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Recent advances in clinical research involving carotenoids

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Abstract - Epidemiological studies show consistent decreased risk of lung cancer and certain other cancers, cataracts, age-related macular degeneration, and coronary heart disease in populations with the highest intakes of carotenoid-rich diets. Intervention studies show reductions in precancerous oral lesions, enhancement in immune parameters, and reduced incidence of cardiovascular events in individuals supplemented with ß-carotene.

INTRODUCTION

Major advances in our knowledge of the function and activities of carotenoids in humans have been seen in the past decade. Cumulative evidence from epidemiological studies shows consistent decreased risk of lung cancer and certain other cancers in populations with the highest intakes of carotenoid-rich diets. Additionally, smokers with precancerous oral leukoplakia had significant reduction in lesion progression following supplementation with ß-carotene and/or antioxidant vitamins. The finding of a 50% reduced incidence of cardiovascular events in physicians, with preexisting disease, who took ß-carotene supplements has focused research on the cardioprotective functions of carotenoids. Recent epidemiological data point also to a role of carotenoids in prevention of cataracts and macular degeneration. Potential mechanisms associated with carotenoid actions include antioxidant and singlet oxygen quenching, provitamin A activity, regulation of gap junction communications between cells, modulation of lipoxygenase activity, enhancement of immune functions associated with increased tumor immunity and modulation of lymphocyte populations in HIV-infected patients.

CANCER PREVENTION

Epidemiology

Cumulative data from over 100 separate epidemiological studies have consistently found that individuals with the highest intakes of carotenoid-rich fruits and vegetables have the lowest risk for many cancers, including those affecting the lung, oral cavity, stomach and esophagus. Serum ß-carotene levels are also inversely related to cancer risk. From 1975 to 1992, forty-six separate epidemiological studies examined the association of dietary and/or serum carotenoids and the risk of lung cancer. Forty-one of the studies found an average two-fold

reduction in risk of lung cancer in individuals with the highest carotenoid intake or highest serum ß-carotene levels (ref. 1). Fourteen of fifteen epidemiological studies published between 1972 and 1993 found a decreased risk of oral, nasal and pharyngeal cancer in the groups with the highest intake and/or serum carotenoid levels. The risk of esophageal cancer in ten out of another fourteen epidemiological studies was also significantly lower in the participants with the highest carotenoid status. From 1963 until 1992, seventeen out of nineteen studies found a decreased risk of stomach cancer associated with high intakes of dietary carotenoids and/or serum ß-carotene (ref. 1).

In contrast to the strong, consistent data concerning lung, head and neck, esophageal and stomach cancers, there are fewer studies and inconsistent findings concerning the association of high intakes of carotenoids and lowered risk of cervical, ovarian and breast cancer. However, there is a strong correlation between high intakes of fruits and vegetables and lower risk of cancer of the reproductive organs (ref. 1, 2).

Intervention Studies: Cancer Prevention

Based upon the promising epidemiological findings, several large intervention trials were initiated by NCI to determine whether ß-carotene supplementation could prevent cancer. The first long-term intervention trial involved 1800 patients with one verified skin cancer biopsy. Patients were given 50 mg ß-carotene or placebo per day for five years and evaluated for subsequent appearance of new skin cancers. Greenberg et al. (ref. 3) published the results of this trial in 1990. ß-Carotene supplementation had no effect on reducing the number of new skin cancers. There are several explanations which have been suggested for the lack of effect of ß-carotene in this study. For instance, the dose may have been too low; the duration may have been too short, ß-carotene may not be effective in blocking progression of skin cells which are well along the cancer process. Skin cancer risk is directly related to lifetime exposure to UV rays found in sunlight. It may be that ß-carotene would be effective in preventing the occurrence of skin cancer rather than recurrence. For example, circulating plasma carotenoids were significantly decreased following exposure to UV light (ref. 4). ß-Carotene supplementation blocked the UV-induced depression in overall immune responses, as determined by delayed type hypersensitivity (DTH) skin tests (ref. 5). As discussed below, optimal immune function may be critical in preventing skin cancer.

