Pure & Appl. Chem., Vol. 57, No. 5, pp. 717–722, 1985.
Printed in Great Britain.

Carotenoids and cancer prevention — experimental and epidemiological studies

Micheline M. Mathews-Roth

Channing Laboratory, Department of Medicine, Harvard Medical School, and Brigham and Women's Hospital, 180 Longwood Avenue, Boston, Mass, 02115, USA.

Abstract - It is now well established that a primary function of carotenoids in photosynthetic organisms and carotenoid-containing non-photosynthetic organisms is to prevent photosensitization by endogenous photosensitizers. This protective ability of carotenoids has been made use of in the administration of beta-carotene and canthaxanthin to relieve the symptoms of patients suffering from certain light-sensitive skin diseases. Recently interest has developed in carotenoids and vitamin A as potential cancer-preventive agents. This paper will review the epidemiological and experimental studies that have given impetus to this suggestion.

INTRODUCTION

At the last Symposium, we discussed the medical applications and uses of carotenoids (1), describing in detail the pigments' function of protecting photosynthetic organisms against photosensitization by their own chlorophyll, and the application of this protective function in the treatment of patients suffering from light-sensitive skin diseases with high doses of carotenoids. In the present paper, we will concentrate on the discussion of a possible new role for carotenoids which was just briefly described in the above mentioned paper - the pigments' possible role as anti-tumor agents.

ANIMAL STUDIES

Over the past thirty years, reports have appeared in the scientific literature of the anti-tumor effects of vitamin A in animal models. This work has been extensively reviewed (2-5) and thus will not be discussed here. Instead, we will concentrate on describing the work concerning beta-carotene.

In view of the success of vitamin A in suppressing tumor development, it is not surprising that investigations were done to see if beta-carotene, the precursor of vitamin A, had an anti-tumor effect. In 1973, Dorogokupla *et al.* induced subcutaneous tumors in rats by injecting 9,10-dimethyl-1,2-benzanthracene (DMBA), and skin tumors in mice by topical application of DMBA. The authors found in both cases that those animals fed a diet supplemented with "unlimited amounts of red carrots" developed tumors at a slower rate than did the animals receiving the unsupplemented diet (6). In that year also, Epstein reported inducing skin tumors by exposing hairless mice to UV-B radiation (290 - 320 nm) and found that those mice which received injections of a solution of beta-carotene beadlets (Roche) developed tumors at a slower rate than did mice injected with a solution of placebo beadlets (7). Dorogokupla *et al.* proposed that the effect of beta-carotene on tumor formation was due to the effect of vitamin A, which was formed from the administered beta-carotene (6).

To determine whether the carotenoid molecule itself has an anti-tumor action, we decided to study the effects on tumor formation of two other carotenoids which have no vitamin A activity: canthaxanthin (4,4'-diketo beta-carotene), which cannot be converted to vitamin A by mammals, and which has been found to be effective in preventing photosensitization both *in vitro* and *in vivo*, and phytoene (7,8,11,12,7',8',11',12'-octahydrolycopene), which we have found to have protective activity against sunburn (8,9). We examined the effects of

these two pigments and of beta-carotene on the development in hairless mice of skin tumors induced by three different methods: 1) UV-B irradiation (7.2 kJ/m^2, 3 times a week), 2) 2 applications of DMBA followed by twice-weekly applications of croton oil, and 3) 1 application of DMBA followed by UV-B irradiation (2.7 kJ/m^2, twice a week). We found that administration of beta-carotene, canthaxanthin or phytoene resulted in a statistically significant delay in the appearance of skin tumors induced by UV-B radiation (8,9). We also found that beta-carotene and canthaxanthin had some effect in delaying the appearance of subsequent tumors, when the administration of these pigments was started after one tumor had developed (10). However, in the case of DMBA-croton oil tumor induction, only beta-carotene was effective in delaying tumor development - canthaxanthin and phytoene had no such effect (8,9). With DMBA application and low-dose UV-B irradiation, both beta-carotene and canthaxanthin administration caused a delay in tumor development, but phytoene had no effect (8,9). In each case, when the administration of a given pigment caused a delay in tumor development, fewer tumors per animal were found in the pigment-treated mice, as compared to the placebo-treated animals (8,9).

