FEBS Letters 401 (1997) 262–266 FEBS 18094

The carotenoids β-carotene, canthaxanthin and zeaxanthin inhibit macrophage-mediated LDL oxidation

Keri L.H. Carpenter*, Carina van der Veen, Rachel Hird, Ian F. Dennis, Tina Ding, Malcolm J. Mitchinson

Division of Cellular Pathology, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK

Received 9 December 1996

Abstract Human monocyte-macrophages were incubated for 24 h in Ham's F-10 medium with human low-density lipoprotein (LDL) in the presence or absence of β-carotene, canthaxanthin or zeaxanthin, at final concentrations of 2.5, 12.5 and 25 mg/l. LDL oxidation, measured by agarose gel electrophoresis, the thiobarbituric acid assay and gas chromatography, was inhibited by each of the carotenoids in a concentration-dependent manner. Canthaxanthin was more effective when incorporated into LDL before addition to the cultures whereas β-carotene and zeaxanthin were more effective when added simultaneously with LDL. The results suggest that dietary carotenoids might help slow atherosclerosis progression.

Key words: Carotenoid; β-Carotene; Zeaxanthin; Canthaxanthin; Low-density lipoprotein; Monocyte-macrophage (human)

1. Introduction

Carotenoids are plant pigments which can act as antioxidants, being radical scavengers and quenchers of singlet oxygen [1,2]. Dietary carotenoids occur in plasma lipoproteins, including low-density lipoprotein (LDL) [3,4]. Epidemiologically, they are associated with protection against ischaemic heart disease [5,6]. LDL oxidation by macrophages is believed to be important in progression of atherosclerotic lesions [7,8], and might be inhibited by carotenoids. The present study assesses the abilities of the carotenoids β-carotene, zeaxanthin and canthaxanthin to inhibit LDL oxidation by human monocyte-derived macrophages (HMM) in vitro. β-Carotene and zeaxanthin are common in vegetables and fruits. Canthaxanthin occurs in the edible mushroom *Cantharellus cinnabarinus* and is a permissible food additive.

Each carotenoid was tested at final concentrations of 2.5, 12.5 and 25 mg/l. Normal β-carotene levels in plasma are in the range 0.1–1.3 mg/l (i.e. 0.2–2.5 μM) [9–12], and zeaxanthin levels are of a similar order of magnitude, depending on diet [11]. Canthaxanthin levels are usually much lower; it was included mainly to help assess structure-activity relationships. The lower two carotenoid concentrations correspond to those attainable by dietary supplementation, based on β-carotene studies [12,13]. The third concentration, 25 mg/l, is non-physiological, being slightly above the upper limit of the supplemented range [12], but was included because LDL oxidation by macrophages is almost certainly much more rapid in vitro than in lesions.

*Corresponding author. Fax: (44) (1223) 333872.
E-mail: klhc@mole.bio.cam.ac.uk

2. Materials and methods

β-Carotene, zeaxanthin and canthaxanthin were gifts of F. Hoffmann-La Roche AG, Basel, Switzerland, and were stored in crystalline form at −70°C under argon. Nominal purities were respectively 100%, 97% and 97% (by spectrophotometry) as supplied. They were pure judged by mass spectrometry (in the electron impact, positive ionisation mode), with ions consistent with the literature [14,15]. High resolution mass spectrometry gave accurate masses of the molecular ions consistent with the theoretical values. Stock solutions of carotenoids in HPLC-grade tetrahydrofuran (THF) (Sigma-Aldrich, Gillingham, Dorset, UK) were freshly made up for each experiment, and were judged pure by HPLC. Carotenoids are highly soluble in THF, and the solutions are water-miscible, forming microemulsions, filtrable through a 0.2 μm filter, in which carotenoid molecules are surrounded by 'cages' of THF molecules [16]. This is an established method of adding carotenoids to cell cultures [16]. Each carotenoid was tested at final concentrations in the cultures of 2.5, 12.5 and 25 mg/l (4.7, 23 and 47 μM for β-carotene, and 4.4, 22 and 44 μM for canthaxanthin and zeaxanthin). None of these carotenoids was cytotoxic to HMM at these concentrations, judged by the [^{3}H]adenine release method [17].

