Prooxidant effects of β-carotene in cultured cells

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Abstract

There is a growing body of interest on the role of β-carotene and other carotenoids in human chronic diseases, including cancer. While epidemiological evidence shows that people who ingest more dietary carotenoids exhibit a reduced risk for cancer, results from intervention trials indicate that supplemental β-carotene enhances lung cancer incidence and mortality among smokers. A possible mechanism which can explain the dual role of β-carotene as both a beneficial and a harmful agent in cancer as well as in other chronic diseases is its ability in modulating intracellular redox status. β-Carotene may serve as an antioxidant or as a prooxidant, depending on its intrinsic properties as well as on the redox potential of the biological environment in which it acts. This review summarizes the available evidence for a prooxidant activity of β-carotene in cultured cells, focusing on biochemical and molecular markers of oxidative stress, which have been reported to be enhanced by the carotenoid.

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1. Introduction

Epidemiological studies indicate that subjects consuming diets rich in carotenoid-containing food, including fruit and vegetables, show a low incidence of chronic diseases, including various cancers and cardiovascular diseases (Mayne, 1996; Ziegler et al., 1996). β-Carotene, one of the large number of naturally occurring carotenoids, thus appears to actively participate in health. However, recent intervention trials indicate that β-carotene supplements are not efficacious in the prevention of cardiovascular disease and major cancers occurring in well-nourished populations (Omenn et al., 1996; The Alpha-tocopherol, Beta-carotene Cancer Prevention Study Group, 1994). In fact, supplemental β-carotene appears to increase, rather than to
reduce, lung cancer incidence and death from cardiovascular diseases in current smokers and in asbestos workers. Possible explanations for this paradox have been hypothesized. It is known that β-carotene is converted to retinoids (vitamin A and its analogs) in humans and the hormone-like effects of these compounds may exert potent influences on cell differentiation, proliferation and development. β-Carotene also acts as an immunomodulatory agent (Bendich and Shapiro, 1986), increasing immune surveillance in carcinogenesis. Moreover, it may enhance gap junction communication (Zhang et al., 1992), restricting clonal expansion of initiated cells. It may also interfere with metabolic pathways involved in the detoxification of chemical carcinogens (Paolini et al., 1999).

However, a possible mechanism which can explain the dual role β-carotene as both a beneficial and a harmful agent in cancer as well as in other chronic diseases is its ability in modulating intracellular redox status. β-Carotene may serve as an antioxidant (Palozza and Krinsky, 1992a; Krinsky, 1993; Sies and Stahl, 1995), inhibiting free radical production, or as a prooxidant (Palozza, 1998; Young and Lowe, 2001), propagating free radical-induced reactions, depending on its intrinsic properties as well as on the redox potential of the biological environment in which it acts. In fact, carotenoid molecule does not possess the structural features commonly associated with chain-breaking antioxidants. The extensive system of conjugated double bonds in its molecule imparts a prooxidant character and makes it very susceptible to attack by free radical species (Burton and Ingold, 1984). This review summarizes the available evidence for a prooxidant activity of β-carotene in cultured cells, focusing on biochemical and molecular markers of oxidative stress enhanced by the carotenoid.

2. Prooxidant role of β-carotene in cells: analysis of oxidative markers

β-Carotene has been shown to possess prooxidant properties in cell models, at least under certain circumstances (high oxygen tension, high carotenoid concentration, unbalanced intracellular redox status) (Palozza, 1998). Its prooxidant effects into the cells may be evidenced as a rough oxidative damage to the cell structures (DNA, lipids and proteins) or, otherwise, as a subtle modulation of redox-sensitive genes and transcription factors.