These experiments suggest that carotenoid pigments, irrespective of their vitamin A activity, can prevent or slow down the growth of skin tumors induced by UV-B alone. These experiments also suggest that the mechanism of tumor induction by DMBA-croton oil application must be different from that of UV-B exposure. As to tumors produced by DMBA and low-dose UV-B radiation, canthaxanthin may not be able to affect initiation by DMBA, but may have an effect on promotion or co-carcinogenesis by UV-B. Phytoene, because of molecular differences between it and either beta-carotene or canthaxanthin, may not be able to exert enough effect on the development of tumors induced by an agent other than UV-B. There are suggestions in the literature that excited species such as free radicals and singlet oxygen may be involved in the development of skin tumors induced by UV-B. It is interesting to note that beta-carotene and canthaxanthin are extremely efficient quenchers of such species: phytoene is less so (11).

Our work has been confirmed and extended by Santamaria *et al.* (12). These workers have reported that beta-carotene and canthaxanthin administered orally to Swiss albino mice will delay the development of tumors induced by the application of benz(a)pyrene alone (BP) or BP plus UV-A radiation (320-400 nm). They have also found that these pigments will delay the development of skin tumors in hairless mice induced by the administration of 8-methoxy psoralen and UV-A radiation. They will be presenting this interesting finding in detail at this Symposium, so we will not discuss it further.

Seifter *et al.* have reported a series of experiments in which they have found that either vitamin A or beta-carotene inhibits the size and/or development of transplanted tumors in mice (13-15). These workers also suggest that beta-carotene and vitamin A administration prevent the involution of the thymus gland, which occurs when transplanted tumors take hold in the host. They have recently reported that administration of beta-carotene or vitamin A can decrease the incidence of DMBA induced tumors in rats (16). The kinds of tumors were not specified. One concern with their studies is that the authors added beta-carotene to the diet of the animals as an alcoholic solution of crystalline beta-carotene. It is difficult to determine whether the added beta-carotene and vitamin A survived oxidation and contributed significantly to the blood and tissue level of these substances.

In recent experiments, we have found that beta-carotene or canthaxanthin at a dose of 100 mg/kg of diet, a bit higher than the 90 mg/kg of diet used by Seifter *et al.*, was not protective against tumors induced by UV-B irradiation. However, a dose level of 3.5 mg carotenoid/5 g of diet, similar to the 2.5 mg/5 g of diet (plus a bit more from gavaging) used by Santamaria *et al.* (12) was protective. In all of our experiments we used beta-carotene beadlets (Roche) as the source of our pigments. The beadlet formulation protects the pigments against oxidation. Indeed we could detect high levels of carotenoids in the skin of the treated animals (8,9 and unpublished observations), and analysis of the supplemented foods contained the specified amounts of pigments. Santamaria *et al.* also used Roche pigment beadlets as the source of carotenoids, as well as freshly prepared solutions of carotenoids in oil for supplemental gavage administration. In this latter case also, little deterioration of the pigments could have occurred.

The experiments described to this point have been concerned with oral administration of carotenoids before and/or during tumor induction. Shamberger

reported the effect of applying topical beta-carotene to haired mice undergoing tumor induction with DMBA and croton oil or resin application. He found an increase of tumor formation in the animals which received beta-carotene applications (17). It is difficult to know what happened to the beta-carotene under these conditions. We have found that beta-carotene applied topically to human skin bleaches quickly (18). Shamberger's finding is also somewhat reminiscent of similar findings with topically applied vitamin A, in which some doses applied topically cause excess tumor formation and other dose levels protect.

