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Short communication

Modulation of UVA light-induced oxidative stress by β-carotene, lutein and astaxanthin in cultured fibroblasts

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Abstract

The ability of β-carotene, lutein or astaxanthin to protect against UVA-induced oxidative stress in rat kidney fibroblasts (NRK) was assessed. Activities of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD), and changes in thiobarbituric acid reactive substances (TBARS) were measured as indices of oxidative stress. Exposure to UVA light at a dose intensity of 5.6 mW/cm^2 for 4 h resulted in a significant decrease in CAT and SOD activities and a significant increase in TBARS. No cytotoxicity, as indicated by lactate dehydrogenase (LDH) release, was observed. β-Carotene (1 μM), lutein (1 μM) and astaxanthin (10 nM) protect against UVA light-induced oxidative stress in vitro with astaxanthin exhibiting superior protective properties.

Keywords: UVA light; β-Carotene; Lutein; Astaxanthin; Rat kidney fibroblasts

1. Introduction

There is evidence that certain antioxidants can protect against photodamage induced by UVA light. Increased dietary antioxidant consumption may be beneficial in prevention of UV light-induced damage in vivo. Most of the studies have focused on α-tocopherol [1] and on the carotenoid β-carotene [2,3] as photoprotective agents with little regard for the possible role of other carotenoids. In fact, β-carotene only represents 10–15% of total carotenoids in the plasma [4]. Little is known about the antioxidant activity, and hence possible photoprotective properties, of naturally occurring carotenoids other than β-carotene. However, the structure of all carotenoids is broadly similar and many are be-

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lieved to have antioxidant properties. Lutein, a major xanthophyll carotenoid, is as abundant as β-carotene in human plasma [4]. It is structurally similar to β-carotene but has an additional hydroxyl group on each terminal β-ionone ring of the molecule making lutein more hydrophilic. Lutein has been shown in in vitro studies to exhibit superior antioxidant properties compared to β-carotene [5]. Astaxanthin, a carotenoid present in marine fish and shellfish is also structurally similar to β-carotene but has an additional hydroxyl and keto group on each β-ionone ring. The antioxidant potential of astaxanthin has been demonstrated both in in vivo [6] and in vitro studies [7]. In a previous study, we demonstrated the efficacy of astaxanthin in inhibiting paraquat-induced oxidative stress in chicken embryo fibroblasts (CEF) [8]. Furthermore, we have shown that astaxanthin is a superior antioxidant compared to α-tocopherol [9] and β-carotene in CEF [10].

The objectives of this study were to establish the effects of UVA radiation on endogenous antioxidant enzyme activities and lipid peroxidation in cells in culture and to evaluate if the addition of carotenoids, namely β-carotene, lutein and astaxanthin would protect against UVA-induced modulation of endogenous antioxidant enzyme activities and lipid peroxidation in these cells.

2. Materials and methods

2.1. Materials

All cell culture media were purchased from GIBCO (Paisley, Scotland). β-Carotene and lutein were obtained from Sigma Chemical Co. (Poole, Dorset, UK). Astaxanthin was generously provided by F. Hoffmann-La Roche (Basel, Switzerland). All other reagents and solvents were of analytical grade and used without purification.

2.2. Incubation of cells with test compounds and exposure to UVA light

Rat kidney (NRK) fibroblasts were purchased from the European Collection of Animal Cell Cultures (Salisbury, UK). NRK fibroblasts were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% newborn calf serum and antibiotics as previously described [11]. Stock solutions of β-carotene and astaxanthin were prepared in absolute alcohol as described previously [8,10]. Similarly, lutein was dissolved in absolute alcohol in the dark. The concentration of lutein was measured spectrophotometrically at 445 nm prior to further dilutions (mmolar extinction coefficient = 145). The final concentration of absolute alcohol in the culture medium was < 1%. A control group containing absolute alcohol was included and no differences were seen between the absolute alcohol control and a control without absolute alcohol. In all experiments, the cells were subjected to a particular exogenous level of antioxidant from the time of initial seeding until the end of the experiment. UVA light exposure was performed with polychromatic light from a 2-tube fluorescent sun lamp which emitted wavelengths between 320–400 nm. The dose intensity was fixed at 5.6 mW/cm^2 as measured with a UVX radiometer. Cells, when confluent, were rinsed twice with 5 ml of pre-chilled PBS. Exposure to UVA light was carried out in a light-proofed chamber and culture dishes were placed on an ice block. The cells were irradiated through 5 ml of PBS in covered dishes as exposure through the dish lids excluded UV wavelengths below 300 nm. Control cells were sham-irradiated, i.e. maintained at the same conditions as the UVA light-exposed cells. NRK cells were exposed to UVA light for 4 h as determined from time course experiments. Lactate dehydrogenase (LDH) release was determined in all in vitro preparations as an index of cytotoxicity [12]. LDH release was expressed as a % of the total LDH released from cells treated with 10% Triton X-100.

