

Anticancer potential of vitamin A and beta-carotene: mechanistic approach

Biswajit Mukherjee*, Miltu Kumar Ghosh, Chowdhury Mobaswar Hossain

Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700 032, India

*Corresponding author's e-mail: biswajit55@yahoo.com

ABSTRACT Cancer modulation by nutritional variation is a subject of recent interest among nutritionists and cancer scientists. The influences of vitamin A and β -carotene on cancer reported in the current literature are reviewed here critically to provide an overview of the latest development in this field and to address the present perspective and future possibilities of the micronutrients to control various forms of cancers. Although their role in cancer prevention is not completely non-controversial, both vitamin A and β -carotene have been found to be effective in various forms of cancer. Despite some contradictory findings, it has been tried here to elucidate the possible cancer-preventive mechanism of β -carotene and vitamin A. Cancer preventive potentials of the compounds have been compared to establish their significances in human health. Nevertheless we conclude that more investigations are required to yield important information and strategies with a large public health impact to bring them into suitable chemopreventive programs.

Keywords: vitamin A, beta-carotene, anticancer agent, chemotherapeutic agent

Introduction

Although it has been known for decades that tumor incidence is affected by nutritional manipulation with various micronutrients, the modulation of cancer with micronutrients is the subject of recent interest among nutritionists and many cancer scientists. Micronutrients like vitamins, carotenoids, flavonoids, trace elements etc. have been found to reduce cancer risk and cancer incidences. However, because of the brevity of this article we remain little selective to discuss the roles of vitamin A and beta-carotene among the various micronutrients, in controlling the risk and incidences of various forms of cancers both in humans and animals. More importantly, it is addressed here to the problem whether or not abatement of cancer is possible with the treatment of these micronutrients. Precisely, the effects of vitamin A and beta-carotene in cancer are briefly summarized, updated and analyzed here to provide an overview of the development in this field as cancer modulation with nutritional variation is a

subject of much interest as well as controversy.

Vitamin A as an anticancer agent – supportive and non-supportive findings along with its toxic aspects

Many animal experiments and some clinical studies indicate that vitamin A (retinol) is effective in preventing or suppressing various forms of cancer namely liver cancer, head and neck cancer, oral cancer, lung cancer etc. Toxicity has been a major concern with vitamin A therapy as a prolonged administration is required in chemoprevention studies during the treatment of cancer. Infante *et al* (1991)¹ reported their laboratory findings in patients receiving high dose of vitamin A as an adjunct for treatment of stage I lung cancer. They studied in a group of 263 patients randomized to either treatment with retinyl palmitate or standard follow up and they observed that there were some inductions in serum gamma glutamyl transferase (GGT) and triglycerides levels in some patients with no other laboratory evidence toxicity

attributable to vitamin A. Finally, they concluded that 3,00,000 IU/ day of retinyl palmitate can be administered as a possible chemotherapeutic agent with reasonable safety for up to two years.

Reports suggest that vitamin A plays a role in the etiology of second tumors in head and neck cancer^{2,3,4,5}. A metabolic product of vitamin A (13'-des-cis- retinoic acid) has been shown to induce protective effect in the development of secondary cancer in patients with epidermoid cancer of buccal cavity, the pharynx or the larynx^{6,7,8} although no effect has been observed on primary cancer⁹. Again, the therapeutic response of vitamin A against advanced cancer is disappointing. Vitamin A has not been consistently effective in bronchial atypia or metaplasia¹⁰. It was showed that vitamin A level was significantly decreased in the hepatocellular carcinoma^{11,12,13}. Vitamin A supplementation was found to reduce cancer incidences in both male and female patients. When vitamin A supplementation was given in both male and female patients higher level of vitamin A during lung cancer and lower level during oral cancer in male patients as compared to their female counterparts was observed in the study. This might be due to the variable Zn levels in males and females, as Zn is already known to play role in absorption of vitamin A^{14, 15,16}. Mobilization of retinol binding protein (RBP)¹⁷, the plasma transport protein of retinol, takes place in liver after the stimulation of liver store of RBP by vitamin A (retinol). Due to the metabolic variation in those patients a deficiency of vitamin A may take place and RBP secretion is specifically inhibited and further availability of retinol becomes less. Suphakaran *et al*¹⁸ showed that vitamin A deficient rats are susceptible to colon cancer¹⁹. Vitamin A deficiency had no effect on the salivary gland carcinogenesis, enhanced urinary bladder, lung, and liver