Beta-carotene, canthaxanthin and phytoene are all lipid-soluble carotenoids. Gainer et al. have been investigating the water-soluble carotenoid, crocetin, and found that this pigment, prepared by them from an extract of saffron, offered protection against DMBA-croton oil induced tumors in haired mice (19). We repeated their experiments, but used instead hairless mice and crystalline crocetin produced synthetically (Roche). The route of administration, vehicle and dose were those used by Gainer et al.. We found only a small effect in delaying tumor development when tumors were induced with DMBA-croton oil applications. We found no effect in preventing the development of skin tumors induced by UV-B alone (20). The same amounts of radiation were used in these experiments as were used in the experiments mentioned above in which beta-carotene, canthaxanthin and phytoene had a protective effect against skin tumor formation. It is not totally clear why crocetin is not as effective as the other pigments. Perhaps the dose used was incorrect. Also, the molecular structure of crocetin differs from the other pigments, and the water-solubility may affect where the pigments localize in the cell.

The several experiments reported here would suggest that the carotenoid molecule itself has some ability to prevent or delay the development of skin tumors induced by UV-B radiation, and in some cases tumors induced by a chemical carcinogen (DMBA or BP) and promoted by UV-B or UV-A. Certainly beta-carotene should have some effect on the inhibition of tumors in organs other than skin which have been shown to be inhibited by vitamin A. Carefully controlled experiments where adequate dose levels of beta-carotene are given, and carotenoid and vitamin A levels in the serum and target organs are measured need to be performed to determine the anti-tumor spectrum of not only beta-carotene but also other carotenoid pigments.

Studies on the mechanism of action of the protective functions of carotenoids have also been performed. Mufson et al. (21) have suggested that beta-carotene can inhibit 12-O-tetradecanoyl-phorbol-13 acetate-induced arachidonic acid and prostaglandin release in chick embryo fibroblasts, but that retinoic acid has a higher activity. Petrunyaka reports that carotenoids are found in molluscan neurons and that the pigments are involved in membrane transport of calcium (22). Dixit et al. have shown that reactive oxygen species are involved in lipid peroxide formation in rat epidermal microsomes, and that beta-carotene added to the cells can inhibit lipid peroxide formation (23). An experiment to detect lipid peroxide formation in vivo in guinea pigs as measured by pentane and ethane formation was performed by Kunert and Tappel (24). They found that beta-carotene administration, as well as vitamin C administration, could protect against in vivo lipid peroxidation. We found that the administration of either beta-carotene or canthaxanthin to porphyric hairless mice could protect against singlet oxygen formation in epidermis, when isolated epidermis was exposed to light (25). These experiments suggest that indeed enough carotenoids can accumulate in skin to function in quenching excited species which may form there. It is also interesting to note that carotenoid treatment of photosensitivity has also been applied to animal diseases: beta-carotene therapy has been found to ameliorate the skin lesions associated with feline solar dermatitis (26).

Some recent investigations have looked at the effect of beta-carotene on some immunological parameters. Leslie and Duby reported that beta-carotene administration increased the activity of T-cell response to the mitogen, concanavalin A (27). Bendich and Shapiro have shown that increased levels of dietary beta-carotene leads to enhanced t-cell response (28).

HUMAN STUDIES

The reports of animal studies suggesting that vitamin A has anti-tumor activity have led to studies to determine whether vitamin A, or its precursor pigment in foodstuffs, beta-carotene, have any such activity in human tumors.

We have found 33 reports in the literature of epidemiological studies concerned with the effect of diet, particularly the ingestion of carotenoid-containing vegetables, on the incidence of cancer (29-61). All but 4 (44, 48, 50, 53) have suggested that there is an inverse relationship between the ingestion of carotenoid-containing vegetables and the incidence of cancer. Most of the studies have been retrospective or prospective dietary studies. However, 12 studies analysed the sera of cancer victims and control populations (50-61). Peto et al. have reviewed many of the studies (62) so we will not attempt to do that here.

Admittedly, it is risky to base conclusions of drug efficacy on dietary intake studies, because recall of specific food intake may be incomplete. Even studies examining serum levels of the compound of interest may be suspect in that control populations may not have been chosen properly. In addition, in dietary studies, there is always the possibility that the actual active compound may not be the component upon which interest is focused, but another component of the foods containing it. Even negative studies of either type do not necessarily mean that the compound of interest has no effect in preventing cancer. The compound may only be effective in certain types of cancer not yet tested, or in sub-groups of a population (such as people with low-normal or deficient blood levels of the compound).