LDL was prepared from fresh human EDTA-plasma pooled from at least four normal volunteers, using the method of Havel et al. [18]. LDL was stored at 4°C with 1 mM EDTA for no longer than 1 month. Directly before use it was dialysed against several changes of phosphate-buffered saline (PBS), the protein assayed, diluted to 1 mg LDL protein/ml with PBS, and EDTA added such that the resultant final concentration of EDTA in the cultures was 5 μM [19]. HMM were prepared by an established procedure [17]. Essentially, venous blood (treated with 2.7 mM EDTA) from normal volunteers was centrifuged on Lymphoprep separation medium (Nycomed AS, Oslo, Norway), and monocytes were purified by adherence to Falcon 24-well plastic tissue culture plates (Becton Dickinson UK Ltd., Cowley, Oxford, UK) in Gibco Macrophage SFM (serum-free medium) (Life Technologies Ltd., Paisley, Scotland, UK). Mononuclear cells were seeded at 3×10^6 cells per well, resulting in ca. $0.5–1.0\times10^6$ monocytes per well after removal of nonadherent cells. For LDL oxidation experiments the medium was changed to serum-free Ham's F-10 medium (ICN Biomedicals Inc., Costa Mesa, CA, USA), supplemented additionally with 3 μM iron(II) sulphate, 10.6 mg/l phenol red and 1 mM glutamine [19,20]. Appropriate amounts of carotenoid stock solutions were either (a) added to LDL, allowed to equilibrate for 18 h at room temperature, and this carotenoid-supplemented LDL subsequently added (without reisolation) to HMM cultures, or (b) added directly to HMM cultures, to which unsupplemented LDL was added simultaneously. The final concentrations in the cultures were 160 μg LDL protein/ml, THF≤0.5% (v/v), carotenoids as stated above. The final volume of the medium was 0.62 ml per well. The controls were culture medium containing LDL in the presence or absence of carotenoids, but no cells. For LDL oxidations in the absence of carotenoid, THF was added at 0.5% (v/v). LDL oxidations were terminated at 24 h by addition of EDTA (1.5 mM) and butylated hydroxytoluene (30 μM). Samples were stored at 4°C for the thiobarbituric acid (TBA) assay and electrophoresis, or at −20°C under argon for analysis by gas chromatography (GC) [19–21].

LDL oxidation was measured in samples of medium by (a) the TBA assay [21], (b) relative mobility on agarose gel electrophoresis (distance migrated by oxidised LDL divided by distance migrated by native LDL) using the Beckman Paragon LipoGel system (Beckman Instruments, Brea, CA, USA) [19,21] and (c) analysis of lipids and

PII S0014-5793(96)01488-3

oxidised lipids by GC [19–21]. Essentially the procedure comprised addition of internal standards, Bligh and Dyer extraction, sodium borohydride reduction, saponification and derivatisation to methyl esters and trimethylsilyl ethers, prior to analysis by GC using a 30 m DB-1 fused silica capillary column (J and W Scientific, Folsom, CA, USA) [19–21]. When LDL is oxidised, its polyunsaturated fatty acids, of which linoleate is the major species, become depleted, and 7β-hydroxycholesterol is produced [19–21].

Purity of carotenoid stock solutions, carotenoid uptake by cells, and levels in medium and in LDL, were measured by reversed-phase HPLC, monitoring at 460 nm, using a 1050 series HPLC system with a ChemStation data system (Hewlett-Packard Ltd., Stockport, Cheshire, UK). The method was an adaptation of that of Nelis and De Leenheer [22], using a Spherisorb ODS-2 column (Phase Separations Ltd., Deeside, Clwyd, UK), 25 cm×4.6 mm, particle size 5 μm, eluting at 1.5 ml/min with isocratic methanol/acetonitrile/dichloromethane in the following volume proportions: 60/30/10 for canthaxanthin, 60/10/30 for β-carotene and 85/10/5 for zeaxanthin. Carotenoids were extracted from the medium, from cell lysates, and from LDL, using ethanol-hexane, with Sudan I (Sigma Chemical, Poole, Dorset, UK) as an internal standard.

The level of incorporation of carotenoid was measured in LDL (1 ml of 1 mg LDL protein/ml in PBS) which had been equilibrated for 18 h at room temperature with canthaxanthin, zeaxanthin or β-carotene (30 μl of a 5 mg/ml solution in THF). This represents the top concentration used in the main part of the study (i.e. equivalent to a final carotenoid concentration of 25 mg/l in the cultures). Unincorporated carotenoid was removed using an anion-exchange column (DEAE Sepharose CL-6B; Sigma) eluted initially with 50 mM Tris buffer (pH 8), and then the LDL with its incorporated carotenoid eluted subsequently using 0.5 M sodium chloride. An aliquot of the eluted LDL was assayed for protein, and another aliquot was extracted with hexane and analysed by HPLC as above to quantitate carotenoids.

