Carotenoids and lung cancer: biochemical aspects

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Abstract: Carotenoids are part of the human diet and a regular low-dose intake of these compounds from natural sources is normally preferred. Carotenoid supplementation in various diseases, including cancer, was described to be useful, but evidence has been obtained that high-dose supplementation of β-carotene may be unsafe, especially to smokers and asbestos-exposed workers, because of a stastically detected increased cancer risk. The negative effect might be mediated by carotenoid breakdown products having a high reactivity towards biomolecules. It has been suggested that these compounds originate from nonenzymatic cleavage of carotenoids by oxidants liberated in large amounts by neutrophils that accumulate in various inflammatory diseases and, in particular, in pulmonary disorders characterized by profound abnormalities in inflammatory pathways, such as those triggered by tobacco smoking. Carotenoid breakdown products, in turn, may affect neutrophil response in different ways that depend on the concentration that is reached by these products in the medium. In vitro studies show that nanomolar and micromolar concentrations of carotenoid derivatives stimulate superoxide production by neutrophils activated by phorbol myristate acetate (PMA), while a slight inhibition is noticed with cells activated by the chemotactic tripeptide N-formyl-Met-Leu-Phe (f-MLP). At higher concentrations, carotenoid breakdown products inhibit superoxide production in the presence of both PMA and f-MLP.

Keywords: Carotenoids • Neutrophils • Prooxidant effect • Lung cancer

1. Introduction

Low intake of vegetables and fruits was consistently associated for a long time with increased risk of cancer including lung cancer in both prospective and retrospective analyses [1-3]. The interest was focused on the potential protective role of β-carotene and evidence from observational and epidemiologic studies rapidly accumulated, which supported an inverse relationship between cancer incidence and β-carotene intake and serum concentration. The question was studied, in particular, for lung cancer and large-scale randomized trials were performed to test the hypothesis that β-carotene supplements protected against this disease. Unfortunately, the trials had disappointing results [4-7]. Indeed, the decreases in risk of lung cancer were generally small and not statistically significant, and could be simply attributed to a healthier lifestyle. Moreover, high-dose β-carotene supplementation was found to increase, not to decrease as anticipated, the risk of lung cancer in high-risk populations, such as smokers and asbestos-exposed workers.

Cigarette smoking is the principal cause of lung cancer, and it tends to be closely associated with less healthy diets, including lower intake of fruit and vegetables [8], as well as with depletion of circulating provitamin A carotenoids [9]. Residual confounding by smoking is a viable explanation for the inverse relationship between carotenoid intake and cancer risk in the observational studies. Indeed, lowered plasma β-carotene concentrations may result in part by enhanced metabolic turnover resulting from smoking-induced oxidative stress [10,11]. Moreover, cigarette smoking history may not be measured in enough detail. Anyhow, the mechanisms by which β-carotene promotes the development of lung cancer in high-risk populations still remains unclear. It was reported [4,5] that β-carotene or a combination of β-carotene and α-tocopherol increased the risk of cancer.

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Carotenoids and lung cancer: biochemical aspects

18-48 months after starting the supplementation. This temporal effect suggests that β-carotene in some way accelerates the progression of more advanced latent lung cancers, and argues against a promotional effect of β-carotene on earlier phases of lung carcinogenesis. Experiments performed in model systems [12] are in line with this hypothesis and suggest that β-carotene does not induce genotoxic effects per se.

It has been suggested that components of cigarette smoke in the presence of relatively high oxygen tension in the lung combine to induce oxidation of β-carotene, resulting in a prooxidant effect [13]. Indeed, at higher oxygen pressure (about 150 mm Hg as present in normal air), carotenoids lose their antioxidant activity and show an autocatalytic, prooxidant effect, while a switch from prooxidant to antioxidant behaviour might be achieved at relatively low oxygen partial tension as well by adding vitamin E and C [14]. Carotenoids may behave as oxygen “carriers” from peroxyl radicals to cellular targets [15], but a study involving human bronchial epithelial cells did not find a direct prooxidant effect of the smoke-induced β-carotene oxidation products [16], suggesting more complex mechanisms for the increased risk of lung cancer.