Certainly the best way to determine whether a particular substance is effective in preventing human cancer is to administer it prophylactically to a large enough number of people at high risk to develop the particular kind of cancer to be studied, the study population being randomized into a group receiving the substance under study and a group receiving placebo. Indeed the National Cancer Institute of the United States' National Institutes of Health is presently funding 6 such studies in which carotenoids are administered to people at high risk for developing certain kinds of cancer (M.A. Sestili, personal communication: data presented with permission). In each case, the study population is divided into groups receiving carotenoid and groups receiving placebo. We will briefly describe the studies. Three studies are concerned with the prevention of non-melanoma skin cancer. The first, in Tanzania, will administer a mixture of beta-carotene and canthaxanthin in doses adjusted to the patients' weight, to albinos, who are at extremely high risk for developing these tumors. The second is determining the effects of administering 30 mg/day of beta-carotene to patients who have had at least one such tumor, and the third skin study is using either beta-carotene alone (15 mg/day) and the same dose of beta carotene in combination with ascorbic acid (4 g/day) and alpha-tocopherol (400 mg/day). In the fourth study, 15 mg/day of beta-carotene is administered to patients who have previously developed at least one adenomatous polyp of the colon, to determine if the administration of the pigment will prevent or slow down the development of subsequent polyps, or colonic cancer. In the fifth study, 20 mg/day of beta-carotene is administered to young men who are heavy smokers to determine if the administration of the pigment will reduce the incidence of lung cancer. The sixth study, which is designed to see if the administration of 50 mg of beta-carotene on alternate days to healthy physicians will decrease the incidence of all types of cancer, will be described in detail by Dr. Hennekens at this Symposium.

All of the 6 placebo-control studies described here are due to run for several years, and may have to be extended beyond their originally planned periods, to assure that sufficient data have been obtained. Thus it will take quite a few more years before it is definitely established whether or not beta-carotene and/or vitamin A have a significant anti-cancer effect in man. A prime requiremnent besides efficacy in cancer prevention for a prophylactic anti-cancer agent to be used in large numbers of people is that it must have little or no toxicity. To date, beta-carotene has proven to be very safe, as evidenced by its widespread use in the treatment of photosensitivity. The only side-effect reported to date is mild gastro-intestinal disturbances. Significant carotenodermia may not develop if low doses of beta-carotene are used: this condition can be controlled by lowering the dose. The dietary epidemiological studies seem to suggest that it is not necessary to ingest extremely large amounts of beta-carotene to obtain an anti-cancer effect. If this bears out to be true, then carotenodermia will not be much of a problem. Recent studies have suggested that beta-carotene had no deleterious effect on the genome (63, 64), thus reinforcing prevailing impressions that this pigment is of low toxicity (1). Thus, if it is found from studies in man such as the ones described here that beta-carotene is effective in preventing the

development of one or more kinds of cancer, this pigment will be an extremely good candidate for use as a cancer-preventive agent for use in large populations. We eagerly await the results of the on-going clinical studies described here, and others which may also be commenced in the near future.