3. Results and discussion

LDL oxidation was much more in the presence of cells than in their absence, and carotenoids generally made little difference to the background level of LDL oxidation in the absence of cells, which was typically: thiobarbituric acid-reactive substances (TBARS) less than 5 nmol/mg LDL protein, relative electrophoretic mobility (REM) less than 1.5, 7β-hydroxycholesterol less than 10 μg/mg LDL protein. Results in the presence of cells are explained below. Each experiment was repeated on three separate occasions, each with a different batch of LDL and a different donor of monocytes. Results varied somewhat, presumably due to individual variations in LDL composition and monocyte activity, but the trend was maintained, and a representative experiment is illustrated in each case.

Canthaxanthin, added to the LDL, inhibited HMM-mediated LDL oxidation in a concentration-dependent manner, judged by TBARS, REM and GC (Fig. 1). However, when canthaxanthin was added to the medium at the same time as the LDL, negligible inhibition was seen in terms of TBARS and REM (Fig. 1a,b), or linoleate (18:2) depletion, though some inhibition of 7β-hydroxycholesterol production was seen (Fig. 1c).

Zeaxanthin produced slight inhibition of LDL oxidation when added to the LDL, and marked, concentration-dependent inhibition when added to the medium at the same time as the LDL (Fig. 2). The apparent differences in behaviour of these two carotenoids, i.e. zeaxanthin being more effective when added to the medium whereas canthaxanthin was more effective when added to the LDL, are in keeping with the polarities of the carotenoids; canthaxanthin is a diketone and therefore less polar than the diol zeaxanthin. However, β-carotene, which is a hydrocarbon, and so even less polar than canthaxanthin, was more effective at inhibiting LDL oxidation when added to the medium than when added to the LDL (Fig. 3).

Simple consideration of polarities thus does not explain all the differences in results. The different carotenoid species might preferentially scavenge different types of radicals; e.g. canthaxanthin might be better at scavenging lipid peroxyl radicals, as in another system [23], whereas β-carotene and zeaxanthin might be better at scavenging thiyl radicals. The carotenoids' ability to quench singlet oxygen is probably irrelevant here. Singlet oxygen would have given rise to cholest-6-en-3β,5α-diol, whereas free radicals produce 7-oxysterols [24], and the latter are formed in LDL oxidation by macrophages or copper [19–21].

The relative antioxidant potencies of the three carotenoids tested were comparable in this study. At 25 mg/l, mean percentage effects of β-carotene (added to the medium), zeaxanthin (added to the medium) and canthaxanthin (added via the LDL) were, respectively: 73%, 75% and 83% inhibition of 7β-hydroxycholesterol production; 54%, 55% and 78% inhibition of depletion of linoleate; 65%, 43% and 56% inhibition of REM; and 63%, 58% and 52% inhibition of TBARS formation. The results imply that the carotenoids are slightly more effective at protecting cholesterol from oxidation than they are at protecting polyunsaturated fatty acids from oxidation, consequent MDA formation and apo B-100 modification. Carotenoids are rigid lipophilic molecules and so might tend to associate with cholesterol, either the unesterified cholesterol in the shell of LDL, or the cholesterol moiety of cholesterol esters in the core of the LDL particle.

The effect of increasing the carotenoid concentration to 50 mg/l was also tested, and for canthaxanthin and zeaxanthin gave increased inhibition of LDL oxidation, but this was not extensively studied as this concentration was far above physiological levels. β-Carotene (added to the medium) gave only marginally more inhibition on increasing to 50 mg/l (93 μM). Canthaxanthin at 50 mg/l (89 μM; added via the LDL), gave 84% inhibition of REM and 84% inhibition of TBARS, whilst zeaxanthin at 50 mg/l (88 μM; added to the medium) gave inhibitions of 57% for REM, 86% for TBARS, and 90% for 7β-hydroxycholesterol. In comparison, 80 μM α-tocopherol gave 90% inhibition of REM under similar conditions [19]; such a plasma level is achievable by dietary supplementation [12,13].