Even though ß-carotene supplementation did not prevent the recurrence of skin cancer, there are several intervention trials which have greater potentials for success. Two studies are of particular importance because of the large study populations and their inclusion of individuals with no signs of cancer. The Physician's Health Study, a randomized, double-blind, placebo-controlled trial, involves 22,000 healthy male physicians who take 50 mg of ß-carotene or a matched placebo every other day, and, from 1988, all participants also take an aspirin (325 mg) on the alternate day. The trial, which began in 1983, will continue until 1995. At present, there are no data concerning the efficacy of ß-carotene supplementation in cancer prevention, but, as discussed below, benefits have been shown in patients with precancerous lesions. The second major double-blind, placebo-controlled intervention trial involves 19,000 Finnish male smokers who have taken daily either 20 mg of beta-carotene, 50 mg of vitamin E, both supplements or placebo for the past five years. The data from the study are expected to be available in early 1994.

Intervention: Precancerous Lesions

In 1988, the first paper was published which suggested that ß-carotene supplementation could enhance regression of oral precancerous lesions and leukoplakia and, at the same time, reduce the development of new lesions. In this study, betel nut chewers in the placebo group had three times the number of new lesions and nine times less regression of existing lesions as the group given ß-carotene and vitamin A supplements (180 mg and 100,000 IU/week, respectively) (ref. 6). Since ß-carotene alone had lower efficacy, it is presumed that the study population had a marginal deficiency of vitamin A. More recently, studies in the US with cigarette smokers have shown that ß-carotene supplementation (30 mg/day for 3-6 months) caused complete or partial remission of lesions in 71% of participants. One other study has reported a response rate of 44% with 90 mg of ß-carotene/day (reviewed in ref. 7). In addition, a 60% response rate has been seen in a third intervention trial in smokers who were given a supplement containing ß-carotene, vitamin C and vitamin E (24 mg, 1000 mg, and 800 IU/day respectively) (ref. 8).

CARDIOVASCULAR DISEASE PREVENTION

Epidemiology

There have been several epidemiological studies which show a reduced risk of cardiovascular disease in populations with the highest intake of carotenoids. In a case-control study, there was a 60% reduced risk of myocardial infarction in 125 individuals with the highest serum ß-carotene levels compared to 125 controls (ref. 9). A prospective cohort study of over 87,000 nurses found a significantly decreased risk of coronary heart disease (34%) in the women with the highest vitamin E or carotenoid intakes (ref. 10). In the third study, an inverse relationship was seen between carotenoid-rich diets and subsequent risk of cardiovascular death in a cohort of 1299 elderly men; there was a 45% reduction in risk (ref. 11). Data from the longitudinal Basel Heart Study showed that individuals with low intakes of ß-carotene and vitamin C had more than a 4-fold increased risk of stroke and almost a two-fold increased risk of heart disease (ref. 12). A recent study found that smokers with the highest intake of carotenoids had a significantly decreased risk of heart disease compared to smokers with low carotenoid intakes; RR = 0.3, CI = 0.11-0.82, p=0.02 (ref. 13).

Intervention

The longest intervention study with ß-carotene supplementation involved 333 physicians who entered the Physicians Health Study in 1983 with existing cardiovascular disease, but with no cardiovascular events. The physicians took either 50 mg of ß-carotene, 325 mg of aspirin, both supplements, or placebos every other day for five years. The group that took both supplements had no incidents of myocardial infarctions (MI). Subjects who took both placebos had 20 MIs. The aspirin ß-carotene placebo group had 7 MIs and the ß-carotene-aspirin placebo group had 10 MIs. There was also a significant, 54% reduction in all subsequent vascular events including non-fatal MI, non-fatal stroke, coronary revascularization and coronary death in the ß-carotene-supplemented groups (ref. 11).

There are several possible mechanisms by which ß-carotene supplementation may reduce cardiovascular disease. Although ß-carotene does not appear to protect LDL from oxidation *in vitro* (ref. 14), ß-carotene decreased the oxidative modification of lipoprotein (a) (ref. 15). Carotenoids have been shown to alter macrophage function (ref. 16). Macrophages are transformed into foam cells which are an integral component of the

atherosclerotic plaque. Macrophages also produce reactive oxygen species as well as reactive cytokines via the lipoxygenase enzyme pathway. Macrophages control many actions of other immune cells. ß-carotene can block the activity of lipoxygenase (ref. 17). Other possible mechanisms of carotenoid cardioprotection could include elevation of HDL concentrations (ref. 18, 19) and gap junction communication among endothelial cells (ref. 20).

