The effect of β-carotene on lung and skin carcinogenesis

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The disturbing outcome of two large epidemiologic intervention studies (1,2), in which large daily doses of dietary β-carotene (BC) were given to current or former cigarette smokers over 4 and 6 years, resulting in 16 and 23% excess incidence of lung cancer, respectively, rightly elicited a great deal of comment (e.g. refs 3–5). Surprisingly, no animal experiments had been reported on the effect of high doses of BC on lung carcinogenesis prior to the human intervention trials. This lack has now been corrected by the work of Wang et al. (6) and Liu et al. (7), which introduced the ferret as an experimental animal. Whereas mice and rats almost completely convert BC to retinoids in the intestine and liver and therefore would transport little to the lungs, ferrets, like humans, absorb it intact into the bloodstream and transport it to the lung as well as to other tissues.

Liu et al. (7) showed that ferrets fed high doses of BC (2.4 mg/kg body wt/day) over 6 months developed alveolar cell proliferation and keratinized squamous metaplasia in their lungs, an effect greatly exacerbated when they were also exposed to cigarette smoke. The concentration of BC in blood and lung increased greatly, yet surprisingly, the level of retinoic acid (RA), presumably derived from BC, decreased to one-sixth that of ferrets fed a low dose of BC (0.43 mg/kg body wt/day). The authors hypothesized that the presence of large amounts of BC in lung tissue resulted in oxidative metabolites or eccentric cleavage products of BC, which would induce cytochrome P450 enzymes. These would then remove RA by oxidatively metabolizing it and thus lower its level in the lung. In addition to the decreased concentration of RA in the high-dose BC ferrets, the investigators observed a decreased level of the RA receptor RARβ [a putative tumor suppressor (6)] and stimulation of the proliferation-signalling proteins (AP-1). No actual lung tumors were observed, however.

The question then arises: was the keratinizing squamous metaplastic state a pre-cancerous lesion that would—given time and the presence of smoke, the presumed carcinogen—be transformed into lung carcinoma? And would BC then influence this conversion, positively or negatively? Answers to these questions would determine whether or not BC should be abandoned as a potential chemopreventive agent against lung cancer.

Some leads towards answers to these questions could perhaps be found by comparing and contrasting the considerable data accumulated on the effect of dietary BC on carcinogenesis in skin or other organs with those on lung carcinogenesis.

Thus, Chen et al. (8) determined the effect of feeding BC at 0.6 (marginal dose), 60 (physiologic dose) or 600 mg/kg diet (pharmacologic dose) to SENCAR mice in a purified, retinoid and carotene-free diet for 45 weeks, while applying a single dose of carcinogen (7,12-dimethylbenz[a]anthracene, DMBA) and 20 weekly applications of a tumor promoter (12-O-tetradecanoylphorbol-13-acetate, TPA) to their skin. The high-dose feeding of BC caused a significantly increased yield of skin papillomas compared with low-dose BC-fed mice. Under slightly different conditions (9), such as a reduction in liver reserves of retinoids, papilloma formation with a high-dose of BC was slightly reduced and the intermediate and low-dose groups had the same incidence of papillomas. However, in both experiments (8,9) conversion to carcinomas was strongly inhibited by high doses of dietary BC. The authors (9) comment on the ‘lack of correlation between papilloma and carcinoma incidence’.

Other reports describe a variable response of papilloma formation to BC: extremely high doses of BC (30 g/kg diet) fed to SENCAR mice following the two-stage (DMBA/TPA) tumor induction procedure caused no increase in papilloma formation, but a limited decrease (10), nor did mice whose skin was irradiated by UVB light, when given a high dose of BC (33 g/kg diet) display an increased papilloma yield, although it delayed their appearance (11). Dietary BC effectively inhibited DMBA-induced salivary gland tumors in rats (12) and dimethylhydrazine-induced colon adenocarcinomas in mice (13). In the latter case, no pre-cancerous hyperplasia was observed, even though a dose at the upper limit of the physiological dose was fed (30 mg/kg diet). In the case of induced stomach cancer in rats (14), on the other hand, the presumably pre-cancerous mucosal dysplasia was enhanced, whereas the transformation of dysplasias to carcinomas was inhibited by dietary BC, an effect exactly parallel to the effect of high doses of BC on skin carcinogenesis (8).