Results from HPLC suggested that uptake of canthaxanthin, zeaxanthin and β-carotene by HMM was approximately of the order of a nanomole per 10^6 cells, in Ham's F-10 medium in the absence of LDL, for a 25 mg/l final concentration in cultures. This is comparable to uptake for various cell lines, using THF as a solvent vehicle for carotenoids [16]. Carotenoids might become associated with cell membranes and/or be taken up into the cytosol. Incorporation of carotenoids into the cell surface membrane might be important in inhibiting cell-mediated LDL oxidation. However, pre-incubation of HMM with zeaxanthin (25 mg/l) for 18 h did not diminish their ability to oxidise LDL (data not shown). Thus scavenging of aqueous-phase radicals (e.g. thiyl radicals) by zeaxanthin seems a more likely explanation of this carotenoid's effect.

The levels of incorporation of canthaxanthin, zeaxanthin and β-carotene into LDL were respectively 198, 129 and 201

nmol/mg LDL protein, for a level of addition equivalent to final concentration in the cultures of 25 mg/l. Thus the comparative inefficiency of β-carotene to inhibit oxidation when added via the LDL, rather than to the medium, was not due

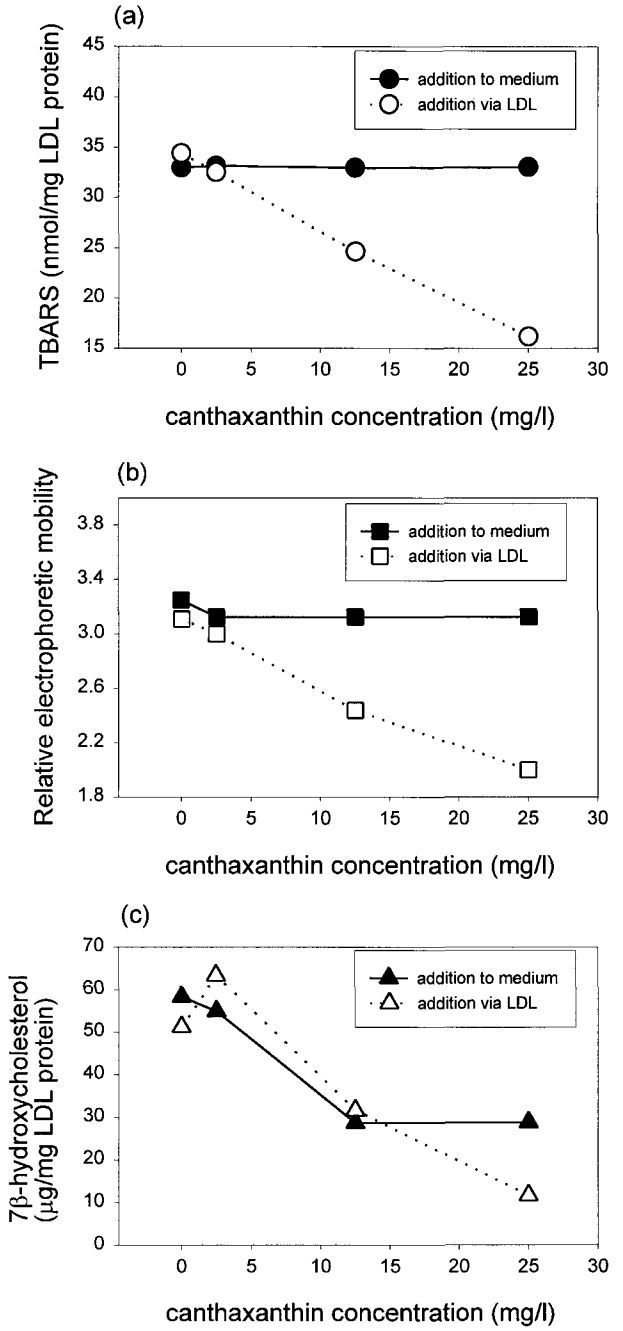

Fig. 1. Effect of canthaxanthin on HMM-mediated oxidation of LDL. (a) Thiobarbituric acid-reactive substances (TBARS) expressed as equivalent nanomoles of malondialdehyde/mg LDL protein (circles), (b) relative electrophoretic mobility (REM) (squares) and (c) 7β-hydroxycholesterol expressed as μg/mg LDL protein (triangles), are plotted against canthaxanthin final concentration expressed as mg/l. Open symbols and dotted lines denote where canthaxanthin was added via the LDL; closed symbols and solid lines denote where canthaxanthin was added directly to the culture medium at the same time as the LDL. For further details see Section 2. TBARS, REM and 7β-hydroxycholesterol were all measured in the same experiment. The data are from one experiment representative of three.

