ANTICANCER RESEARCH *25*: 3767-3772 (2005)

The Photoreceptor Protector Zeaxanthin Induces Cell Death in Neuroblastoma Cells

MAURO MACCARRONE[1,2], MONICA BARI[1], VALERIA GASPERI[1] and BARBARA DEMMIG-ADAMS[3]

[1]*Department of Biomedical Sciences, University of Teramo, Piazza A. Moro 45, 64100 Teramo;*
[2]*IRCCS Center for Experimental Neurobiology "C. Mondino", Rome, Italy;*
[3]*Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309-0334, U.S.A.*

Abstract. *Background: The dietary carotenoid zeaxanthin protects against age-related eye disease by preventing apoptosis in photoreceptor cells. This study examined the effect of zeaxanthin on neuroblastoma cells in which apoptosis can be induced with lipid peroxidation products. Since zeaxanthin can inhibit lipid peroxidation and β-carotene inhibits lipoxygenase (LOX) activity, it was of concern that zeaxanthin might inhibit apoptosis in these cancer cells. Materials and Methods: Apoptosis-resistant CHP100 neuroblastoma cells were treated with zeaxanthin. Apoptosis was assessed via an immunoassay for histone-associated DNA fragments and cytofluorimetric analysis of apoptotic body formation. The effect of zeaxanthin on the activity of two model LOXs and LOX-mediated lipid peroxidation in liposomes was assessed. Results: Zeaxanthin strongly induced apoptosis in neuroblastoma cells. Consistent with this finding, zeaxanthin did not inhibit LOX activity. Conclusion: Zeaxanthin is a remarkable dietary factor that is able to induce apoptosis in neuroblastoma cells while being able to prevent apoptosis in healthy cells.*

The crucial role of some minor food compounds in human physiology (*e.g.* vitamins) has long been recognized. However, the realization that a multitude of dietary factors is capable of modulating the expression of key regulatory genes in humans is much more recent (see 1). Most of these dietary factors are synthesized by plants or algae (the latter being the source of fish oils) and are, therefore, sometimes referred to as phytochemicals. These phytochemicals modulate the expression of human genes that regulate fundamentally important processes such as cell division, programmed cell death, and immune responses/ inflammation (see *e.g.* 1-3). These central processes and, in turn, imbalances in their regulation, play a key role in all major human diseases including cancer, pro-inflammatory diseases (*e.g.* heart disease, diabetes, age-related blindness), and autoimmune diseases (4). These diseases involve seemingly opposite problems, *i.e.* either too little or too much programmed cell death. While runaway cell division and/or insufficient cell death is a key feature of, for instance, cancer, excessive cell death is involved in other diseases such as heart disease, diabetes, age-related blindness and neurodegenerative diseases. Can dietary factors ameliorate both of these contrasting conditions? Or do compounds that trigger the health of unwanted cells exacerbate conditions involving excess cell death?

There is evidence that among phytochemicals, phenolics (3,5) and the polyunsaturated fatty acids of fish oil (6), for example, do indeed have the notable ability to 'work both ways', triggering programmed cell death of unwanted cells while aiding in the survival of needed cells. The present report adds new insight into a similarly remarkable role of another phytochemical, the yellow pigment zeaxanthin, that belongs to the subclass of oxygen-containing carotenoids known as xanthophylls.

Zeaxanthin is synthesized by and functions in a protective role in plants exposed to stressful environmental conditions (7). Zeaxanthin protects against the formation of potentially destructive reactive oxygen species in leaves exposed to intense sunlight alone or moderate levels of sunlight in the presence of environmental conditions unfavorable for plant growth (1). This photoprotective process is necessary for plant fitness (8); aerobic photosynthesis could not exist without it. Zeaxanthin facilitates the harmless dissipation of excess energy absorbed by chlorophyll (1) *via* a mechanism that involves a reversible electron exchange between chlorophyll and zeaxanthin (with zeaxanthin transiently reducing chlorophyll; 9). Zeaxanthin's close isomer lutein also plays a role, albeit a minor one, in this dissipation

Correspondence to: Dr. Mauro Maccarrone, Department of Biomedical Sciences, University of Teramo, Piazza A. Moro 45, 64100 Teramo, Italy. e-mail: mmaccarrone@unite.it

Key Words: Apoptosis, lipoxygenase, carotenoid, lipid peroxidation, phytochemical.