and colon carcinogenesis. Vitamin A deficiency was found to cause squamous metaplasia²⁰⁻²⁵. But Nauss *et al*²⁶ reported that there was no indication that either vitamin A deficiency or supplementations influence the formation of preneoplastic lesions on methylbenzyl nitrosamine-induced oesophageal carcinogenesis in Sprague-Dawley rats²⁷. Excess of vitamin A inhibited mammary carcinogenesis in rats (but not in mice), as well as carcinogenesis in fore stomach, liver and urinary bladder. Astorg *et al* (28) reported that an excess of dietary vitamin A (70,000 IU/ kg) diet had no influence on the number and sizes of preneoplastic foci during rat hepatocarcinogenesis initiated with diethylnitrosamine (200 mg/kg) and promoted with 2-acetylaminofluorine, phenobarbital and 2/3rd partial hepatectomy. Maiorana and Gullino observed that there was no significant incidence of mammary carcinoma between control and retinyl acetate fed mice²⁹. Though there was some activity of vitamin A in decreasing lesion accounts in actinic keratoses, there was no reduction of occurrence of recurrent lesions in vitamin A treated skin cancer patients.

Ferozi *et al*³⁰ studied the action of vitamin A on the formation of DNA adducts by aflatoxin B-1 in *in-vitro* reaction catalyzed by rat liver microsomes. Retinol, retinal, all-trans-retinoic acid and 2-retinyl esters were found to inhibit the DNA adduct formation in a dose dependant manner. All-trans-retinol has been found to inhibit formation of aflatoxin B-1 DNA adduct in a dose dependent manner throughout a concentration range of 34-122 micromole by 40 to 80%. Low vitamin A levels can be of results not only of low dietary intake but also of interference of vitamin A metabolism by agents like ethanol, carbon tetra chloride and similar compounds, which are claimed to be risk factors for development of liver fibrosis³¹.