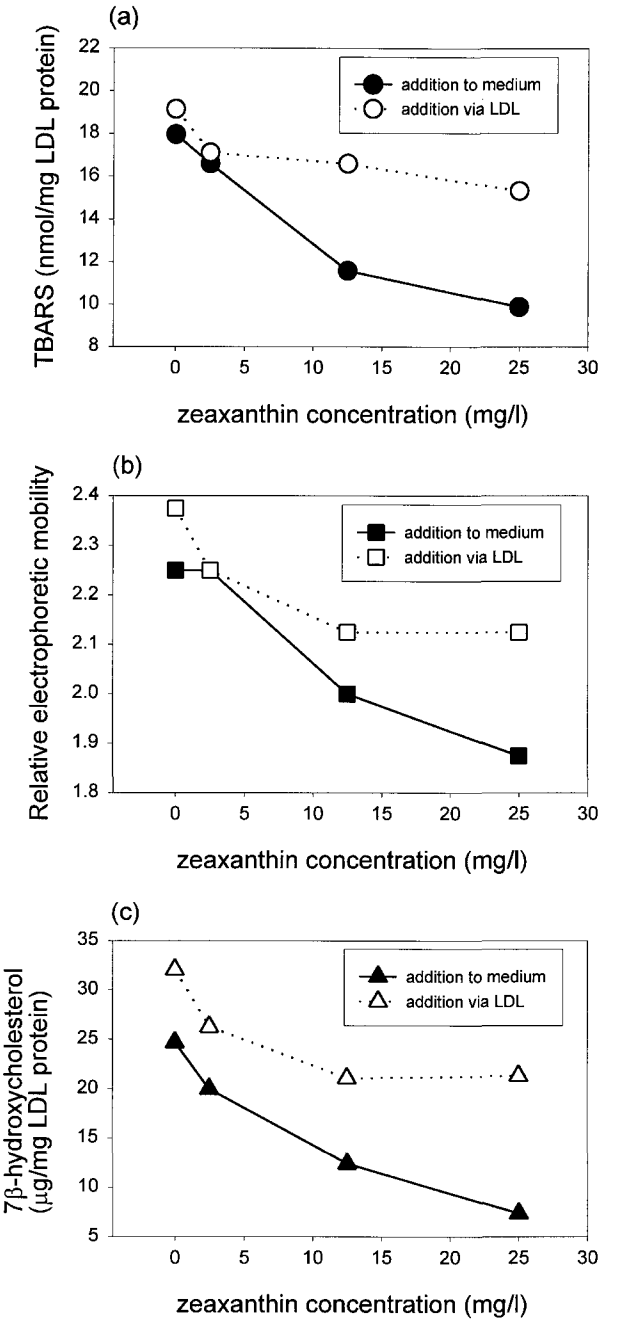

Fig. 2. Effect of zeaxanthin on HMM-mediated oxidation of LDL. Other details are as in the legend to Fig. 1.

to lack of incorporation. The lower level of incorporation of zeaxanthin than canthaxanthin into LDL is in keeping with the former being more effective at inhibiting LDL oxidation when added to the medium, whereas the latter was more effective when added via the LDL.

Levels of β-carotene, canthaxanthin and zeaxanthin, measured by HPLC, declined in the extracellular culture medium during oxidation of LDL (data not shown). Likewise, falls in the lower levels of endogenous carotenoids were reported in copper-catalysed LDL oxidation [3]. In the present study, depletion was more marked in the situation where the carotenoid was acting more effectively as a radical scavenger, i.e. β-carotene and zeaxanthin were more depleted when added di-