2.1. Increase in reactive oxygen species production

Although several findings suggested that β-carotene is able to inhibit the formation of free radicals (Palozza and Krinsky, 1992a), a recent evidence shows that the carotenoid, at relatively high concentrations, can also increase it in different tumor cell lines (Palozza et al., 2001, 2002a, 2003). In these studies, reactive oxygen species (ROS) production was measured by different fluorescent probes, such as dichlorofluorescein diacetate (DCF-DA) and dihydrorhodamine (DHR), which detect ROS production in whole cytoplasm or mitochondria, respectively. It is noteworthy that cell type deeply modifies the ability of β-carotene to act as a prooxidant. The caro-
tenoid exhibits prooxidant effects at 2.5 μM in LS-174 cells and at 50 μM in WiDr adenocarcinoma cells (Palozza et al., 2001, 2003), presumably because of the different cell capability in incorporating the carotenoid (Palozza et al., 2002b). In addition, the degree of cell differentiation seems to deeply alter β-carotene ability in enhancing ROS production. β-Carotene is a much more potent inducer of ROS in undifferentiated than in differentiated human leukemic (HL-60) cells (Palozza et al., 2002a). This effect is presumably due to the fact that during differentiation, HL-60 cells acquired more resistance to oxidative stress, since they increased the content and/or the activity of antioxidants and modified the cell distribution of antioxidants (Shen et al., 1994). Several mechanisms have been proposed to explain how β-carotene induces an overproduction of ROS in the cells. They include: processes of autooxidation of carotenoid metabolites (Murata and Kawanishi, 2000), alterations in iron levels (Garcia-Casal et al., 1998), in the activity of cytochrome P450 enzymes (Paolini et al., 2001), and changes in cell antioxidant defences, as described below.

2.2. Impairment of cell antioxidant status

Numerous findings suggest that β-carotene treatment may influence tocopherol status in cell membranes (Palozza, 1998). Carotenoids have been reported to increase the loss of α-tocopherol induced by different sources of free radicals in isolated membranes (Palozza and Krinsky, 1992b) as well as in intact cells (Palozza et al., 1997). It has been shown that the presence of β-carotene induces an endogenous consumption of α-tocopherol in both normal and tumor thymocytes isolated from BALB/c mice and exposed to the action of xanthine/xanthine oxidase. The increased consumption of α-tocopherol observed in these studies can be the result of two different processes. α-Tocopherol may protect β-carotene from its oxidation and/or from the formation of β-carotene-derived peroxy radicals.

Recently, it has been shown that β-carotene is able to decrease the levels of reduced glutathione (GSH) and to increase that of oxidized glutathione (GSSG) in cultured HL-60 cells at concentrations ranging from 10 to 20 μM (Palozza et al., 2002a).

In cultured human oral carcinoma cells, β-carotene decreases the activity of both superoxide dismutase (SOD) and glutathione-transferase (Schwartz et al., 1993). Such a reduction does not occur if the cells are incubated with a combination of β-carotene and α-tocopherol. In chicken embryo fibroblasts, β-carotene modulates the activity of SOD, catalase and GSH-Px in a concentration-dependent manner (Lawlor and O’Brien, 1995). At a high concentration (10 μM), the carotenoid increases SOD and catalase activities and decreases GSH-Px activity, whereas at a low concentration (0.1 μM), it induces opposite effects. Moreover, β-carotene prevents paraquat-induced elevation of catalase and SOD activities and the reduction of GSH-Px at low, but not at high concentrations (Lawlor and O’Brien, 1997). The modulation of the antioxidant enzymes by β-carotene also varies under different $p_{O_2}$ (Lawlor and O’Brien, 1995).
2.3. Increase in lipid oxidation

Several studies show that β-carotene may enhance lipid peroxidation products, at least under certain circumstances (high $p_{O_2}$, high carotenoid concentration, cell chronic oxidative status) (Palozza, 1998). Increases in spontaneous and free radical-induced malondialdehyde production by β-carotene have been reported in both isolated membranes (Palozza et al., 1995) and intact cells (Palozza et al., 1997). Prooxidant effects are observed using β-carotene but also other carotenoids, such as lycopene. In particular, in human foreskin fibroblasts (Hs68 cells), it has been reported that lycopene may increase TBAR production induced by the lipid-soluble radical generator 2,2′-azobis(2,4-dimethylvaleronitrile) (Yeh and Hu, 2000). This effect is dose-dependent and is not observed when other generators of free radicals, such as the system ferric nitrilotriacetate and the water-soluble 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH), are used. β-Carotene behaves similarly under the same in vitro oxidative conditions, suggesting that the kind of the oxidant used may be also an important determinant in the prooxidant activity of carotenoids.