2.3. Procedures

Cell sonicates were prepared for enzyme analysis as previously outlined [9]. CAT activity in cell sonicates was determined on the same day as harvesting using the method of Baudhuin et al. [13]. The remaining supernatants were stored overnight at −20°C until SOD activity was determined [14]. TBARS (nmol MDA/mg protein)

Table 1
Protective effect of β-carotene, lutein and astaxanthin against UVA light-induced modulations in catalase (CAT), superoxide dismutase (SOD) activities and TBARS levels in rat kidney cells in culture

	CAT (U/mg protein)	SOD (U/mg protein)	TBARS (nmol MDA/mg protein)
β-Carotene (μM)			
Control[a]	8.59 ± 0.37	10.28 ± 0.84	3.68 ± 0.23
0	4.76 ± 0.02*	3.39 ± 0.92*	7.04 ± 0.15*
0.01	5.43 ± 0.56*	4.02 ± 1.06*	6.64 ± 0.45*
0.1	6.32 ± 0.09*	6.89 ± 1.62*	4.39 ± 0.25
0.5	7.11 ± 0.64*	9.30 ± 1.28	4.49 ± 0.05
1.0	7.85 ± 0.21	9.80 ± 0.98	4.22 ± 0.46
LSD ($P<0.05$)	1.12	3.22	0.87
Lutein (μM)			
Control[a]	7.35 ± 0.53	9.78 ± 0.35	2.34 ± 0.25
0	4.05 ± 1.02*	3.83 ± 1.84*	5.30 ± 0.67*
0.01	4.77 ± 0.80*	6.68 ± 1.05*	6.01 ± 0.18*
0.1	5.47 ± 0.56	8.38 ± 0.52	4.02 ± 0.30*
0.5	6.13 ± 0.81	7.05 ± 1.58*	3.92 ± 0.18*
1.0	8.63 ± 0.34	10.34 ± 0.67	2.60 ± 0.65
LSD ($P<0.05$)	2.37	1.77	1.58
Astaxanthin (nM)			
Control[a]	7.58 ± 0.25	9.78 ± 0.54	4.88 ± 0.49
0	3.84 ± 0.56*	3.81 ± 1.85*	9.30 ± 0.81*
0.1	5.82 ± 0.53*	5.90 ± 1.20*	8.97 ± 1.23*
1	6.08 ± 0.73*	7.16 ± 0.07*	5.43 ± 0.51
5	7.06 ± 0.49	7.06 ± 1.60*	5.32 ± 0.29
10	7.67 ± 0.08	9.73 ± 0.48	3.56 ± 0.17
LSD ($P<0.05$)	1.42	2.04	1.94

MDA, malondialdehyde; LSD, least significant difference. Data are expressed as means ± S.E.M.
[a] Control cells not exposed to UVA light and not supplemented with carotenoids. Cells were exposed to UVA light (5.6 mW/cm^2) for 4 h.
* Significantly different from control cells ($P<0.05$) one-way ANOVA, $n=6$ for all groups.

were measured as an index of lipid peroxidation as previously outlined [11]. To prepare cell sonicates for measurement of β-carotene, lutein and astaxanthin the incubation medium was rapidly aspirated and 0.5 ml of the appropriate chromatographic solvent was added at 0°C to the cell layer. The cells were removed from the dishes by scraping and placed on ice. Extraction and quantification of the carotenoids from cells followed the method of Sheehy et al. [15].

2.4. Statistical analysis

Results are presented as mean values and standard errors of the means. Data were analysed by one-way analysis of variance (ANOVA) followed by least significant difference (LSD). The level of statistical significance was taken as $P < 0.05$.