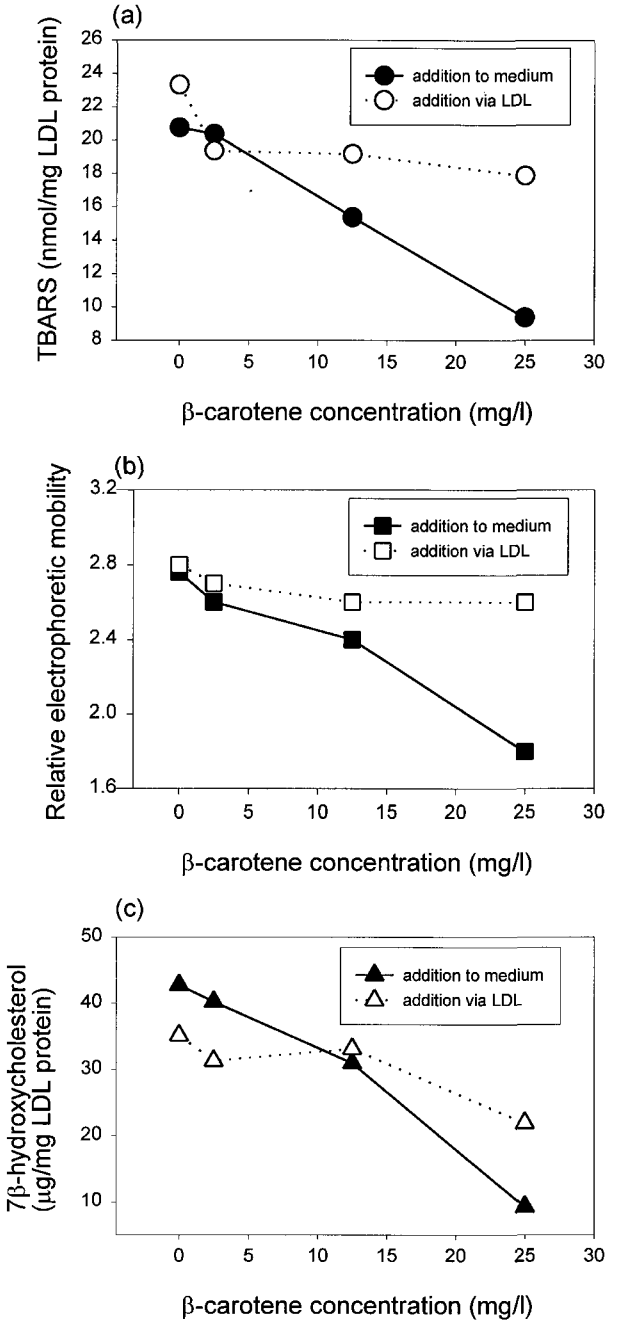

Fig. 3. Effect of β-carotene on HMM-mediated oxidation of LDL. Other details are as in the legend to Fig. 1.

rectly into the medium than via the LDL, whereas canthaxanthin was more depleted when it was added via the LDL.

Previous studies have investigated β-carotene, but no other carotenoids, for potential inhibition of LDL oxidation. Some ex vivo measurements of LDL from volunteers receiving oral β-carotene supplements and of LDL supplemented with β-carotene in vitro revealed no prolongation of the lag phase (234 nm absorbance) of copper-catalysed LDL oxidation [9,12,13]. However, Jialal and colleagues found that addition of β-carotene in vitro inhibited LDL oxidation mediated by copper or HMM [25]. The lack of effect of β-carotene in some studies [9,12,13] might be due to harshness of the in vitro oxidation conditions, obliterating any protective effect of β-carotene, as carotenoids are susceptible to breakdown under oxidative stress.

Lipid peroxyl radicals (ROO•), thiyl radicals (RS•), and superoxide radical cation ($O_2^{\bullet-}$) are believed important in macrophage-mediated LDL oxidation [8], and there is evidence that β-carotene can scavenge these radicals by forming β-carotene-radical adducts [1,26,27]. Canthaxanthin and zeaxanthin may behave likewise. All three carotenoids slowed peroxidation of methyl linoleate, canthaxanthin being the most potent of the three [23]. Mechanisms of radical scavenging by carotenoids are complex and incompletely understood, although the common feature appears that the carotenoid molecule itself becomes a radical, the unpaired electron of which is highly delocalised over the conjugated polyene chromophore, thereby having a stabilising effect [2]. The efficacy of individual carotenoids for radical scavenging depends on the particular system, presumably because multiple factors are involved, regarding both the radical being scavenged and the carotenoid itself, including steric considerations, electronic charge distribution, nature of solvent and microenvironment [23,28–30]. Thus a dietary intake of mixed carotenoids might be better than one dominated by an individual carotenoid, since in vivo a variety of radicals and local microenvironments will be encountered. Similar behaviour of β-carotene and its 3,3′-diol, zeaxanthin, in this and other studies, is in keeping with similarities in electron density profiles, and consequently similar chromophores, of these two compounds [2]. For canthaxanthin, the electron-withdrawing conjugated keto groups at C-4 and C-4′ cause substantial changes in electron density along the polyene chain, especially near the end groups [2], partly explaining why canthaxanthin behaves differently from zeaxanthin and β-carotene [23,28].