2.4. Increase in DNA oxidation

Although some studies have shown that carotenoids are effective in protecting cells from oxidative DNA damage, some others fail to show it (Collins, 2001). In particular, it has been reported that β-carotene is able to enhance the susceptibility of HepG2 cells to the DNA damaging effects of H$_2$O$_2$, measured as formation of DNA strand breaks (Woods et al., 1999). Moreover, in this study, pre-treatment with the carotenoid enhances the H$_2$O$_2$-induced cytotoxicity. The failure of β-carotene and lycopene to protect human cells against the DNA damaging effects of H$_2$O$_2$ has been recently demonstrated for HT29 cells (Lowe et al., 1999). In addition, consumption of vegetables do not enhance resistance to oxidative damage induced by H$_2$O$_2$ in human lymphocytes (Pool-Zobel et al., 1997). It has also been shown that whereas β-carotene treatment decreases the number of sister chromatide exchanges induced by H$_2$O$_2$ in Chinese hamster ovary cells, it significantly increases the number of H$_2$O$_2$-induced chromosome aberrations (Cozzi et al., 1997). Moreover, it has been also reported an enhancement of the clastogenic effects of bleomycin by β-carotene in CHO cells (Salvadori et al., 1994). Finally, β-carotene causes a concentration-dependent DNA breakdown, although this effect is evidenced only at high $p_{O_2}$ (Zhang and Omaye, 2001). In this case, α-tocopherol and/or ascorbic acid only exert a limited protection towards the prooxidant effects of β-carotene on DNA. Recently, it has been shown an increased DNA breakage and increased levels of 8-OH-dG in both purified calf thymus DNA and DNA isolated from human Hs68 fibroblasts by oxidized β-carotene and lycopene (Yeh and Hu, 2001).

2.5. Increase in protein oxidation

Although at the moment no data are available in cell models, it has been recently demonstrated that β-carotene is able to enhance AAPH-induced oxidation of human molecules.
serum albumin, measured as carbonyl formation. Such an effect is modulated by the oxygen tensions as well as by the concomitant presence of \( \alpha \)-tocopherol and/or ascorbic acid (Zhang and Omaye, 2000). Interestingly, high concentrations of \( \beta \)-carotene produce more protein oxidation in the presence of high \( p_{O_2} \). A mixture of \( \beta \)-carotene, \( \alpha \)-tocopherol and ascorbic acid provides better protective effects on protein oxidation than the compounds given alone. In addition, Andersen and co-workers, using \( \text{Fe}^{2+} \)-mediated oxidation of heme proteins as an early indicator of oxidative stress, find that \( \beta \)-carotene exhibits limited protection or a prooxidant effect with respect to a pronounced vitamin E antioxidant activity (Andersen and Andersen, 1993).