When comparing BC action on lung and skin carcinogenesis, the question may be asked: do high doses of BC fed to experimental animals reach the target tissues? In ferrets, high-dose feeding (2.4 mg/kg body wt/day) resulted in 12 ± 1.2 µg/g lung tissue (7); in mice fed a pharmacologic dose (600 mg/kg diet), BC appeared in skin at a low, but still substantial level of 0.51 ± 0.28 µg/g (15).

In a comparison of the effect of BC on lung and skin carcinogenesis, the conversion of BC to retinoids must be
considered. This process is especially active in mice and rats as compared with ferrets. The results of administration of retinoids on lung cancer in experimental animals have been variable (16). This is not surprising, considering the distinct kinds of tumors of the lung that could be induced by carcinogens: squamous (epithelial) tumors, adenocarcinomas, large-cell carcinomas and small-cell cancers. Squamous metaplasia of the upper respiratory tract in experimental animals appears to be similar to the pre-neoplastic changes observed in the lungs of heavy smokers (17). The incidence of respiratory carcinoma induced in hamsters by benzo[a]pyrene has been effectively reduced by intragastric administration of large doses of 13-cis-RA (18). Studies with rats (19) failed to obtain a reduction of 3-methylcholanthrene-induced pre-cancerous lung nodules with 13-cis-RA in rats.

For comparison, mice treated with DMBA and TPA in the two-stage skin carcinogenesis procedure, while being fed three levels of RA (marginal, physiological and pharmacological), produced a similar incidence of papillomas for all three dose levels (20). In that respect, the effect of RA differed from that of BC, as the latter caused an increased papilloma yield under similar conditions (8). The conversion of papillomas to carcinomas, on the other hand, was powerfully inhibited by the pharmacologic dose, although not by the lower doses of RA (21).

Dietary RA, when fed to mice in pharmacologic doses, or when applied topically to the skin, strongly enhanced papilloma formation following a single application of DMBA (without TPA treatment), suggesting a tumor promoter activity for this retinoid (22). The stimulation of papilloma yield by high doses of BC in the two-stage carcinogenesis procedure (DMBA/TPA) (8) led to the thought that BC might have tumor-promoter properties. A test for tumor promoting activity of BC was made (9) by a single application to the skin of mice of DMBA, followed by weekly topical applications of BC for 42 weeks: BC showed no tumor promoter activity in this procedure.

The two-stage carcinogenesis procedure does not, of course, apply to lung carcinogenesis. Nonetheless, a co-carcinogenic activity, akin to what could be considered tumor promotion for skin carcinogenesis, has been postulated for the lung by Paolini et al. (23,24). They observed the induction of cytochrome P450 enzymes in lungs of rats fed high doses of BC (500 mg/kg body wt). These enzymes would then enhance the metabolic oxidation of RA and thereby lower its level in the lungs, as shown by Liu et al. (7). In consequence,RARβ declined, leading to a cascade of proliferation signals (7). In a cell culture bioassay of carcinogenesis with BALB/c3T3 cells, Perocco et al. (25) reported the enhancement of benzo[a]pyrene-induced transformation when the cells were treated with BC. The authors suggested that the cytochrome P450 enzymes induced by the BC might convert the benzo[a]pyrene to its active form. In this sense, BC could be regarded as a tumor promoter in lung tissue.

That the effect of BC on lung carcinogenesis may differ from that on other tissues or organs was hinted at in an epidemiologic study in humans (26). Patients who had been treated curatively for early stages of oral cavity, pharynx or larynx squamous cell carcinoma and were given high doses of BC (50 mg/day) for 51 months showed a trend towards lower second head and neck cancer risk, and a trend towards increased lung cancer risk. Neither of these trends, however, was statistically significant.

In conclusion, the keratinized squamous state of lung tissue in ferrets resulting from high dietary BC is pre-neoplastic, representing a reversible change in differentiation. This state is distinct from, and not parallel to, the neoplastic skin papillomas capable of malignant conversion that is susceptible to inhibition by high doses of dietary BC (8,9). One would not, then, expect that the potential smoke-induced lung tumors in ferrets would be inhibited by continued treatment with high doses of BC. This conclusion is in tune with the epidemiologic findings (1,2), which show that long-term, high-dose dietary BC does not inhibit smoke-induced lung tumors in humans. On the other hand, one should note that low levels of dietary BC alleviated smoke-induced squamous metaplasia in lungs of ferrets (7). Therefore, provided the appropriate dose level of BC could be ascertained, BC may yet find application for treatment of smoke-induced lung carcinogenesis.

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References

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