CATARACT PREVENTION

Cataract removal is the most frequently performed surgery among the elderly in the US, with an annual cost of over $3.5 billion. Cataracts are more prevalent in individuals exposed to UV and in smokers. Several studies have recently found a significant association between high intakes of carotenoid-rich diets and lowered risk of cataracts. In one study, the risk of developing a cortical cataract was several times greater in the group with the lowest serum carotenoid levels than in the group with the highest level (ref. 21). In another study, those in the lowest third of serum concentrations of both ß-carotene and vitamin E had a 2.6-fold increased risk of needing cataract surgery compared to the groups with the higher serum levels (ref. 22). In a prospective, case-control study involving over 50,000 nurses, those with the highest intake of total vitamin A had a 39% lower risk of cataract compared to women with the lowest intakes. Spinach, an excellent source of several carotenoids, was a major predictor of lower risk (ref. 23). Although ß-carotene is not found in the lens or other tissues of the eye, two other carotenoids found in the eye, lutein and zeaxanthin, can quench singlet oxygen and may indirectly reduce the oxidative stress on lens proteins. ß-Carotene may reduce the overall systemic oxidative risk, and indirectly reduce cataract risk.

MACULAR DEGENERATION PREVENTION

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in individuals over 65 years old in the Western world. A large, multi-center case-control study recently reported that the risk of AMD was significantly reduced in the groups with the median and highest intakes of carotenoids (50% and 70% reductions, respectively), with the greatest reduction seen with ß-carotene intake compared to that of five other dietary carotenoids (ref. 24).

IMMUNE FUNCTION

Two major functions of the immune system are to ward off infections and to prevent cancer. With regard to cancer prevention, the immune system is composed of several cells which can recognize and destroy tumor cells and virally infected cells. Immune responses often decline with age and there is a greater risk of cancer and infection in the elderly. Supplementation of healthy elderly individuals for 2 months with 45 or 60 mg/day of ß-carotene increased certain of the indices of immune cell activation (ref. 25). In a recent placebo-controlled intervention trial, elderly subjects were given daily for one year a multivitamin preparation which contained 16 mg of ß-carotene, i.e., approximately eight times the standard level of intake. The supplemented group had significantly fewer infections than the placebo group. Responses to vaccines were also improved in the supplemented group (ref. 26). Further research is

required to determine whether it was ß-carotene in the supplement which was responsible for the beneficial effects.

Recently, a placebo-controlled, double-blind crossover study examined the effects of high dose ß-carotene supplementation on the immune cells of HIV-infected patients. Supplementation with 180 mg/day for one month caused a significant increase in total white blood cells, and the percent change in helper T lymphocytes, resulting in a significant improvement in the helper/suppressor ratio. The majority of the patients showed improvement during ß-carotene supplementation, whilst the majority of patients given the placebo worsened (ref. 27). In earlier, non-controlled studies, HIV-infected patients were given lower doses of ß-carotene and no improvements in helper:suppressor ratios were seen, but there were increases in NK cells and in other markers of lymphocyte activation in one study (ref. 28), a decrease in CD8 suppressor cells in another study (ref. 29) and an improvement in well-being in a third study (ref. 30). The difference between the studies may reflect the decreased intestinal absorption of many micronutrients found in HIV infection. HIV-infected patients may require more than 60-120 mg/day of ß-carotene to raise helper T lymphocyte levels (ref. 31).

Of importance is the recent finding by Ward *et al*. (ref. 32) that HIV-infected patients from the United States had significantly decreased serum vitamin A levels, which could be increased with supplementation. Vitamin A supplementation significantly increased the helper T lymphocyte levels in vitamin A-deficient children in Indonesia and increased the helper:suppressor ratio from 0.97 to 1.32 (ref. 33). Low serum vitamin A levels were associated with low helper T lymphocyte levels and increased risk of mortality in IV-drug using, HIV-infected U.S. adults (ref. 34). It is possible, therefore, that ß-carotene supplementation increased vitamin A levels as well as ß-carotene levels, and the resulting immune cell changes were due to vitamin A. On the other hand, Loya *et al*. (ref. 35) showed that a carotenoid could block the HIV reverse transcriptase enzyme without affecting human cells. ß-Carotene was not reported to have been tested in this system, however, the ability of antioxidants to reduce HIV replication has recently been reported (ref. 36). Thus, even though the mechanism of action of ß-carotene on immune parameters in HIV-infected patients is yet to be elucidated, the findings are sufficiently promising to warrant further research.