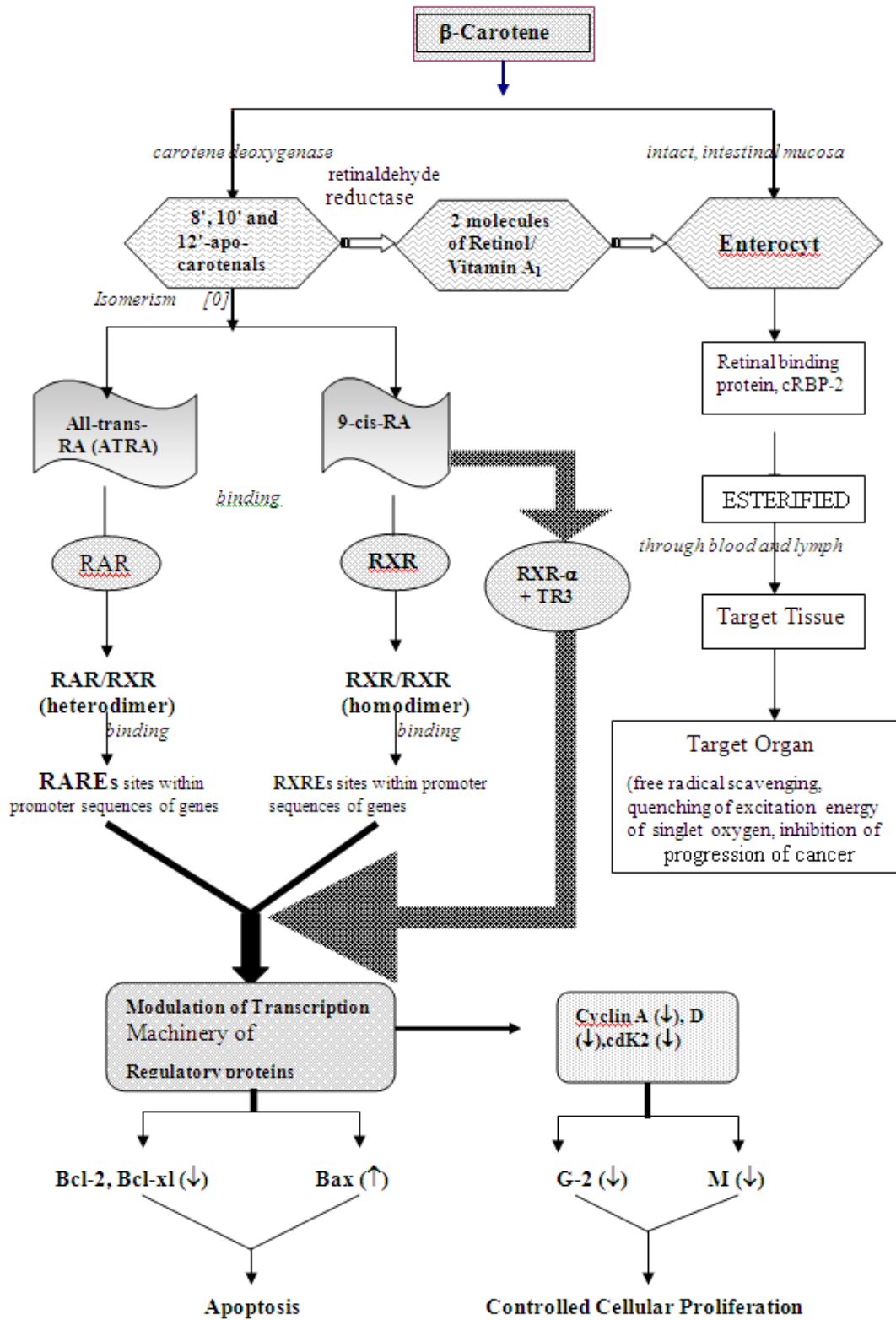


Figure 1: Mode of anticancer action of vitamin A and beta-carotene

Metabolic aspects of vitamin A

All-trans-retinoic acids, 13-cis-retinoic acids are the vitamin A derivatives commonly used in the treatment of cancer (32). Patients taking these compounds often show side effects resembling the symptoms of hypovitaminosis A, namely, night blindness and decreased plasma retinol levels. As dietary vitamin A deficiency is not suspected, interference with normal vitamin A metabolism seems to be the cause of it. At micro-molar concentrations, all these retinoic acids were found to inhibit intestinal lecithin:retinol acyltransferase and to a lesser extent hepatic lecithin:retinol acyltransferase and intestinal retinal reductase³³⁻³⁷. The ability of these molecules to inhibit retinal reduction and retinol esterification suggests ability to interfere with normal vitamin A metabolism, particularly during absorption³⁸.

Possible mode of anticancer action by vitamin A

Gad (1994) (39) examined the influence of vitamin A on detoxification enzymes like non-specific carboxylesterase and glutathion S-transferase and he concluded that vitamin A has a potential role in the regulation of detoxification enzymes⁴⁰. Role of vitamin A on the biotransformation may be critical in delaying or inhibiting the cancerous process. Vitamin A status was found to play role in regulating GSHT activity in hepatic cytosolic fraction of rats treated with aflatoxin B-1³⁸. Activity of GSHT was low in vitamin A deficient rats and increased progressively with increasing supplementation of vitamin A³⁸.

Hauswirth and Brizuela showed in their study that carcinogens like methylcholanthrene, 2-acetylaminofluorine had a higher hepatic microsomal cytochrome P-450 level in vitamin A deficient rats⁴¹. Thus vitamin A and its metabolites may play a crucial role in metabolizing various carcinogens to less harmful chemicals and they, thereby, provide some anticarcinogenic effects.

Moreover, vitamin A is known to be a potent inducer of apoptosis⁴².

Beta-carotene undergoes metabolism to retinol (vit. A₁), which is required for normal cell differentiation of stem cells in epithelial tissue⁴³. This molecule is reported to protect various forms of cancer including epithelial cancer of skin, gastric cancer cells, lung, bladder, breast, etc.⁴⁴. Beta-carotene in the intestinal mucosa converts into 8', 10' and 12'-apo-carotenals by asymmetric cleavage with the enzyme carotene deoxygenase⁴⁵. This is then oxidized into two isomers of retinoic acid. Two major active metabolites all-trans-retinoic acid and 9-cis-retinoic acid have high affinity for ligands to the protein receptor RARs and 9-cis-retinoic acids also possess high affinity for the RXRs⁴³. These two receptors proteins form homodimer (RXR/RXR) and heterodimer (RXR/RAR)⁴⁴. They function as retinoic acid inducible transcriptional regulatory protein and bind to DNA sequences probably within the promoter region of genes to control the gene function. The parts of these sequences are commonly known as retinoic acid response element (RARE) and retinoid X response element (RXRE)^{45,47}. Thus they play an important role in the transcriptional control of various genes by causing DNA to be inaccessible to the transcriptional machinery. The abnormal transcriptional regulation of the retinoic receptors (RARs) α , β and γ results in various pathophysiological conditions including cancer. Loss of normal RAR function in the presence of physiological levels of retinoic acid is reported to be associated with various forms of cancer. Translocation involving RAR- α gene is reported to be a hallmark of acute promyelocytic leukaemia^{47,49}. Again a loss in RAR- β expression occurs in a variety of premalignant and malignant lesions^{50,51,52}. Various transcriptional factors like COUP-TF and two orphan receptors have been reported to regulate RAR- β expression in malignant cells^{53,54} (Figure 1). The orphan receptor TR3 is deeply involved in the regulatory

