer 2000;37:173–8.
[13] Obermuller-Jevic UC, Francz PI, Frank J, Flaccus A, Biesalski HK. Enhancement of the UVA induction of haem oxygenase-1 expression by beta-carotene in human skin fibroblasts. FEBS Lett 1999;460:212–6.
[14] Offord EA, Gautier JC, Avanti O, Scaletta C, Runge F, Kramer K, et al. Photoprotective potential of lycopene, beta-carotene, vitamin E, vitamin C and carnosic acid in UVA-irradiated human skin fibroblasts. Free Radic Biol Med 2002;32:1293–303.

[15] Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. N Engl J Med 1997;337:1419–28.
[16] Knekt P, Kumpulainen J, Jarvinen R, Rissanen H, Heliovaara M, Reunanen A, et al. Flavonoid intake and risk of chronic diseases. Am J Clin Nutr 2002;76:560–8.
[17] Mennen LI, Sapinho D, de Bree A, Arnault N, Bertrais S, Galan P, et al. Consumption of foods rich in flavonoids is related to a decreased cardiovascular risk in apparently healthy French women. J Nutr 2004; 134:923–6.
[18] Sies H, Stahl W. Nutritional protection against skin damage from sunlight. Annu Rev Nutr 2004;24:173–200.
[19] Avail PK, Metal A, Hara Y, Elmets CA, Katiyar SK. Green tea polyphenols prevent ultraviolet light-induced oxidative damage and matrix metalloproteinases expression in mouse skin. J Invest Dermatol 2004;122:1480–7.
[20] Depeint F, Gee JM, Williamson G, Johnson IT. Evidence for consistent patterns between flavonoid structures and cellular activities. Proc Nutr Soc 2002;61:97–103.
[21] Burda S, Oleszek W. Antioxidant and antiradical activities of flavonoids. J Agric Food Chem 2001;49:2774–9.
[22] Phan TT, Wang L, See P, Grayer RJ, Chan SY, Lee ST. Phenolic compounds of *Chromolaena odorata* protect cultured skin cells from oxidative damage: implication for cutaneous wound healing. Biol Pharm Bull 2001;24:1373–9.
[23] Kahraman A, Inal ME. Protective effects of quercetin on ultraviolet A light-induced oxidative stress in the blood of rat. J Appl Toxicol 2002;22:303–9.
[24] Kanno S, Shouji A, Asou K, Ishikawa M. Effects of naringin on hydrogen peroxide-induced cytotoxicity and apoptosis in P388 cells. J Pharmacol Sci 2003;92:166–70.
[25] Kootstra A. Protection from UV-B-induced DNA damage by flavonoids. Plant Mol Biol 1994;26:771–4.
[26] Erden Inal M, Kahraman A. The protective effect of flavonol quercetin against ultraviolet A induced oxidative stress in rats. Toxicology 2000;154:21–9.
[27] Jagetia GC, Reddy TK. The grapefruit flavanone naringin protects against the radiation-induced genomic instability in the mice bone marrow: a micronucleus study. Mutat Res 2002;519:37–48.
[28] Scharffetter K, Wlaschek M, Hogg A, Bolsen K, Schothorst A, Goerz G, et al. UVA irradiation induces collagenase in human dermal fibroblasts in vitro and in vivo. Arch Dermatol Res 1991;283:506–11.
[29] Vile GF, Tyrrell RM. UVA radiation-induced oxidative damage to lipids and proteins in vitro and in human skin fibroblasts is dependent on iron and singlet oxygen. Free Radic Biol Med 1995;18(4):721–30.
[30] Yeh S-L, Huang C-S, Hu M-L. Lycopene enhances UVA-induced DNA damage and expression of heme oxygenase-1 in cultured mouse embryo fibroblasts. Eur J Nutr 2004 [in press].
[31] Khopde SM, Priyadarsini KI, Mukherjee T, Kulkarni PB, Satav JG, Bhattacharya RK. Does beta-carotene protect membrane lipids from nitrogen dioxide? Free Radic Biol Med 1998;25:66–71.
[32] Rundhaug JE, Pung A, Read CM, Bertram JS. Uptake and metabolism of beta-carotene and retinal by C3H/10T1/2 cells. Carcinogenesis 1998;9:1541–5.
[33] Kanaze FI, Gabrieli C, Kokkalou E, Georgarakis M, Niopas I. Simultaneous reversed-phase high-performance liquid chromatographic method for the determination of diosmin, hesperidin and naringin in different citrus fruit juices and pharmaceutical formulations. J Pharm Biomed Anal 2003;33:243–9.
[34] Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988;175:184–91.
[35] Collins AR, Ma AG, Duthie SJ. The kinetics of repair of oxidative DNA damage (strand breaks and oxidised pyrimidines) in human cells. Mutat Res 1995;336:69–77.
[36] Vile GF, Tyrrell RM. UVA radiation-induced oxidative damage to lipids and proteins in vitro and in human skin fibroblasts is dependent on iron and singlet oxygen. Free Radic Biol Med 1995;18: 721–30.
[37] Shih M-K, Hu M-L. UVA-induced oxidative damage to rat liver nuclei: reduction of iron ions and the relationship between lipid peroxidation and DNA damage. Mutat Res 1999;438:125–32.
[38] Luy H, Frenk E, Applegate LA. Ultraviolet A-induced cellular membrane damage in the photosensitivity dermatitis/actinic reticuloid syndrome. Photodermatol Photoimmunol Photomed 1994;10:126–33.
[39] Palozza P, Luberto C, Calviello G, Ricci P, Bartoli GM. Antioxidant and prooxidant role of beta-carotene in murine normal and tumor thymocytes: effects of oxygen partial pressure. Free Radic Biol Med 1997;22:1065–73.
[40] Yeh SL, Hu ML. Induction of oxidative DNA damage in human foreskin fibroblast Hs68 cells by oxidized beta-carotene and lycopene. Free Radic Res 2001;35:203–13.
[41] Yeh S-L, Hu M-L. Antioxidant and pro-oxidant effects of lycopene in comparison with beta-carotene on oxidant-induced damage in Hs68 cells. J Nutr Biochem 2000;11:548–54.
[42] Lin SY, Tsai SJ, Wang LH, Wu MF, Lee H. Protection by quercetin against cooking oil fumes-induced DNA damage in human lung adenocarcinoma CL-3 cells: role of COX-2. Nutr Cancer 2002;44: 95–101.
[43] Bors W, Heller W, Michel C, Saran M. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. Methods Enzymol 1990;186:343–55.
[44] Cao G, Sofic E, Prior RL. Antioxidant and prooxidant behavior of flavonoids: structure–activity relationships. Free Radic Biol Med 1997;22:749–60.
[45] Hou L, Zhou B, Yang L, Liu ZL. Inhibition of human low density lipoprotein oxidation by flavonols and their glycosides. Chem Phys Lipids 2004;129:209–19.
[46] Trombino S, Serini S, Di Nicuolo F, Celleno L, Ando S, Picci N, et al. Antioxidant effect of ferulic acid in isolated membranes and intact cells: synergistic interactions with alpha-tocopherol, beta-carotene, and ascorbic acid. J Agric Food Chem 2004;52:2411–20.