0250-7005/2005 $2.00+.40

process (10). In addition, zeaxanthin serves in photoprotection *via* a second, poorly understood mechanism that involves an inhibition of lipid peroxidation (11,12). Zeaxanthin and lutein – neither of which can be synthesized by humans – apparently have a host of beneficial effects when consumed by humans as well. Epidemiological studies have identified inverse links between zeaxanthin/lutein and a wide range of human diseases, including age-related eye disease, various cancers and other conditions (1,13). However, the underlying mechanisms for these apparent protective effects have remained poorly understood.

Although zeaxanthin clearly is the primary protective xanthophyll in plants, greater attention has been paid to lutein in human studies. This is, however, not necessarily based on the available evidence, but may simply be due to the fact that lutein is commercially available whereas zeaxanthin is not. In the human eye, zeaxanthin and lutein form the yellow spot of the retinal macula, with greater zeaxanthin/lutein ratios in the areas of highest light exposure, and the zeaxanthin/lutein ratio also increases from blood plasma to retina (reviewed in 13). Individuals suffering from age-related eye disease (*e.g.* AMD for age-related macular degeneration) have lesser xanthophyll densities throughout their retinas, and dietary zeaxanthin and lutein levels are inversely linked to the risk for AMD as well as cataracts (reviewed in 13). A breakthrough in the understanding of the functioning of retinal zeaxanthin was recently made when it was shown that zeaxanthin prevents programmed cell death (apoptosis) of retinal photoreceptor cells in an animal model (14,15). On the other hand, studies with human cancer cell lines provided evidence that lutein can stimulate apoptosis of human breast cancer cells (transformed human mammary epithelium; 16) and leukemia cells (T-lymphoblast Jurkat cells; 17). Lutein furthermore selectively induced apoptosis in mouse tumor cells, but decreased apoptosis in cancer-fighting immune cells (blood leukocytes) of tumor-bearing mice (18).

It is presently unknown how xanthophylls may exert these remarkable effects on different cell types. Since zeaxanthin can apparently modulate lipid peroxidation in plants (11,12) as well as in humans (*e.g.* in epithelial cells of the lens; 19), reactions associated with lipid peroxidation could be involved. Zeaxanthin protects lipids against photosensitized, singlet oxygen-catalyzed peroxidation *in vitro* and, while this ability is enhanced by tocopherol (Vitamin E), zeaxanthin has the more potent, primary effect (20,21). In both plants and animals, lipids can be oxidized by several classes of enzymes, including lipoxygenases (LOXs). Moreover, plant and animal lipoxygenases produce lipid derivatives with both pro- and anti-apoptotic functions (22,23). For example, inhibition of LOX activity (blockage of 12-LOX expression) prevented apoptosis of neurons and was discussed as a new target for the treatment of Alzheimer's disease (24), whereas inhibition of LOX (5-LOX and 12-LOX) in leukemia cells induced apoptosis (25). Furthermore, various phytochemicals acting as antioxidants, such as α-tocopherol (vitamin E) and red wine resveratrol, have been shown to inhibit the activity of lipoxygenases (26). The carotenoid β-carotene was also capable of inhibiting LOX activity (27-30).

The present study examines the effect of zeaxanthin – as an inhibitor of photoreceptor cell death in the eye – on neuroblastoma, a childhood cancer affecting neurons and the eye that is rather resistant to programmed cell death (31). A neuroblastoma cell line was chosen in which 5-LOX activity is present, and apoptosis had previously been stimulated by treatments with 5-LOX products (32). In addition, we examined whether or not zeaxanthin may have a direct effect on isolated model lipoxygenases over a range of zeaxanthin concentrations.