2. Carotenoids and Neutrophils

There exists a variety of conditions to generate potentially-toxic β-carotene degradation products (Fig. 1). One of these possibilities – under inflammatory conditions probably a major contributor – is the metabolism of stimulated neutrophils. It has already been suggested by our group [15] that stimulated neutrophils are able to degrade β-carotene and generate a number of carotenoid breakdown products, including highly reactive aldehydes and epoxides (Fig. 2). These compounds exert prooxidative actions (Fig. 3), especially in the nuclei and mitochondria [17], that may be responsible, at least in part, for the harmful effects of high-dose supplementation of β-carotene in patients suffering from extraordinary oxidative stress. Neutrophil activation relies on the generation of toxic oxygen products from molecular oxygen through a series of electron transfers (Fig. 4). Oxidant production begins with a cytoplasmic membrane-associated NADPH oxidase, which reduces molecular oxygen to superoxide (O2−). Most O2− dismutates to form hydrogen peroxide and O2. The hydrogen peroxide is converted to hypochlorous acid (HOCl) through the reaction catalysed by the heme protein myeloperoxidase that constitutes about 5% of the overall protein content in activated neutrophils [18]. As revealed in vitro, the reaction of HOCl with β-carotene is slow as compared to other reducing agents [19]. Nevertheless, interaction of HOCl with more lipophilic constituents, such as carotenoids, seems to be realistic under conditions where neutrophils accumulate extensively [21], since myeloperoxidase is rapidly released in the surrounding medium from stimulated neutrophils [20], catalysing the formation of relatively large amounts of HOCl. Smokers compared to non-smokers show a highly increased number of inflammatory cells in lung tissues. The number of neutrophils found in a biopsy specimens of smokers was about 12 cell mm−2 (i.e., about 4×10^4 cell mL−1), while non-smokers had...
only about 2 cell mm\(^{-2}\) [22]. Calculations reveal that 2-4×10\(^4\) leukocyte mL\(^{-1}\) produce 1-1.4 nmol mL\(^{-1}\) HOCl during 1 h [23,24] that could degrade a significant amount of β-carotene, which is present in the plasma at a concentration of about 3 nmol mL\(^{-1}\) in subjects consuming 24 mg β-carotene per day [25].

The release of cytotoxic β-carotene breakdown products could exacerbate the inflammatory response in lung tissues, contributing to the development of cancer [26]. Indeed, pulmonary diseases that are associated with the greatest risk for lung cancer are characterized by abundant and deregulated inflammation [27-29]. Pulmonary disorders such as chronic obstructive pulmonary disease, which can be induced by tobacco smoking, are characterized by profound abnormalities in inflammatory pathways [30]. For example, among the cytokines, growth factors, and mediators released in these lung diseases and in the developing tumor microenvironment, interleukin (IL)-1β, prostaglandin (PG) E2, and transforming growth factor (TGF)-β have been found to have deleterious properties that simultaneously pave the way for both epithelial–mesenchymal transition and destruction of specific host cell–mediated immune responses against tumor antigens [31-33].

Carotenoid breakdown products, in turn, may affect neutrophil response in different ways that depend on the concentration that is reached by these products in the medium (Fig. 5). Experiments with purified suspensions of human neutrophils [34] show that stimulation of superoxide production can be induced by nanomolar and micromolar concentrations of carotenoid derivatives with aliphatic chains of different length (retinal, β-ionone, mixture of carotenoid cleavage products), but not by carotenoids lacking of the carbonyl moiety. It is noteworthy that this stimulatory effect of carotenoids can be observed only with cells activated by phorbol myristate acetate (PMA), while a slight inhibition of superoxide production is noticed if the chemotactic tripeptide \(\text{N-}^\text{formyl-Met-Leu-Phe}\) (f-MLP) is employed to trigger a cell response. At slightly higher concentrations, carotenoids inhibit superoxide production in the presence of both PMA and f-MLP. Under these conditions, carotenoid oxidation products and derivatives lacking the carbonyl moiety exert similar inhibitory effects.

Activation of apoptosis may contribute to reduced neutrophil responsiveness that has been observed when incubating the cells in the presence of relatively high concentrations of carotenoid breakdown products. It is quite hard to predict the effect of abnormal activation of neutrophil apoptosis on lung carcinogenesis. Neutrophil apoptosis results in the loss of expression of adhesion molecules and responsiveness to external stimuli, so that the cells become functionally isolated from the environment. Since specific changes in the plasma membrane allow neutrophil recognition and ingestion by macrophages, apoptosis results in neutrophil...

**Figure 3.** Overview of the biological effects of β-carotene breakdown products.
Carotenoids and lung cancer: biochemical aspects

It has been reported [34,38] that the addition of 5-20 µM retinal or mixture of β-carotene breakdown products to unstimulated neutrophil suspensions causes a remarkable increase of intracellular caspase-3 activity, which is regarded as a biochemical marker of cell apoptosis [39].