process of ATRA. It has been observed that TR3/RXR- α heterodimer formation in the nucleus⁵⁵ and subsequent translocation in the cytoplasm down regulate antiapoptotic protein like Bcl-2 and Bcl-xl^{56,57} and induces apoptotic protein Bax that results in ATRA induced apoptosis in the cancerous cell⁵⁸. It was also found that ATRA, 9-cis-retinoic acid reduce the expression of apoptotic proteins and cell cycle protein cyclin A, D1, cdk4 and cdk2 which causes large growth inhibition by slow down G₂ and M phases of cell division⁵⁹⁻⁶¹.

Although controversies regarding the effectiveness of vitamin A against various forms of cancers exist, the findings are suggestive of a potential role of vitamin A against chemical induced carcinogenesis. Evidences in human and laboratory animals support cancer protective effect of vitamin A. Further research should be conducted to elucidate the exact mechanism of vitamin A to provide more insight of its cancer protective effect.

Beta-carotene as an anticancer agent-supportive and non-supportive findings along with its toxic aspects

The discovery of beta-carotene in the later 1970s as a possible anticancer agent opened a new avenue in the field of cancer chemoprevention. Deficiency of beta-carotene along with other antioxidants associated with some cancers suggests that low antioxidant activity in diet might allow neoplastic changes to continue. There are several reports that diets rich in vegetable and fruits can provide some protection against a number of cancers, not only of the digestive tract but also of respiratory, genital, and other forms of cancers. Role of beta-carotene, a well-known carotenoid, as a possible cancer protective agent has been claimed by many workers and an apparent primary mechanism of effect as an antioxidant has been suggested⁶². Although carotenoids do not have hormone like properties of retinol, they do have potent antioxidant effect and could thus reduce cancer risk by preventing oxidative tissue damage. The population of Linxian, China,

has been known to have one of the highest rates of oesophageal / gastric cardia cancer in the world and recent intervention studies from the particular geographic area have indicated that supplementation with beta-carotene, vitamin E and selenium reduces cancer risk (63,64). A French group has demonstrated that low serum beta-carotene level is a risk factor in various forms of cancer. The cancer sites that were associated with serum beta-carotene level in this epidemiological study were leukemia, lymphoma, central nervous system, bone and renal tumors. On the other hand, Finish alpha-tocopherol- beta-carotene cancer prevention trial (ATBC) resulted in a higher lung cancer risk in long-term smokers than in the control group. It could be possible that those long-term smokers had developed an irreversible damage in the mechanistic pathway of beta-carotene to provide chemoprevention. Further, cytotoxic and genotoxic effects of the beta-carotene breakdown products may be responsible for the occurrence of carcinogenic effects found in the ATBC and CARET trials⁶⁵. Intervention studies are of course, needed to test this hypothesis. The paradoxical effect of beta-carotene on smoke induced lung cancer from animal studies has shown to be due to the production of a number of transient metabolites including P450 enzymes that result in destruction of retinoic acid, diminished retinoid signaling and enhanced cell proliferation. In addition to this, beta-carotene is eccentrically cleaved into metabolites that facilitate the binding of smoke derived carcinogens to DNA⁶⁶. Beta-carotene has been reported to provide anti-carcinogenic action during the development of hepatocarcinogenesis in rats⁶⁷. It has been found to be most effective in long-term study when beta-carotene was administered for a prolonged period of time as compared to during initiation or promotional stages of 2-acetylaminofluorine-induced hepatocarcinogenesis. Again beta-carotene has been reported to be more effective in initiation phase than in promotional phase of carcinogenesis when beta-carotene