3. Results

3.1. Antioxidant potential of β-carotene, lutein and astaxanthin (Tables 1 and 2)

CAT and SOD activities was significantly decreased ($P < 0.05$) compared to control cells following 4 h of exposure to UVA light and a significant increase ($P < 0.05$) in TBARS level occurred. However, the levels of LDH release in UVA exposed NRK were well below any suggestion of significant cytotoxicity (i.e. %LDH release > 5%, data not shown). β-Carotene at a level of 1 μM and lutein at 0.1 μM returned CAT activity to control values. On the other hand, much lower levels of astaxanthin (5 nM) were required to restore CAT to control. Incorporation of

Table 2
β-Carotene and lutein content of rat kidney cells grown in β-carotene (β-C) or lutein-enriched media with or without exposure to UVA light

Treatment	Content (μg/mg protein)	Treatment	Content (μg/mg protein)
β-Carotene			
Control[a]	0.89 ± 0.12	UVA[a]	0.39 ± 0.04
Control + 0.01 μM β-C	1.13 ± 0.17	UVA + 0.01 μM β-C	0.89 ± 0.11
Control + 0.1 μM β-C	3.81 ± 0.18	UVA + 0.1 μM β-C	3.53 ± 0.07
Control + 0.5 μM β-C	28.83 ± 0.19	UVA + 0.5 μM β-C	24.62 ± 0.69
Control + 1.0 μM β-C	30.49 ± 0.59	UVA + 1.0 μM β-C	26.12 ± 0.70
Lutein			
Control[a]	nd	UVA[a]	nd
Control + 0.01 μM lutein	nd	UVA + 0.01 μM lutein	nd
Control + 0.1 μM lutein	nd	UVA + 0.1 μM lutein	nd
Control + 0.5 μM lutein	0.31 ± 0.07	UVA + 0.5 μM lutein	0.16 ± 0.02
Control + 1.0 μM lutein	0.58 ± 0.02	UVA + 1.0 μM lutein	0.50 ± 0.03

[a] Control cells and UVA light-exposed control cells were grown in media not supplemented with β-C or lutein. Cells were exposed to UVA light (5.6 mW/cm²) for 4 h. β-Carotene or lutein content of the cells were determined by HPLC analysis. Data are expressed as mean ± S.E.M.; $n = 4$ for all treatments.
nd, not detectable.

carotenoids into the UVA exposed cells also restored the SOD activity. Levels of 0.5 μM β-carotene, higher levels of lutein (1 μM) and much lower concentrations of astaxanthin (10 nM) were required. Supplementation with β-carotene (0.1 μM), lutein (1 μM) and astaxanthin (1 nM) also prevented the UVA-induced increase in TBARS. The β-carotene and lutein content of NRK cells was determined by HPLC analysis (Table 2). These data indicated that β-carotene and lutein were incorporated into cells. The carotenoid levels measured in the UVA exposed cells were lower than their corresponding controls. The nanomolar levels of astaxanthin with which cells were enriched in these studies were too small to be detected by our HPLC method. However, Lawlor and O'Brien [8] demonstrated uptake of astaxanthin at higher levels (1–10 μM) in CEF.

4. Discussion

Increased uptake of β-carotene, lutein or astaxanthin by the NRK cells from carotenoid enriched growth media improved their ability to withstand UVA light-induced modulations in the antioxidant enzymes CAT and SOD. Chopra et al. [5] demonstrated, in an in vitro cellular system, that lutein was a more effective antioxidant than β-carotene. On the other hand, β-carotene was found to be more effective than lutein in inhibiting methylcholanthrene-induced transformation of 10T1/2 cells in culture [16]. In this study the potential of lutein and β-carotene was similar and astaxanthin was more effective in restoring the UVA light-induced alteration in antioxidant enzyme activities and lipid peroxidation. The enhanced antioxidant properties of astaxanthin have previously been reported [7,8]. The decrease in carotenoid content of cells exposed to UVA light (Table 2) may be due to an increase in carotenoid consumption following ROS generation. A decrease in the carotenoid content of plasma in vivo was observed in human subjects following exposure to UV light [17].

The identification of compounds which can modulate UV light-induced skin degradation would be of assistance in determining strategies to reduce oxidative damage to skin. Human studies investigating administration of dietary antioxidants as photoprotective agents have generated conflicting results. Oral administration of β-carotene (180 mg/day) was effective as a therapy in the treatment of light sensitivity diseases such

as erythropoietic protoporphyria (EPP) and UV light-induced oxidative damage [18]. Dietary supplementation with β-carotene and/or cantaxanthin at low doses caused a significant decrease in the number of UVB light induced skin tumours in hairless mice [19]. On the other hand, oral supplementation with β-carotene (150 mg/day) did not protect against photosuppression of delayed-type sensitivity in normal human subjects [20]. β-Carotene was ineffective in preventing the occurrence of a secondary carcinoma in patients who previously had skin cancer [3].