Carotenoid radicals are short-lived and oxidative breakdown eventually produces non-radical products, with resultant bleaching due to breaking of the chromophore [2]. Carotenoids are reportedly better radical scavengers at low pO_2 [1], which may be relevant in tissues such as artery walls, arterial blood having a pO_2 of 95 mm Hg (i.e. 12.5% oxygen) [31], cf. 20.9% oxygen for atmospheric air. Within the artery wall, and within lesions, pO_2 may or may not be lower than that of blood [32,33]. Carotenoids can also act as pro-oxidants, especially at high concentration and high pO_2 [1,2]. In vivo, α-tocopherol and carotenoids might be complementary, being respectively effective at high and low pO_2 [1].

LDL oxidation in vitro is probably much harsher than in atherosclerotic lesions, which evolve over decades, whereas experiments take only 24 h. Moreover the overall degree of oxidation in the lesion appears to be much less than attained by LDL oxidation in vitro, judged by the levels of oxidised lipids [20,34,35], e.g. the level of 7β-hydroxycholesterol in lesions is ca. 0.1 μg/100 μg cholesterol, whereas it is about 100-fold higher in LDL oxidised by HMM or copper for 24 h [20,34,35]. Studies of LDL isolated from atherosclerotic lesions suggest only a modest degree of oxidation [36]. However, 'hot spots' of oxidation might occur in microenvironments not apparent on analysis of bulk lesion contents.

Although carotenoids are less abundant than α-tocopherol in plasma lipoproteins, they may still play a complementary role in scavenging radicals, slowing lipoprotein oxidation in the lesion and thus retarding lesion progression. Cholesterol oxidation in lesions is also caused by cytochrome P-450 sterol 26-hydroxylase (also termed sterol 27-hydroxylase) [34,35];

the effects of carotenoids on this enzyme are untested. Besides their antioxidant properties, carotenoids have other biological activities, including inhibition of cell proliferation and enhancement of the immune system [37], which might affect atherosclerosis progression.

This study showed that β-carotene, canthaxanthin and zeaxanthin all inhibited HMM-mediated LDL oxidation in vitro, supporting the idea that diets rich in carotenoids might help slow the progression of arterial disease.

Acknowledgements: We thank Drs. W. Schüep and J. Schierle (Hoffmann-La Roche AG, Basle, Switzerland) for the kind gift of carotenoids, Dr. C.M. Fitzsimmons, Dr. S.J. Hardwick, Mrs. C.E. Marchant and Mrs. N.S. Law (Pathology Department, University of Cambridge) for advice, Dr. J.A. Ballantine (University of Wales, Swansea, UK) for mass spectrometry, and the Ministry of Agriculture, Fisheries and Food (UK) and the British Heart Foundation for financial support.