administration (during these two phases) were compared. It probably indicates that beta-carotene may be better suited as a prophylactic agent than as a chemopreventive one. This idea may be supported with the findings of an Italian study group who demonstrated significant residual protective effect of beta-carotene in gastric cancer risk. There are reports, which stated that beta-carotene supplementation leads to markedly decreased serum concentration of beta-carotene and tocopherol. Goodman *et al*⁶⁸ reported the effectiveness of beta-carotene supplementation on serum concentration of alpha-tocopherol in 2319 participants enrolled in carotene and retinol efficacy trial. The participants received beta-carotene (30 mg/day) and vitamin A (25,000 IU/day) for up to 6 years. They concluded for their findings that long-term supplementation with this combination of β -carotene and vitamin A does not decrease serum concentration of A-tocopherol and low levels of β -carotene have been demonstrated in various forms of cancer. The preneoplastic lesions induced by diethylnitrosamine (DENa) in resistant hepatocytes model in rat were found to decrease to a statistically significant level by beta-carotene⁶⁹. Again, carrot feeding resulted in a significant delay in hepatoma formation in DENa treated rats. In contrast, no discernible effects on the growth of rats with Morris transplantable hepatoma were observed following dietary administration with beta-carotene for 6 weeks. Administration of large dose of synthetic beta carotene had no effect on mesothelioma and lung cancer in human subject⁷⁰.

Possible anticancer mechanism of action by beta-carotene

Beta-carotene is one of the most efficient substances known for quenching the excitation energy of singlet oxygen and for trapping certain organic free radicals⁷¹⁻⁷⁵. The properties of beta carotene on enhancing gap junctional communication and inhibitory lipid peroxidation in chemically induced neoplastic

transformation in 10T1/2 cells and as a chain breaking anti-oxidant in the lipid phase by neutralizing peroxy radicals have obvious implications for controlling cancer growth (Figure 1). The free radical scavenging nature of beta-carotene and its immediate involvement in trapping singlet oxygen providing an overall increased reducing environment in the hepatic tissues entails the anticancer potential of long-term exposure to beta-carotene.

Metabolic aspects of beta-carotene

The enzymatic cleavage of β -carotene has been recently established to be a central cleavage and to produce retinal through action of the enzyme β -carotene 15, 15'-dioxygenase in the small intestine⁷⁶. β -carotene is not efficiently metabolized to vitamin A (retinol) and it is mostly absorbed intact along with the metabolized parts. In the intestinal mucosa, retinal is reduced to vitamin A (retinol) by retinaldehyde reductase and free retinol is taken up by the enterocytes, perhaps involving both diffusion and protein-mediated facilitated transport. In cells, retinol is complexed with cellular retinol-binding protein type-II and this complex is believed to help re-esterification process of retinol by the enzyme retinol acyltransferase and free retinol is also esterified by acyl-CoA and acyltransferase and these esters are then incorporated into chylomicrons and finally secreted into the lymph^{77,78} and transported to the target tissues (Figure 1). It may be suggested that the different metabolic patterns of vitamin A and β -carotene play significant roles in cancer modulation by these compounds at variable degrees.

Beta-carotene and vitamin A – a comparison

Beta-carotene even when administered in high doses for long periods of time does not cause toxicity^{79,80} on the other hand, high doses of vitamin A and retinoids, if used as possible prophylactic agents for cancer prevention could lead to acute hepatotoxicity and produce other adverse effects. Amongst more than 600 different

carotenoids so far discovered, only 10% of them are metabolized to vitamin A and beta carotenes, a common constituent of carrots, green vegetables and fruits appears to be a powerful anti-oxidant acting as a "provitamin A" ^{81,82}. Dietary administration of beta-carotene has been found to reduce incidences, appearance, numbers and sizes of visible hyperplastic hepatic neoplastic nodules in experimental animals. Certain drugs stimulate hepatic metabolizing enzymes and reduce vitamin A levels. Beta-carotene-induced vitamin A depletion was observed when rats were fed basal diet with more than their vitamin A requirements as active vitamin A. This depletion was prevented by beta-carotene. This suggests that beta-carotene has metabolic advantages over active vitamin A. Such an effect of beta-carotene may help to explain the epidemiological findings that the reduced risk of lung cancer appears to be stronger with beta-carotene than with active vitamin A ⁸³.

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Conclusions

In spite of some contradictory findings, it may be stated at the end that cancer-preventive potentials of beta-carotene and vitamin A suggest a promising area of chemoprevention in reducing the risk of cancer in humans. But more investigations are required to yield important information and strategies with a large public health impact. The micronutrients like vitamin A and beta carotene individually or their metabolites or with a combination of other compounds prevent, delay or even reverse the process of development of various forms of cancer and thus reduces the incidence of and mortality due to this dreadful disease. Again when the two types of these compounds are compared, beta-carotene seems to be more advantageous and promising in terms of its anticancer potential than vitamin A. More clear understanding of the relevant biological and biochemical basis of these micronutrients is needed to bring them into suitable chemopreventive programs.

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