Materials and Methods

Cell culture and treatment, and determination of apoptosis. Adherent human CHP100 neuroblastoma cells were cultured, as previously reported (33), in a 1:1 mixture of MEM (Eagle's minimal essential medium plus Earle's salts) and Ham's F-12 media (Flow Laboratories Ltd., Herts, UK), supplemented with 15% heat-inactivated fetal bovine serum, sodium bicarbonate (1.2 g/l), 15 mM Hepes buffer, 2 mM L-glutamine and 1% non-essential amino acids. CHP100 cells were cultured at 37˚C in a 5% CO_2 atmosphere, and were trypsinized (0.05% trypsin/0.02% EDTA) before treatment. Cells were treated with zeaxanthin (or dimethylsulfoxide vehicle in the controls) by adding this substance directly to the culture medium. Pure zeaxanthin was obtained as a gift from Hoffman La Roche (Basel, Switzerland); its purity was confirmed by high-pressure liquid chromatography using a previously described column (34) that provides baseline separation of zeaxanthin and, when present, lutein.

Apoptosis was estimated 48 h after treatment (33) by using a cell-death detection ELISA kit (Boehringer Mannheim, Mannheim, Germany), based on the evaluation of DNA fragmentation by an immunoassay for histone-associated DNA fragments in the cell cytoplasm (33). In addition, apoptosis was evaluated by cytofluorimetric analysis, performed in a FACScalibur Flow Cytometer (Becton Dickinson, Lincoln Park, NJ, USA), as described before (33). This latter technique quantifies apoptotic body formation in dead cells by staining with propidium iodide (100 μg/ml) and recording orange fluorescence (FL2 channel) in order to evaluate the percentage of hypodiploid events through the Cell Quest software (Becton Dickinson).

Lipoxygenase isolation and assay. Lipoxygenase-1 (LOX-1) was purified from soybean (*Glycine max*) seeds as reported (35), and was then subjected to a further step of purification by fast-protein liquid chromatography (FPLC, size exclusion column Superdex-200; Pharmacia, Uppsala, Sweden), using an AKTA Explorer apparatus (Pharmacia). 5-Lipoxygenase (5-LOX) was purified to homogeneity from barley (*Hordeum vulgare*) by immunoaffinity chromatography, as reported earlier (36).

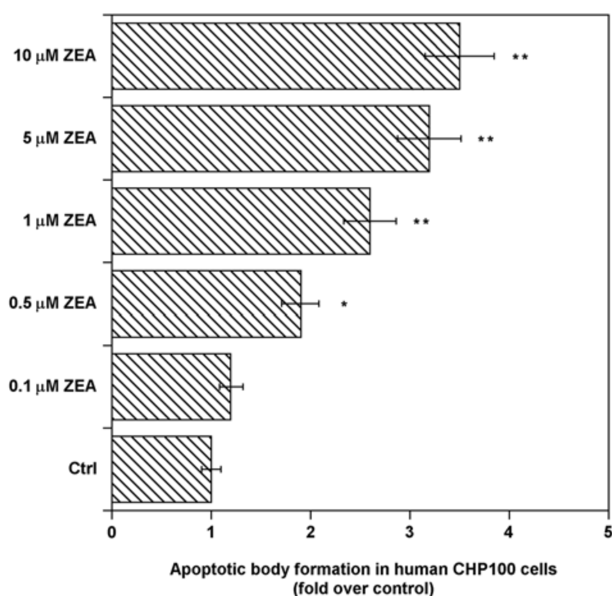

Figure 1. *Induction of apoptosis by zeaxanthin in human CHP100 neuroblastoma cells. Apoptosis was evaluated as DNA fragmentation by an immunoassay for histone-associated DNA fragments in the cell cytoplasm. ZEA, zeaxanthin. **denotes p<0.01 and *denotes p<0.05 versus controls.*

Lipoxygenase activity was assayed spectrophotometrically at 25˚C in 100 mM sodium phosphate buffer (pH 7.0) by recording the formation of conjugated hydroperoxides from linoleic acid at 234 nm (35).

Preparation of liposomes. Negatively-charged, unsaturated phosphatidylcholine liposomes were prepared using linoleic acid and a liposome kit (Sigma Chemical Co., St. Louis, MO, USA), as reported previously (35). Liposome suspensions (100 μl, corresponding to 2 μmol phosphatidylcholine) were incubated for 30 min at 25˚C with 2 μM LOX-1, after which time membrane lipids were isolated and the oxidative index, *i.e.* the $A_{234/205}$ ratio (37), was measured as previously described (38).