Exposure to UV light has been shown to depress human immune responses and also increase the risk of skin cancer, as discussed previously. Specifically, UV exposure caused a significant reduction in circulating total lymphocytes and helper T lymphocytes, resulting in an inversion of the helper:suppressor ratio (ref. 37). UV light also decreases antioxidant enzyme levels in the skin while increasing lipid peroxide levels. White *et al*. (ref. 4) reported a significant decrease in circulating plasma carotenoids following exposure to UV light. In a placebo-controlled trial, ß-carotene supplementation (30 mg/day for 7 weeks) blocked the UV-induced depression in overall immune responses, as determined by delayed type hypersensitivity (DTH) skin tests (ref. 5). The number of DTH responses was significantly decreased, but returned to baseline by the end of the study. In contrast, the vigor of the response, as measured by the induration diameter was still significantly reduced in the placebo group at the end of the study.

UV light, either from lamps or sunlight, activated human HIV gene expression in transgenic mice (ref. 38). The immune system of HIV-infected patients is severely compromised because the virus attacks helper T lymphocytes. Exposure to UV could adversely affect HIV-infected

patients in two ways: by activating the viral genetic material or by further reducing immune responses.

CIGARETTE SMOKING: EFFECTS ON ß-CAROTENE STATUS

Tobacco use is linked to more than 70% of oral cancers and lung, head and neck, esophageal and stomach cancers are all associated with cigarette smoking. Cigarette smokers and tobacco chewers have significantly lower serum levels of ß-carotene, vitamin C and several other micronutrients, than do nonsmokers who consume comparable levels of the micronutrients (ref. 39). However, smokers with the highest intake of ß-carotene-rich foods showed a significant reduction in risk of these cancers in a number of studies (ref. 2). In a placebo-controlled, double-blind study, smokers given 20 mg/day for 14 weeks had a significant reduction in abnormal cells in their sputum, but no change in the level of DNA damage in circulating white blood cells (ref. 40, 41). Abnormal sputum cells may be an early sign of precancerous oral, bronchial or lung lesions, which are precursors of cancer. ß-Carotene supplementation (30 mg day for 2 months) reduced the progression of oral precancerous lesions in the mouths of 70% of smokers in one intervention trial (ref. 7) and, at the same time, enhanced NK cell receptors and <u>in vitro</u> killing of tumor cells by NK cells. Lymphocyte proliferation is also depressed and has been shown to be increased when smokers are given 20 mg/day of ß-carotene for 14 weeks (ref. 42).

Surveys demonstrate that the average dietary intake of ß-carotene is approximately 1.5 mg/day. However diets recommended by the National Cancer Institute and the American Cancer Society for healthy adults contain about 5-6 mg/day of ß-carotene (ref. 43). Individuals, such as smokers, exposed to greater oxidative stresses, may require even higher levels of intake of ß-carotene and other antioxidant nutrients. Thus, food fortification and supplementation should also be considered as options for increasing intakes of ß-carotene, especially for population groups at risk of oxidative stress.

Table 1. Survey of clinical research involving carotenoids

EPIDEMIOLOGY
Lower risk with highest intakes of carotenoids
CANCER
CARDIOVASCULAR DISEASE
CATARACT
MACULAR DEGENERATION
INTERVENTION STUDIES
Beneficial effects of ß-carotene supplementation
PRECANCEROUS LESION REGRESSION
IMMUNE FUNCTION ENHANCEMENT
REDUCTION IN SECONDARY CARDIOVASCULAR EVENTS

CONCLUSIONS

The data presented in this review, summarized in Table 1, clearly point to the clinical importance of ß-carotene. The epidemiological studies

indicate that ß-carotene and other dietary carotenoids are associated with the prevention of cancer, cardiovascular disease, cataracts, AMD, and enhancement of immune function.

Intervention trials, especially the double-blind, placebo-controlled studies with ß-carotene as the sole active agent have shown beneficial effects in a number of important, clinically relevant parameters. Of equal importance, no adverse effects have been reported. The safety of ß-carotene is well established (ref. 44).

Dietary requirements for ß-carotene appear to be increased in smokers and those exposed to UV light, and may be related to the higher oxidative burden associated with these activities. Individuals with other life-styles or environmental exposures may also have higher than normal levels of oxidative stress and may require higher than recommended intakes of ß-carotene to attain the clinically relevant benefits documented in this review.

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