References

[1] Burton, G.W. and Ingold, K.U. (1984) Science 224, 569–573.
[2] Britton, G. (1995) FASEB J. 9, 1551–1558.
[3] Esterbauer, H., Gebicki, J., Puhl, H. and Jürgens, G. (1992) Free Radical Biol. Med. 13, 341–390.
[4] Clevidence, B.A. and Bieri, J.G. (1993) Methods Enzymol. 214, 33–46.
[5] Kardinaal, F.M., Aro, A., Kark, J.D., Riemersma, R.A., van't Veer, P., Gomez-Aracena, J., Kohlmeier, L., Ringstad, J., Martin, B.C., Mazaev, V.P., Delgado-Rodriguez, M., Thamm, M., Huttunen, J.K., Martin-Moreno, J.M. and Kok, F.J. (1995) Arterioscler. Thromb. Vasc. Biol. 15, 726–732.
[6] Riemersma, R.A., Wood, D.A., MacIntyre, C.C.A., Elton, R.A., Gey, K.F. and Oliver, M.F. (1991) Lancet 337, 1–5.
[7] Witztum, J.L. and Steinberg, D. (1991) J. Clin. Invest. 88, 1785–1792.
[8] Jessup, W. and Leake, D.S. (1995) in: Oxidative Stress, Lipoproteins and Cardiovascular Dysfunction (Rice-Evans, C. and Bruckdorfer, K.R., Eds.) pp. 99–129. Portland Press, London.
[9] Gaziano, J.M., Hatta, A., Flynn, M., Johnson, E.J., Krinsky, N.I., Ridker, P.M., Hennekens, C.H. and Frei, B.H. (1995) Atherosclerosis 112, 187–195.
[10] The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group (1994) New Engl. J. Med. 330, 1029–1035.
[11] Cooney, R.V., Franke, A.A., Hankin, J.H., Custer, L.J., Wilkens, L.R., Harwood, P.J. and Le Marchand, L. (1995) Cancer Epidemiol. Biomarkers Prevent. 4, 207–215.
[12] Reaven, P.D., Khouw, A., Beltz, W.F., Parthasarathy, S. and Witztum, J.L. (1993) Arterioscler. Thromb. 13, 590–600.
[13] Princen, H.M.G., van Poppel, G., Vogelsang, C., Buytenhek, R. and Kok, F.J. (1992) Arterioscler. Thromb. 12, 554–562.
[14] Lusby, W.R., Khachik, F., Beecher, G.R. and Lau, J. (1992) Methods Enzymol. 213, 111–128.
[15] Enzell, C.R., Francis, G.W. and Liaaen-Jensen, S. (1969) Acta Chem. Scand. 23, 727–750.
[16] Cooney, R.V., Kappock, T.J., Pung, A. and Bertram, J.S. (1993) Methods Enzymol. 214, 55–68.
[17] Müller, K., Hardwick, S.J., Marchant, C.E., Law, N.S., Waeg, G., Esterbauer, H., Carpenter, K.L.H. and Mitchinson, M.J. (1996) FEBS Lett. 388, 165–168.
[18] Havel, R.J., Eder, H.A. and Bragdon, J.H. (1955) J. Clin. Invest. 34, 1345–1353.
[19] Marchant, C.E., van der Veen, C., Law, N.S., Hardwick, S.J., Carpenter, K.L.H. and Mitchinson, M.J. (1996) Free Radical Res. 24, 333–342.
[20] Carpenter, K.L.H., Wilkins, G.M., Fussell, B., Ballantine, J.A., Taylor, S.E., Mitchinson, M.J. and Leake, D.S. (1994) Biochem. J. 304, 625–633.
[21] Marchant, C.E., Law, N.S., van der Veen, C., Hardwick, S.J., Carpenter, K.L.H. and Mitchinson, M.J. (1995) FEBS Lett. 358, 175–178.
[22] Nelis, H.J.C.F. and De Leenheer, A.P. (1983) Anal. Chem. 55, 270–275.
[23] Terao, J. (1989) Lipids 24, 659–661.
[24] Teng, J.I. and Smith, L.L. (1973) J. Am. Chem. Soc. 95, 4060–4061.
[25] Jialal, I., Norkus, E.P., Cristol, L. and Grundy, S.M. (1991) Biochim. Biophys. Acta 1086, 134–138.
[26] Everett, S.A., Dennis, M.F., Patel, K.B., Maddix, S., Kundu, S.C. and Willson R.L. (1996) J. Biol. Chem. 271, 3988–3994.
[27] Truscott, T.G., McGarvey, D., Lambert, C., Hill, T., Tinkler, J., Conn, P., Böhm, F., Land, E.J. and Schlach, W. (1995) Biochem. Soc. Trans. 23, 252S.
[28] Miller, N.J., Sampson, J., Candeias, L.P., Bramley, P.M. and Rice-Evans, C.A. (1996) FEBS Lett. 384, 240–242.
[29] Woodall, A.A., Britton, G. and Jackson, M.J. (1995) Biochem. Soc. Trans. 23, 133S.
[30] Hill, T.J., Land, E.J., McGarvey, D.J., Schlach, W., Tinkler, J.H. and Truscott, T.G. (1995) J. Am. Chem. Soc. 117, 8322–8326.
[31] Millhorn, H.T. and Pulley, P.E. (1968) Biophys. J. 8, 337–357.
[32] Hajjar, D.P., Farber, I.C. and Smith, S.C. (1988) Arch. Biochem. Biophys. 262, 375–380.
[33] Klinowski, J., Korsner, S.E. and Winlove, C.P. (1982) Cardiovasc. Res. 16, 448–456.
[34] Carpenter, K.L.H., Taylor, S.E., Ballantine, J.A., Fussell, B., Halliwell, B. and Mitchinson, M.J. (1993) Biochim. Biophys. Acta 1167, 121–130.
[35] Carpenter, K.L.H., Taylor, S.E., van der Veen, C., Williamson, B.K., Ballantine, J.A. and Mitchinson, M.J. (1995) Biochim. Biophys. Acta 1256, 141–150.
[36] Steinbrecher, U.P. and Lougheed, M. (1992) Arterioscler. Thromb. 12, 608–625.
[37] Schwartz, J.L. (1993) Methods Enzymol. 214, 226–256.