Results

In preliminary experiments, treatment with zeaxanthin augmented apoptosis of neuroblastoma cells as triggered by etoposide, a frequently used inducer of apoptosis (not shown). In addition and more significantly, treatment with zeaxanthin alone resulted in a significant and strong induction of programmed cell death in human CHP100 neuroblastoma cells over a concentration range of 0.5-10 μM zeaxanthin (Figures 1 and 2). This was independently assessed as (a) DNA fragmentation *via* an immunoassay for histone-associated DNA fragments in the cell cytoplasm (Figure 1) and (b) apoptotic body formation after staining and visualization *via* cytofluorimetric analysis (Figure 2). Both of these features are hallmarks of programmed cell death.

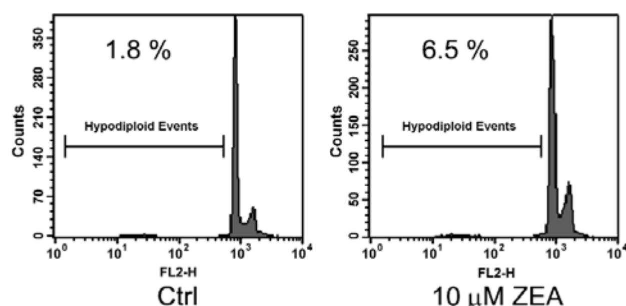

Figure 2. *Induction of apoptosis by zeaxanthin in human CHP100 neuroblastoma cells. Apoptosis was evaluated as hypodiploid events by cytofluorimetric analysis. Ctrl, control; ZEA, zeaxanthin.*

Furthermore, the effect of zeaxanthin on the activity of two model lipoxygenases, soybean LOX-1 (a 15-LOX) and barley 5-LOX, was assessed in liposomes (Figures 3 and 4). In fact, 15-LOX and 5-LOX are the most common isoforms involved in mammalian cell apoptosis (reviewed by 22,23). 15-LOX executes programmed organelle degradation *via* its involvement in the formation of "pore-like" structures in organellar membranes (39). In addition, 5-LOX is the main lipoxygenase in neuronal cells (reviewed by 40) and soybean LOX-1 (a 15-LOX) is frequently used as the prototype for studying the homologous family of lipoxygenases from different species (41). The effect of zeaxanthin on lipid peroxidation by LOX was assessed, firstly, by following the conversion of lipoxygenase substrate linoleic acid (LA) to the corresponding hydroperoxide (Figure 3). Zeaxanthin did not have a significant effect on the activity of either of these lipoxygenases (Figure 3). In addition, the oxidative index of liposomes in the presence of LOX-1 was determined from the absorption of LA hydroperoxides *versus* a reference wavelength at three different concentrations of LA. There were again no significant effects of zeaxanthin over a range of concentrations from 3 to 20 μM at either of the three LA concentrations (Figure 4).

Discussion

The strong induction of apoptosis in neuroblastoma cells by zeaxanthin demonstrated in the present study shows that zeaxanthin is capable of inducing apoptosis of these ‘unwanted’ cells – while preventing apoptosis of ‘needed’ cells such as photoreceptor cells of the eye (14,15). The pro-apoptotic effect of zeaxanthin is remarkable in light of the fact that neuroblastoma cells are rather resistant to apoptosis. Neuroblastoma cells are frequently characterized by decreased levels of inducers of apoptosis, such as caspase-8, and aberrations in other apoptotic regulators (31,42).