A smaller effect on the intracellular caspase-3 level is exerted by β-apo-8'-carotenal and β-ionone. Electrophoretic analysis of neutrophil chromatin extracts shows that activation of caspase-3 activity is accompanied by an increase in DNA fragmentation, while a significant decrease in DNA fragmentation can be obtained by adding z-VAD, a broad-spectrum caspase inhibitor. These results are in good agreement with previous reports [40], indicating that carotenoids induce apoptosis in a T-lymphoblasts in a time- and concentration-dependent manner with the lowest effective concentration of about 3 µM. T-Lymphoblast apoptosis is clearly evident in the presence of 20 µM carotenoids and after 24-h incubation; β-carotene and its cleavage products are more cytotoxic than lycopene. In contrast, β-carotene breakdown products did not show proapoptotic activity on hepatocytes in primary culture, suggesting that there might be a cell and tissue dependent response [41].

It is noteworthy [38] that the proapoptotic activity of carotenoid breakdown products on neutrophils is affected neither by uric acid, an efficient antioxidant that has recently emerged as a peroxynitrite scavenger [42,43], nor by ascorbic acid that has been reported to exert a synergistic protective effects from oxidative stress in model systems [44]. Moreover, a pronounced increase of neutrophil apoptosis (instead of an increase of cell viability, as expected) is observed for combinations of α-tocopherol and carotenoid breakdown products. The up-regulating effect of α-tocopherol was not observed in the presence of retinol that markedly stimulates apoptosis itself, whereas increase in caspase-3 activity was induced by a concomitant addition of α-tocopherol and β-ionone, a cyclohexenyl breakdown product of β-carotene with a shorter aliphatic chain. The effect of carotenoid cleavage products on neutrophil viability was partially influenced by the addition of methanol. This suggests that solvents, which may influence the repartition of lipophilic vitamins between different compartments in the cells, should be considered to explain, at least in part, the effects exerted by carotenoid derivatives in combination with other compounds.

The mechanisms that cause cell apoptosis in the presence of β-carotene breakdown products and α-tocopherol are still unclear. It has been reported [40] that α-tocopherol did not protect T-lymphoblasts.
from apoptosis induced by carotenoids. Moreover, the antioxidant BO-653 led to a slight but statistically significant increase of cell apoptosis when it was used alone, and to a dramatic increase of apoptosis when it was used in combination with β-carotene. It was suggested that a second pathway by which carotenoids might induce apoptosis is via the formation of retinoic acid or retinoic acid analogues [45,46]. Other mechanisms, which were taken into account to explain cell apoptosis, include direct DNA damage [45] and down-regulation of cyclooxygenase-2 activity [47] and epidermal growth-factor receptors [48].

3. Conclusion

At least two practical conclusions can be made, based on the knowledge that high-dose supplementation of β-carotene or other carotenoids is potentially toxic. Firstly we must avoid high-dose supplementation if possible, and secondly we should guarantee safe conditions of high-dose supplementation whenever such a supplementation is unavoidable or useful in preventing diseases. The assumption that one can detoxify the organism by taking high doses of β-carotene or other carotenoids, due to their antioxidant properties, should not be used as a justification for cigarette smoking or drug abuse. The primary medical aim in these cases is to prevent smoking and other types of abuse, instead of prescribing antioxidants. Similarly, one should discourage the use of sun creams and skin sprays containing potentially toxic levels of carotenoids. In fact, if the increased cancer risk is due to the presence of β-carotene degradation products, one can easily understand that this mechanism, which is valid in the inflamed lung tissue, is also operative on the skin exposed to the sun, where increased numbers of leukocytes can be found if inflammation is ongoing [49-52].

When the supplementation of high doses of carotenoids is imperative, such as in patients with cystic fibrosis, where the treatment obviously contributes to prolong their life expectancy, the drug administration should be accompanied with the monitoring of β-carotene plasma concentrations [53] to prevent critically high levels. In these situations, an increased oxidative stress in the organism should be avoided or, if it occurs, it should be treated. For example, the dosage should be decreased in patients with infection in upper air ways or in another place of the body. The upper plasma levels, which should be a part of β-carotene supplementation guidelines, are inferred from longitudinal studies of diseases that may involve these kinds of mechanisms [54-57]. In any case, the so-called "antioxidative network", i.e., the complex system of interacting antioxidants, should be balanced [58-61]. The strengthening of this complex system of antioxidants may contribute both by reducing the rate of β-carotene breakdown product formation by activated neutrophils and by detoxifying these potentially harmful compounds. For practical reasons, this means supplementation of additional antioxidants such as tocopherols, ascorbate or other compounds with similar effects.

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Carotenoids and lung cancer: biochemical aspects

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