ANTIOXIDANT VITAMINS AND CHEMOPREVENTION

Harbans Lal, Rajesh Pandey and S.K. Aggarwal

Department of Biochemistry, Pt. B.D. Sharma Postgraduate Institute of Medical Sciences, Rohtak (Haryana)

ABSTRACT

Free radicals play an important role in human carcinogenesis and the mechanism of their counteraction by antioxidant vitamins has been reviewed. It seems more likely that these vitamins work in concert rather than acting singly. Currently available data are compatible with the notion that these vitamins act as chemopreventives against some important cancers, e.g. carotenoids for lung cancer, ascorbic acid for salivary gland cancer, tocopherols for head and neck cancers etc. Thus, a greater consumption of fruits and vegetables should be encouraged as they are the natural sources of these chemopreventive antioxidants along with other protective factors packaged by nature. However, much work still remains to be done to establish the role of antioxidant vitamins in carcinogenesis.

KEY WORDS: Carcinogenesis, chemoprevention, antioxidant vitamins, carotenoids, vitamin C, vitamin E.

A number of factors such as chemical carcinogens, environmental pollutants, viruses and radiation, either singly and / or in combination, result in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which have been proposed to play a role in carcinogenesis (1,2).

The ROS is a collective term for oxygen free radicals [i.e. superoxide ($O_2^{\cdot-}$), hydroxyl ($OH^{\cdot}$), peroxy ($ROO^{\cdot}$) and alkoxyl ($RO^{\cdot}$)] as well as for some non-radicals [hypochlorous acid (HOCl), ozone (O_3), peroxyxynitrite ($ONOO^{\cdot}$), singlet oxygen (1O_2) and hydrogen peroxide (H_2O_2)] which are either oxidizing agents and / or are

converted into radicals. The RNS include nitric oxide ($NO^{\cdot}$) and nitrogen dioxide ($NO_2^{\cdot}$) radicals, $ONOO^{\cdot}$ and the reaction products of $NO^{\cdot}$ with ROS. The reaction of a radical with a non-radical produces another free radical, which can participate in chain reaction and cause damage to cellular macromolecules such as lipids, proteins and nucleic acids. Production of malondialdehyde (MDA) by lipid peroxidation, DNA base changes etc. are indicative of such damage (1). With the involvement of the vital signaling pathways regulating cell replication and differentiation, cell growth becomes more and more uncontrolled. Through the sequential stages of initiation, promotion and progression, ultimately, the cancer cell is formed (2).

Address for correspondence :

Dr. Harbans Lal

15/8 FM, Medical Enclave, Rohtak - 124001

Defensive mechanisms against oxidative stress may be extracellular as well as intracellular. Extracellular components include vitamin C, bilirubin, urate etc. (3). Intracellular antioxidants include both enzymatic as well as non-enzymatic components. Superoxide dismutase (SOD) which scavenges $O_2^{\cdot-}$ and catalase which detoxifies H_2O_2 are amongst the important enzymes present intracellularly; glutathione, vitamin E and carotenoids are some of the non-enzymatic intracellular antioxidants (3).

Chemoprevention

The strategy of prevention is a much more satisfying approach than therapeutic intervention, although the two are not mutually exclusive. Chemoprevention is defined as the introduction of selected natural as well as synthetic substances into the diet for the purpose of reducing cancer incidence, though natural supplements are superior to synthetic forms (4,5). The usefulness of the supplementation of certain micronutrients as chemopreventives has been confirmed experimentally. It has been shown that there is a significant inverse correlation between mutagen sensitivity scores (assessed by bleomycin-induced chromosomal breaks in cultured peripheral blood lymphocytes) and plasma levels of α -carotene, total carotenoids and ascorbic acid. Also, a significant inverse association has been shown to exist between monthly mean plasma levels of carotenoids and monthly mean chromosomal breaks. Therefore, mutagen sensitivity may be influenced by plasma levels of certain nutrients and could potentially be modified by dietary interventions and / or micronutrient supplementation (6,7). An inverse relationship has also been found between red

blood cell polyamine levels and plasma retinol, lutein, lycopene, β -cryptoxanthin, total carotenoids and α -tocopherol and it has been suggested that this may be used as a biomarker in cancer prevention trials (7).

Antioxidant vitamins

As discussed above, several micronutrients especially various vitamins and / or their precursors may have an important role in chemoprevention. Vitamin A and carotenoids, vitamin E and vitamin C have been defined as antioxidants and the natural dietary inhibitors of mutagenesis and carcinogenesis (8). They can inhibit the formation of ROS/RNS, increase their detoxification and also interfere with the process of multistep carcinogenesis (Fig.1). Although various other vitamins such as B1, B2, B3, B6, B12, niacin, folate and vitamin D may act as chemopreventives, no antioxidant function has been assigned to them, yet (9,10).