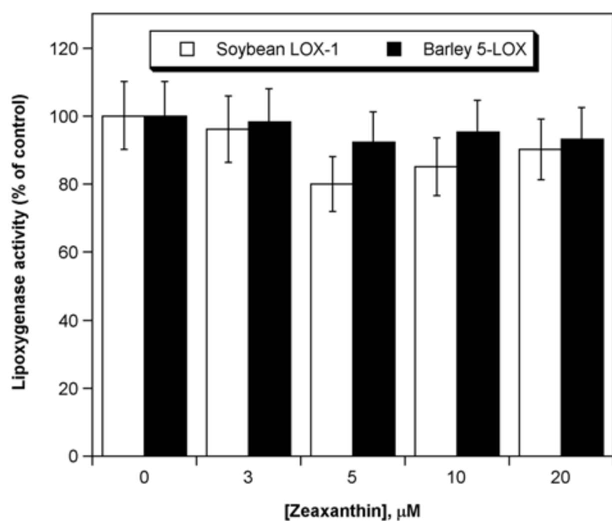

Figure 3. *Effect of zeaxanthin on lipoxygenase activity. Effect of zeaxanthin on purified soybean lipoxygenase-1 (LOX-1) or barley 5-lipoxygenase (5-LOX).*

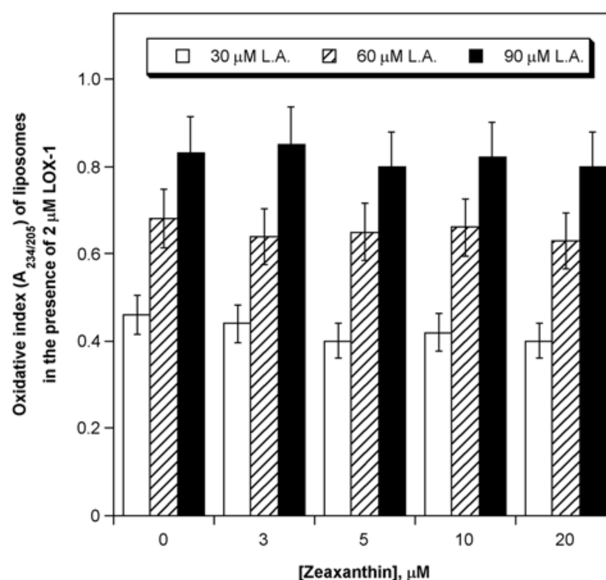

Figure 4. *Effect of zeaxanthin on membrane lipid peroxidation. Effect of zeaxanthin on LOX-1-mediated peroxidation of linoleic acid embedded in synthetic liposomes. Lipid peroxidation was assessed as oxidative index ($A_{234/205}$ ratio), and was tested at three different concentrations of linoleic acid.*

Zeaxanthin may possibly act *via* similar mechanisms as lutein. In the previous studies of effects of lutein on various cancer cell lines, lutein was found to increase the levels of the pro-apoptotic proteins p53 and/or bax, while decreasing the levels of the anti-apoptotic protein bcl-2 in both humans (16) and mice (18). The p53 tumor suppressor induces the expression of bax that, in turn, catalyses the release of cytochrome c from mitochondria which acts as a pro-apoptotic signal (43). The bcl-2 protein is the anti-apoptotic antagonist of bax in the mitochondrial membrane and inhibits the release of cytochrome c (43).

There is evidence that LOX and lipid peroxidation products can be components of the signal transduction pathway involved in modulating these apoptotic regulators (reviewed by 22,23). In certain human neuroblastoma cells (SK-N-BE type), an increase in p53, as well as two other, recently discovered proteins with p53-like properties (p63 and p73), could be induced by treatments with a major lipid peroxidation product, 4-hydroxynonenal (HNE) (44). HNE thus inhibited tumor cell proliferation (44). Furthermore, 5- and to a lesser extent 12- and 15-HpETE (hydroperoxy-eicosatetraenoic acid), that are products of 5-, 12- and 15-LOX, respectively, were able to induce apoptosis in CHP100 neuroblastoma cells (32), as well as in other cancer cells, including the SK-N-BE type (45).

Since zeaxanthin inhibits lipid peroxidation in various systems (20,21; see also 11,12,19), it was of interest to examine its effects on lipoxygenases. Several other phytochemicals, such as tocopherols (Vitamin E; 26), red wine resveratrol (26) and β-carotene (27-30) have been demonstrated to be able to inhibit the activity of lipoxygenase, apparently by being able to access the catalytic cavity of LOX and reducing its catalytic iron. The activity of LOX is dependent on the oxidized form of its catalytic iron, Fe(III), and LOX is inactivated by reduction of this iron to the inactive form Fe(II), as demonstrated both *in vitro* as well as for 5-LOX activity in human CHP100 neuroblastoma cells (46).