REFERENCES

1. M.M. Mathews-Roth, in G. Britton and T.W. Goodwin, eds, Carotenoid Chemistry and Biochemistry, p. 207, Pergamon Press, Oxford (1982).
2. M.B. Sporn, M.M. Dunlop, D.L. Newton and J.M. Smith, Fed. Proc. 35, 1332-1338 (1976).
3. M. Sporn, and D. Newton, Fed. Proc. 38, 2528-2534 (1979).
4. R. Boutwell, Selected Abstracts on Vitamin A in Cancer Biology - International Cancer Research Data Bank (ICRDB) National Cancer Institute, Bethesda, MD (1979).
5. W. Bollag, Ann. N. Y. Acad. Sci. 359, 9-23 (1981).
6. A.C. Dorogokupla, E.G. Troitzkaia, L.K. Adilgireieva, S.F. Postolnikov, Z.P. Chekrygina, Zdravookhr. Kazakh. 10, 32-34 (1973).
7. J.H. Epstein, Photochem. Photobiol. 25, 211-213 (1973).
8. M.M. Mathews-Roth, in J.D. Nelson and C. Grassi, eds., Current Chemotherapy and Infectious Disease, p. 1503, American Society For Microbiology, Washington, DC, (1980).
9. M.M. Mathews-Roth, Oncology 39, 33-37 (1982).
10. M.M. Mathews-Roth, Photochem. Photobiol. 37, 509-511 (1983).
11. M.M. Mathews-Roth, T. Wilson, E. Fujimori and N.I. Krinsky, Photochem. Photobiol. 19, 217-222 (1974).
12. L. Santamaria, A. Bianchi, A. Arnabaldi, L. Andreoni and P. Bermond, Experientia 39, 1043-1045 (1983).
13. E. Seifter, G. Rettura, J. Padawer, F. Strattford, P. Goodwin and S.M. Levinson, Jour. Nat. Cancer Inst. 71, 409-417 (1981).
14. E. Seifter, G. Rettura, J. Padawer and S.M. Levinson, Jour. Nat. Cancer. Inst. 68, 835-840 (1982).
15. G. Rettura, F. Strattford, E. Seifter, Jour. Nat. Cancer Inst. 69, 73-77 (1982).
16. E. Seifter, G. Rettura, S.M. Levinson, Fed. Proc. 43, 662 (1984).
17. R.J. Shamberger, Jour. Natl. Cancer Inst. 47, 667-672 (1971).
18. M.M. Mathews-Roth, M.A. Pathak, T.B. Fitzpatrick, L.C. Harber and E.H. Kass, Jour. Amer. Med. Assoc. 228, 1004-1008 (1974).
19. J.L. Gainer, D.A. Wallis, J.R. Jones, Oncology 11, 222-224 (1976).
20. M.M. Mathews-Roth, Oncology 39, 362-364 (1982).
21. R.A. Mufson, D. DeFeo and I.B. Weinstein, Mol. Pharm. 16, 569-578 (1979).
22. V.V. Petrunyaka, Cell. and Mol. Neurobiol. 1, 11-20 (1982).
23. R. Dixit, H. Mukhtar and D.R. Bickers, Jour. Invest. Derm. 81, 369-375 (1983).
24. K.J. Kunert and A.L. Tappel, Lipids 18, 271-274 (1983).
25. M.M. Mathews-Roth Photochem. Photobiol. 40, 63-68 (1984).
26. R.A. Irving, Amer. Jour. Vet. Med. 43, 2067-2069 (1982).
27. C.A. Leslie and D.P. Duby, Fed. Proc. 41 381 (1982).
28. A. Bendich and S. Shapiro, Fed. Proc. 43, 787 (1983).
29. R.B. Shekelle, S. Liu, W.J. Raynor, M. Lepper, C. Maliza and A.H. Rossof, Lancet II, 1185-1190 (1981).
30. C. Mettlin and S. Graham, Amer. Jour. Epidemiol. 110, 255-263 (1979).
31. R.G. Phillips, Cancer Res. 35, 3513-3522 (1975).
32. E. Bjelke Internat. Jour. Cancer 15, 561-565 (1975)
33. T. Hirayama, Nutr. Cancer 1, 67-81 (1979).
34. R. MacLennan, J. DaCosta, N.L. Day, C.H. Law, Y.K. Ng, and K. Shanmugarainan, Int. Jour. Cancer 20, 854-860 (1977).
35. C. Mettlin, S. Graham and M. Swenson, Jour. Nat. Cancer Inst. 62, 1435-1438 (1979).
36. A. Gregor, P.M. Lee and F.J.C. Roe, Nutr. Cancer 2, 93-97 (1980).
37. S. Graham, C. Mettlin, J. Marshall, R. Priore and T. Rzepka, Amer. Jour. Epidemiol. 112, 422 (1980).
38. C. Mettlin, S. Graham, R. Priore, J. Marshall and M. Swanson, Nutr. Cancer 2, 143-147 (1981).
39. P. Cook-Mozaffani, Brit. Jour. Cancer 39, 293-409 (1979).
40. E. Bjelke, in R.L. Clark, R.W. Cumley, J.E. McCay and M.M. Copeland, eds., Oncology 1970 - Proceedings of the Tenth International Cancer Congress: V, p. 320 Year Book of Medicine, Chicago (1971).
41. J.C. Paymaster, L.D. Sanghri and R. Gangadharan, Cancer 21, 279-288 (1968).
42. W. Haenszel, M. Kurihara and M. Segi, Jour. Nat. Cancer Inst. 49, 969-