2.6. Modulation of redox-sensitive genes

Recent evidence demonstrates that a prooxidant mechanism and a modulation of redox-sensitive genes by \( \beta \)-carotene can be involved in its inhibition of tumor cell growth. Concerning this, we recently observed a link between changes in intracellular redox potential and cell growth by \( \beta \)-carotene in different tumor cells (Palozza et al., 2001). The increase in ROS production and/or the levels of oxidized glutathione induced by the carotenoid in human colon adenocarcinoma (Palozza et al., 2001) and leukemia (Palozza et al., 2002a) cells are highly coincident with its ability to induce apoptosis and to arrest cell cycle progression. In these cells, the carotenoid is also able to decrease the expression of Bcl-2 (Palozza et al., 2002a,b), a protein whose antiapoptotic effects has been, at least partially, explained by its antioxidant properties (Kane et al., 1993). In addition, in HL-60 cells, the carotenoid also induces an increased expression of p21WAF-1, which is implicated in the arrest of cell cycle progression at G1 phase (Palozza et al., 2002a). A recent finding suggests that this protein is regulated by oxidative stress through a mechanism independent on p53 activation (Esposito et al., 2000). As a support to the hypothesis of the involvement of a prooxidant mechanism in the growth-inhibitory effects of \( \beta \)-carotene, we also found that \( \alpha \)-tocopherol minimizes the effect of the carotenoid on cell growth, apoptosis and Bcl-2 expression in a dose-dependent manner (Palozza et al., 2002a). In another study, it has been demonstrated that the growth-inhibitory effects of the carotenoid in SCC-25 tumor cells are decreased by an oxygen-poor environment, in which the prooxidant character of the molecule is minimized (Schwartz, 1993). \( \beta \)-Carotene is also able to induce an oxidative stress in tumor oral cells, which results in an increased expression of stress proteins involved in apoptosis, such as the heat-shock protein (hsp)70 and/or hsp90 (Schwartz et al., 1990). Both 9-\textit{cis} and all-\textit{trans} \( \beta \)-carotene are able to induce an intracellular accumulation of hsp70 in cervical dysplasia-derived cells and the treated cells showed morphological changes indicative of apoptosis (Toba et al., 1997). The carotenoid has been also suggested to reduce the expression of mutant p53, which has been associated with the exposure to cigarette smoke (Schwartz, 1993) and to stimulate the expression of tumor necrosis factor (Abdel-Fatth et al., 1993).

Another mechanism underlying the prooxidant effect of \( \beta \)-carotene may be its ability to stimulate the overproduction of free radical species through the induction
of carcinogen-metabolizing enzymes (Paolini et al., 1999, 2001; Perocco et al., 1999). The induction of transformation by benz(a)pyrene and cigarette-smoke condensate in BALB/c 3T3 cells is markedly enhanced by the presence of β-carotene in either acute or chronic treatment. Such an enhancement has been related to the boosting effect of the carotenoid on P450 apparatus (Perocco et al., 1999). Moreover, carotenoids have been also suggested to modulate tumor growth acting as potent inducers of phase II detoxifying enzymes, such as glutathione S-transferase (GST) and quinone reductase (Sharoni et al., 2002), as well as of cellular defensive enzymes such as heme-oxygenase-1 (Obermuller-Jevic et al., 1999). In human skin fibroblasts enriched with the carotenoid and exposed to UV-light (Obermuller-Jevic et al., 1999), the increase in heme-oxygenase-1 expression by β-carotene is entirely suppressed by vitamin E, but only moderately by vitamin C.

2.7. Modulation of redox-sensitive transcription factors

The NF-κB pathway is generally thought to be a primary oxidative stress response pathway involved in cell proliferation and apoptosis (Schulze-Osthoff et al., 1995; Sen and Packer, 1996; Mercurio and Manning, 1999; Bowie and O’Neill, 2000). We have recently reported that β-carotene, administrated at concentrations found to induce growth-inhibitory and prooxidant effects, increases the DNA-binding activity of nuclear proteins at NF-κB site in leukemic as well as in colon adenocarcinoma cells (Palozza et al., 2003). In these cells, the ability of treatments with α-tocopherol or NAC to diminish both β-carotene-induced NF-κB DNA-binding activity and ROS production further supports the hypothesis that the carotenoid regulates this transcription factor through a prooxidant mechanism. It has been recently reported that NF-κB is activated by certain apoptotic stimuli and that some of the NF-κB target genes, such as c-myc, are implicated in apoptosis induction (La Rosa et al., 1994). According with this, we have recently demonstrated that β-carotene is able to increase the expression of c-myc and that such an increase is directly related to apoptosis induction (Palozza et al., 2003). Interestingly, it has been suggested that β-carotene is also able to modulate the activation of AP-1, which is known to be another redox-sensitive transcription factor involved in the regulation of cell growth (Wang et al., 1999; Lotan, 1999; Tibaduiza et al., 2002).