The present study, therefore, examined the effect of zeaxanthin on two major LOX forms, *i.e.* LOX purified from soybean (LOX-1; a 15-LOX) and barley (5-LOX). No significant effects of zeaxanthin on the two LOX forms tested after incorporation of their LA substrate into a liposome system were identified over a range of zeaxanthin concentrations. This indicates that zeaxanthin apparently does not operate *via* direct effects on these LOX types (5- and 15-LOX). This result is consistent with the present finding that zeaxanthin does not inhibit CHP100 neuroblastoma cell apoptosis, that can also be induced by a range of LOX products. In this neuroblastoma cell system, an inhibition of LOX would not be expected to induce apoptosis. In turn, there is also no evidence that zeaxanthin further stimulates LOX activity.

The absence of an effect of zeaxanthin on purified 5-LOX seems to rule out that this compound may induce apoptosis *via* a LOX-dependent pathway in CHP100 cells that express 5-LOX activity (32). Furthermore, the lack of an effect of

zeaxanthin on purified 15-LOX makes it unlikely that this phytochemical modulates LOX-dependent apoptotic pathways in mammalian cells. This finding indicates that the role of zeaxanthin is different from that of β-carotene (with its previously demonstrated complex effect on LOX activity). The results presented here do not exclude the possibility that zeaxanthin may modulate the levels of lipid peroxidation products *via* LOX-independent mechanisms *in vivo*.

In summary, further investigation into the precise mechanisms of the notable ability of the xanthophylls zeaxanthin and lutein to modulate apoptosis in contrasting patterns in unwanted *versus* healthy cells is warranted. However, while the signal transduction networks involved remain to be identified, it is clear that zeaxanthin can be added to the list of remarkable phytochemicals that have the ability to kill cancer cells while promoting the survival of healthy cells.

Acknowledgements

We are grateful to Dr. Volker Ebbert for performing the purity check of zeaxanthin *via* HLPC, and Dr. Marco Ranalli (University of Rome "Tor Vergata", Rome, Italy) for performing the cytofluorimetric analyses. We also thank Dr. William Adams for helpful comments on the manuscript.

References

1 Demmig-Adams B and Adams WW III: Antioxidants in photosynthesis and human nutrition. Science *298*: 2149-2153, 2002.

2 Lapillonne A, Clarke SD and Heird WC: Polyunsaturated fatty acids and gene expression. Curr Opin Clin Nutr Metabol Care *7*: 151-156, 2004.

3 Surh Y-J: Cancer chemoprevention with dietary phytochemicals. Nature Rev Cancer *3*: 768-780, 2003.

4 Lavrovsky Y, Chatterjee B, Clark RA and Roy AK: Role of redox-regulated transcription factors in inflammation, aging and age-related diseases. Exp Gerontol *35*: 521-532, 2000.

5 Youdim KA, Spencer JPE, Schroeter H and Rice-Evans C: Dietary flavonoids as potential neuroprotectants. Biol Chem *383*: 503-519, 2002.

6 Seo T, Blaner WS and Deckelbaum RJ: Omega-3 fatty acids: molecular approaches to optimal biological outcomes. Curr Opin Lipidol *16*: 11-18, 2005.

7 Demmig-Adams B and Adams WW III: The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends Pl Sci *1*: 21-26, 1996.

8 Külheim C, Agren J and Jansson S: Rapid regulation of light harvesting and plant fitness in the field. Science *297*: 91-93, 2002.

9 Holt NE, Zigmantas D, Valkunas L, Li X-P, Niyogi KK and Fleming GR: Carotenoid cation formation and the regulation of photosynthetic light harvesting. Science *307*: 433-436, 2005.

10 Pogson BJ, Niyogi KK, Björkman O and DellaPenna D: Altered xanthophyll compositions adversely affect chlorophyll accumulation and nonphotochemical quenching in *Arabidopsis* mutants. Proc Natl Acad Sci USA *95*: 133324-13329, 1998.

11 Havaux M and Niyogi KK: The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. Proc Natl Acad Sci USA *96*: 8762-8767, 1999.