In summary, our findings suggest that carotenoids other than β-carotene, and in particular, astaxanthin may be of importance as biological antioxidants. Further studies are necessary to investigate the potential of this xanthophyll, and others of similar structure, to protect against ultraviolet-induced cellular damage.

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References

[1] Black HS, Chan JT. Suppression of ultraviolet light-induced tumour formation by dietary antioxidants. J Invest Dermatol 1975;65:412–4.

[2] Mathews-Roth MM, Krinsky NI. Carotenoids affect development of UVB-induced skin cancer. Photochem Photobiol 1987;46:507–9.

[3] The skin cancer prevention study group, Greenberg ER, Baron JA, Stukel TA, Stevens MM, Mandel JS, Spencer SK, Elias PM, Lowe N, Nierenberg DW, Bayrd G, Vance JC, Freeman DH, Clendenning WE, Kwan T. A clinical trial of beta carotene to prevent basal-cell and squamous cell cancers of the skin. N Engl J Med 1990;323:789–95.

[4] Thurnham DI. Carotenoids: functions and fallacies. Proc Nutr Soc 1994;53:77–87.

[5] Chopra M, Wilson RL, Thurnham DI. Free radical scavenging ability of lutein in vitro. In: Canfield LM, Krinsky NI, Olson JA, editors. Carotenoids in Human Health. New York: Ann NY Acad Sci, 1993:61–7.

[6] Kurashige M, Okimasu E, Inoue M, Utsumi K. Inhibition of oxidative injury of biological membranes by astaxanthin. Physiol Chem Phys Med NMR 1990;22:27–38.

[7] Palozza P, Krinsky NI. Astaxanthin and canthaxanthin are potent antioxidants in a membrane model. Arch Biochem Biophys 1992;297:291–5.

[8] Lawlor SM, O'Brien NM. Astaxanthin: antioxidant effects in chicken embryo fibroblasts. Nutr Res 1995;15:1695–704.

[9] Lawlor SM, O'Brien NM. Development of an in vitro cell culture model to investigate the induction and quantification of oxidative stress and its inhibition by α-tocopherol. Toxicol In Vitro 1994;8:67–73.

[10] Lawlor SM, O'Brien NM. Modulation of oxidative stress by β-carotene in chicken embryo fibroblasts. Br J Nutr 1995;73:841–50.

[11] Wilson AM, Sisk RM, O'Brien MN. Modulation of cholestan-3β,5α,6β-triol toxicity by butylated hydroxytoluene, α-tocopherol and β-carotene in newborn rat kidney cells in vitro. Br J Nutr 1997;78:479–92.

[12] Vassault A. Lactate dehydrogenase. In: Bergmeyer HU, editor. Methods of Enzymatic Analysis. New York: Academic Press, 1983:118–26.

[13] Baudhuin P, Beaufay H, Rahman-Li Y, Sellinger OZ, Wattiaux R, Jacques P, De Duve C. Tissue fractionation studies. Intracellular distribution of monoamine oxidase, aspartate aminotransferance, alanine aminotransferase, D-amino acid oxidase and catalase in rat liver tissue. Biochem J 1964;92:179–84.

[14] McCord JM, Fridovich I. Superoxide dismutase. An enzyme function for erythrocuprein (hemocuprein). J Biol Chem 1969;244:6049–55.

[15] Sheehy PJA, O'Mahony E, O'Sullivan-Corbett K, Salvatella M. Simultaneous determination of retinol, α-, γ- and δ-tocopherol, 6 carotenoids and internal standards in plasma by reverse-phase HPLC. FLAIR Meeting, Montreaux, 1994.

[16] Bertram JS, Pung A, Churley M, Kappock TJ, Wilkins LR, Cooney RV. Diverse carotenoids protect against chemically induced neoplastic transformation. Carcinogene 1991;12:671–8.

[17] White WS, Kim C, Kalkwarf HJ, Bustos P, Roe DA. Ultraviolet light-induced reductions in plasma carotenoid levels. Am J Clin Nutr 1988;47:879–83.

[18] Mathews-Roth MM, Pathak MA, Fitzpatrick TB, Harber LH, Kass EH. Beta carotene therapy for erythropoietic protoprophyria and other photosensitivity diseases. Arch Dermatol 1977;113:1229–32.

[19] Mathews-Roth MM, Krinsky NI. Carotenoids affect development of UVB induced skin cancer. Photochem Photobiol 1987;46:507–9.

[20] Fuller CJ, Faulkner H, Bendich A, Parker RS, Roe DA. Effect of β-carotene supplementation on photosuppression of delayed-type hypersensitivity in normal young men. Am J Clin Nutr 1992;56:684–90.