3. Interactions of β-carotene with antioxidants: a further evidence for a prooxidant activity of β-carotene

The cooperative interactions of β-carotene with antioxidants seem to be an important factor in determining the antioxidant–prooxidant role of the carotenoid in cell models. The presence of antioxidants may limit the prooxidant character of the carotenoid. In isolated cell membranes, α-tocopherol inhibits the increase in lipid peroxidation induced by high β-carotene concentrations (Palozza et al., 1995). In addition, in different tumor cells, the prooxidant effects of β-carotene are completely reverted by the concomitant addition of α-tocopherol (Palozza et al., 2001, 2002a).
In particular, the tocopherol is able to minimize the increase in ROS production induced by high concentrations of \( \beta \)-carotene in both human colon adenocarcinoma (Palozza et al., 2001) and leukemia cells (Palozza et al., 2002a). Moreover, the tocopherol is also able to arrest the loss of reduced glutathione and the increase of oxidized glutathione induced by the carotenoid in HL-60 cells (Palozza et al., 2002a). It also reverts the effect of the carotenoid on the activation of NF-kB in several cell models (Palozza et al., 2003). It is interesting to note that the prevention of the prooxidant effects of the carotenoid by \( \alpha \)-tocopherol or other antioxidants, such as \( N \)-acetyl cysteine, is always accompanied by a loss of the effects of \( \beta \)-carotene on cell growth and apoptosis induction.

Several data in the literature demonstrate that the concomitant presence of \( \beta \)-carotene and antioxidants may also induce synergistic effects in cell oxidative processes. This hypothesis is clearly supported by the finding that combined additions of \( \beta \)-carotene and antioxidants result in an enhanced protection of the cells toward oxidative stress (Leibovitz et al., 1990; Chen and Tappel, 1995). In particular, in cell membranes, a combination of \( \beta \)-carotene and \( \alpha \)-tocopherol results in the inhibition of free radical-induced lipid peroxidation significantly greater than the sum of the individual inhibitions (Palozza and Krinsky, 1992a,b). This synergistic interaction is also accompanied by an increased loss of \( \alpha \)-tocopherol. This finding suggests that \( \alpha \)-tocopherol might be consumed to retard the formation of carotenoid-radical adducts and/or their further degradation to autooxidation products. It is also possible that \( \alpha \)-tocopherol is consumed as a consequence of an increased lipid peroxidation due to \( \beta \)-carotene, without any direct interaction with this molecule. Although no much is known on the mechanisms of these interactions, it has been suggested that tocopherols may reduce carotenoid-radical cations formed during oxidative processes (Edge and Truscott, 1997; Mortensen et al., 1998). In agreement with these findings, it has been recently reported that a synthetic antioxidant, which combines into a single molecule the chroman head of tocopherols and a fragment of lycopene, consisting of a polyisoprenyl sequence of four conjugated double bonds, provides a higher antioxidant efficiency than \( \alpha \)-tocopherol and lycopene, alone or in combination (Palozza et al., 2002c), also suggesting the possibility of oxidative intramolecular cooperations between carotenoids and tocopherols.

4. Conclusions

Although the direct involvement of a prooxidant mechanism in tumor promotion by \( \beta \)-carotene has not been demonstrated, several points may be highlighted: (1) mutagenic or procarcinogenic effects of the carotenoid are evidenced mainly at doses which usually exceed the dietary intake and which, therefore, may enhance its prooxidant character; (2) procarcinogenic effects of \( \beta \)-carotene are evidenced in tissues, such as lung, in which the oxygen tension is so high to promote effective prooxidant effects of carotenoids; (3) a lack of adequate antioxidant defence and/or a chronic oxidative stress, as those observed in chronic smokers, result in increased procarcinogenic effects by the carotenoid; (4) the administration of carotenoids with
fruits and vegetables instead of carotenoid supplements is rarely associated with procarcinogenic effects. This can be related to the fact that such a food contains combinations of antioxidant nutrients, which can limit the prooxidant effects of carotenoids.

In view of these considerations, further studies should be continued to get proper information regarding the role of β-carotene and other carotenoids as prooxidants and their involvement in cancer process.

References