12 Havaux M, Dall'Osto L, Cuine S, Guiliano G and Bassi R: The effect of zeaxanthin as the only xanthophyll on the structure and function of the photosynthetic apparatus in *Arabidopsis thaliana.* J Biol Chem *279*: 13878-13888, 2004.

13 Mares-Perlman JA, Millen AE, Ficek TL and Hankinson SE: The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease. Overview. J Nutr *132*: 518S-524S, 2002.

14 Thomson LR, Toyoda Y, Langner A, Delori FC, Garnett KM, Craft NE, Nichols CR, Cheng KM and Dorey CK: Elevated retinal zeaxanthin and prevention of light-induced photoreceptor cell death in quail. Invest Ophthalmol Vis Sci *43*: 3538-3549, 2002.

15 Thomson LR, Toyoda Y, Delori FC, Garnett KM, Wong ZY, Nichols CR, Cheng KM, Craft NE, and Dorey CK: Long term dietary supplementation with zeaxanthin reduces photoreceptor death in light-damaged Japanese quail. Exp Eye Res *75*: 529-542, 2002.

16 Sumatran VN, Zhang R, Lee DS and Wicha MS: Differential regulation of apoptosis in normal *versus* transformed mammary epithelium by lutein and retinoid acid. Cancer Epidemiol. Biomark Prev *9*: 257-263, 2000.

17 Müller K, Carpenter KLH, Challis IR, Skepper JN and Arends MJ: Carotenoids induce apoptosis in the T-lymphoblast cell line Jurkat E6.1. Free Rad Res *36*: 791-802, 2002.

18 Chew BP, Brown CM, Park JS and Mixter PF: Dietary lutein inhibits mouse mammary tumor growth by regulating angiogenesis and apoptosis. Anticancer Res *23*: 3333-3339, 2003.

19 Chitchumroonchokchai C, Bomser JA, Glamm JE and Failla ML: Xanthophylls and α-tocopherol decrease UVB-induced lipid peroxidation and stress signalling in human lens epithelial cells. J Nutrition *134*: 3225-3232, 2004.

20 Wrona M, Korytowksi W, Ròzanowska M, Sarna T and Truscott TG: Cooperation of antioxidants in protection against photosensitized oxidation. Free Rad Biol Med *35*: 1319-1329, 2003.

21 Wrona M, Ròzanowska M and Sarna T: Zeaxanthin in combination with ascorbic acid or α-tocopherol protects APRE-19 cells against photosensitized peroxidation of lipids. Free Rad Biol Med *36*: 1094-1101, 2004.

22 Maccarrone M, Melino G and Finazzi-Agrò A: Lipoxygenases and their involvement in programmed cell death. Cell Death Differ 8: 776-784, 2001.

23 Tang DG, La E, Kern J and Kehrer JP: Fatty acid oxidation and signaling in apoptosis. Biol Chem *383*: 425-442, 2002.

24 Lebeau A, Terro F, Rostene W and Pelaprat D: Blockade of 12-lipoxygenase expression protects cortical neurons from apoptosis induced by beta-amyloid peptide. Cell Death Diff *11*: 875-884, 2004.

25 Grichenko OE, Shaposhnikova VV, Kudryavtsev AA and Korystov YN: Apoptosis in p388 leukemia cells induced by specific inhibitors of 5- and 12-lipoxygnease and the product of cyclooxygenase, prostaglandin E-2. Biol Bulletin *31*: 221-225, 2004.

26 Maccarrone M, Lorenzon T, Guerrieri P and Finazzi-Agrò A: Resveratrol prevents apoptosis in K562 cells by inhibiting lipoxygenase and cyclooxygenase activity. Eur J Biochem *265*: 27-34, 1999.

27 Lomnitski L, Bar-Natan R, Sklan D and Grossman S: The interaction between β-carotene and lipoxygenase in plant and animal systems. Biochim Biophys Acta *1167*: 331-338, 1993.

28 Lomnitski L, Grossman S, Bergman M, Sofer Y and Sklan D: *In vitro* and *in vivo* effects of β-carotene on rat epidermal lipoxygenases. Int J Vit Res *67*: 407-414, 1997.