988 (1972).
43. R. Ziegler, L.E. Morris, W.J. Blout, L.M. Pattern, R. Hoover and J. Fraumeri, Jour. Nat. Cancer Inst. 67, 1199-1206 (1981).
44. S. Graham, B. Haughey, J. Marshall, R. Price, T. Byers, T. Rzepka, Jour. Nat. Cancer Inst. 70, 687-698 (1983).
45. F.L. Wynder Cancer 16, 1461-1496 (1963).
46. P. Correa, C. Cuello, L.F. Fajardo, W. Haenszel, O. Bolanos, and B. de Ramirez, Jour. Nat. Cancer Inst. 70, 673-678 (1983).
47. H.B. Stahelein, E. Buess, F. Rosel, L.K. Widmer, and G. Brubacher, Lancet I, 394-395 (1982).
48. P.G. Smith and H. Jick, Cancer 42, 808-811 (1978).
49. S. Graham, H. Dayal, M. Swanson, A. Mittelman and G. Wilkinson, Jour Nat. Cancer Inst. 61, 709-714 (1978).
50. W.C. Willet, B.F. Polk, B.A. Underwood, M.J. Stampfer, S. Pressel, B. Rosner, J.W. Taylor, K. Schneider and C.G. Hames, New Engl. Jour. Med. 310, 430-434 (1984).
51. T.K. Basu, D. Donaldson, M. Jenner, D.C. Williams and A. Sakula, Brit. Jour. Cancer 33, 119-121 (1976).
52. S. Atukorala, T.K. Basu, J.W.T. Dickerson, D. Donaldson, and A. Sakula, Brit. Jour. Cancer 40, 927-931 (1979).
53. M.H. Cohen, A. Primack, L.E. Broder and L.R. Williams, Cancer Lett. 4, 51-54 (1977).
54. K. Ibrahim, N.A. Jafary, S.J. Zuberi, Clin. Oncol. 3, 203-207 (1977).
55. N. Wald, M. Idle, J. Boreham and A. Bailey, Lancet II, 813-815 (1980).
56. J.D. Kark, A.H. Smith, B.R. Switzer and C.G. Hams, Jour. Nat. Cancer Inst. 66, 7-16 (1981).
57. I.D. Carpel and D.C. Williams, I.C.R.S. Med. Sci. 7, 361 (1979).
58. J.C. Abels, A.T. Gorham, G.T. Park and C.P. Rhoads, Jour. Clin. Invest. 20, 749-764 (1941).
59. P. Clifford Proc. Roy. Soc. Med. 65, 682-686 (1972)
60. P.N. Wahi, U. Kehar and B. Lahin, Brit. Jour. Cancer 19, 642-660 (1965).
61. P.N. Wahi, B. Lahiri, U. Kehar and S. Arora, Indian Jour. Pathol. Bacteriol. 5, 10-16 (1962).
62. R. Peto, R. Doll, J.E. Buckley and M.B. Sporn, Nature 290, 201-208 (1981).
63. R.B. Haveland-Smith, Mut. Res. 91, 285-290 (1981).
64. T. Kada, T. Tutikawa and Y. Sudaie, Mut. Res. 16, 165-174 (1972).