29 Trono D, Pastore D and Di Fonzo N: Carotenoid dependent inhibition of durum wheat lipoxygenase. J Cereal Sci *29*: 99-102, 1999.

30 Pastore D, Trono D, Padolino L, Simone S, Valenti D, Di Fonzo N and Passarella S: Inhibition by α-tocopherol and L-ascorbate of linoleate hydroperoxidation and β-carotene bleaching activity in durum wheat semolina. J Cereal Sci *31*: 41-54, 2000.

31 Borriello A, Roberto R, Della Ragione F and Iolascon A: Proliferate and survive: cell division cycle and apoptosis in human neuroblastoma. Haematologica *87*: 196-214, 2002.

32 Maccarrone M, Salucci ML, Melino G, Rosato N and Finazzi-Agrò A: The early phase of apoptosis in human neuroblastoma CHP100 cells is characterized by lipoxygenase-dependent ultraweak light emission. Biochem Biophys Res Commun *265*: 758-762, 1999.

33 Maccarrone M, Lorenzon T, Bari M, Melino G and Finazzi-Agrò A: Anandamide induces apoptosis in human cells *via* vanilloid receptors. Evidence for a protective role of cannabinoid receptors. J Biol Chem *275*: 31938-31945, 2000.

34 Gilmore M and Yamamoto HY Resolution of lutein and zeaxanthin using a non-endcapped, lightly carbon-loaded C-18 high-performance liquid-chromatography column. J Chromatography *543*: 137-145, 1991.

35 Maccarrone M, Salucci ML, van Zadelhoff G, Malatesta F, Veldink G, Vliegenthart JF and Finazzi-Agrò A: Tryptic digestion of soybean lipoxygenase-1 generates a 60 kDa fragment with improved activity and membrane binding ability. Biochemistry *40*: 6819-6827, 2001.

36 Van Aarle PG, de Barse MM, Veldink GA and Vliegenthart JFG: Purification of a lipoxygenase from ungerminated barley. Characterization and product formation. FEBS Lett *280*: 159-162, 1999.

37 Kühn H, Belkner J, Wiesner R and Brash AR: Oxygenation of biological membranes by the pure reticulocyte lipoxygenase. J Biol Chem *265*: 18351-18361, 1990.

38 Maccarrone M, Navarra M, Catani V, Corasaniti MT, Bagetta G and Finazzi-Agrò A: Cholesterol-dependent modulation of the toxicity of HIV-1 coat protein gp120 in human neuroblastoma cells. J Neurochem *82*: 1444-1452, 2002.

39 Van Leyen K, Duvoisin RM, Engelhardt H and Wiedmann MA: function for lipoxygenase in programmed organelle degradation. Nature *395*: 392-395, 1998.

40 Manev H, Uz T, Sugaya K and Qu T: Putative role of neuronal 5-lipoxygenase in an aging brain. FASEB J *14*: 1464-1469, 2000.

41 Brash AR: Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. J Biol Chem *274*: 23679-23682, 1999.

42 Kim S, Kang JH, Evers BM and Chung DH: Interferon-gamma induces caspase-8 in neuroblastoma without affecting methylation of caspase-8 promoter. J Pedriatic Surg *39*: 509-515, 2004.

43 Jiang XJ and Wang XD: Cytochrome C-mediated apoptosis. Ann Rev Biochem *73*: 87-106, 2004.

44 Laurora S, Tamagno E, Briatore F, Bardini P, Pizzimenti S, Toaldo C, Reffo P, Costelli P, Dianzani MU, Danni O and Barrera G: 4-Hydroxynonenal modulation of p53 family gene expression in the SK-N-BE neuroblastoma cell line. Free Rad Biol Med *38*: 215-225, 2005.

45 Maccarrone M, Catani MV, Finazzi-Agrò A and Melino G: Involvement of 5-lipoxygenase in programmed cell death of cancer cells. Cell Death Differ *4*: 396-402, 1997.

46 Maccarrone M, Corasantini MT, Guerrieri P, Nistico G and Finazzi-Agrò A: Nitric oxide-donor compounds inhibit lipoxygenase. Biochem Biophys Res Commun *219*: 128-133, 1996.

Received August 26, 2005
Accepted September 1, 2005