Carotenoids are lipophilic compounds that can inhibit free radical reactions, may be by reducing $ROO^{\cdot}$ (11). They also contribute towards immunoenhancement by limiting oxidative membrane damage and altering membrane structure or integrity (12). Among the biologically occurring carotenoids, lycopene (11) is the most efficient quencher of 1O_2 . However, it is β -carotene which is the most prominent and extensively studied. Though β -carotene is not strictly a vitamin, it is commonly included in the "antioxidant vitamin group" (13). At low partial pressures of oxygen (pO_2) found in physiological environments, it has the potential for acting as a lipid-soluble chain-breaking antioxidant, complementing the role of vitamin E which is

Fig. 1: Role of Antioxidant Vitamins (+) against Free Radical Induced Carcinogenesis

effective at high pO_2 . Long-term β -carotene supplementation increases the levels of serum non- β -carotene carotenoids, suggesting a possible biologic interaction between β -carotene and other carotenoids (14).

Vitamin E includes different compounds, basically tocopherols and tocotrienols, of which RRR- α -tocopherol is the most important, *in vivo* (3). Its reactivity with organic peroxy radicals accounts for its antioxidant activity and may also include quenching of 1O_2 (15).

Vitamin C is the most important antioxidant in extracellular fluids and can efficiently scavenge various oxygen-derived free radicals (11). Besides, it also spares other important antioxidants and may protect against membrane

damage by increasing the activity of tocopherol (16). It also guards against oxidative DNA damage in human sperms (11).

The action of the above antioxidant vitamins however, may not be independent of each other. Niki and associates (3) have reported that the cooperative interaction between vitamin C and vitamin E may be quite probable, that between vitamin E and β -carotene may be possible, whereas that of vitamin C and β -carotene is improbable. A possible interaction between the antioxidant vitamins is shown in Fig. 2.

These vitamins may also have other biological activities, which are related to their cancer preventive properties such as immune stimulation, inhibition of nitrosamine formation,

FIG.2: INTERACTION OF ANTIOXIDANT VITAMINS IN THE DETOXIFICATION OF THE PEROXYL RADICAL (ROO•)

enhancement of cell to cell communication and an influence on the metabolic activation of carcinogens (17).

Role of antioxidant vitamins in various types of cancer

Several epidemiological and experimental studies have established a protective effect of the increased consumption of vegetables and fruits (rich in antioxidant vitamins) in various types of cancer. Vegetarians have been shown to have significantly higher levels of plasma vitamin A, β -carotene, vitamin C and molar ratio of vitamin E / total lipids (indicating better protection of polyunsaturated fatty acids against peroxidation) confirming the favourable effects of vegetarian nutritional habits (18).

Lung cancer: Lung cancer has been shown to be associated with low mean plasma carotene levels. Moreover, significantly increased mortality risk from lung cancer has also been shown to be associated with low plasma levels of carotene, retinol, vitamin C and vitamin E (19). Carotenoids are protective against various histological types of lung cancer but chiefly against squamous cell carcinoma (20). Retinoids may cause regression of bronchial metaplasia, a potentially premalignant lesion (2).

On the other hand, β -carotene supplementation with or without vitamin A or E has been shown to result in an increased risk of lung cancer in smokers (11, 21). To explain these discrepancies the following suggestions have been made (21) — (i) in smokers pro-oxidant activities of β -carotene prevail which leads to enhanced tumor progression; (ii) the absorption, bioavailability and distribution of β -carotene is

different when given as a single compound than as a vegetable or fruit; and (iii) additional carotenoids and other protective plant components act together, ensuring a beneficial effect which cannot be simulated by intervention with supplements.

Thus, although there may be no additional protective effect of antioxidant vitamin supplementation beyond that provided through dietary intake, daily consumption of a variety of fruits and vegetables may offer the best dietary protection against lung cancer (19).

Head and neck cancer: Cancers of the head and neck region usually refer to tumors of the larynx, cervical trachea, hypo - and oropharynx, nose and sinuses, ear, lips, oral cavity, salivary glands and thyroid gland. Both etiologically and behaviourally, these lesions are different and that accounts for their variable prognosis, e.g. the increasing amount as well as duration of tobacco in the form of smoking, chewing or snuff is an important risk factor for cancer of the oral cavity than the other parts in the head and neck region (22).

Plasma vitamin E levels have been found to be reduced in patients of head and neck cancer irrespective of the site, TNM stage, histopathology or character of lesion (23). Further, vitamin E and β -carotene have been stated to protect against second primary cancers often occurring in these patients after successful treatment (24).

Serum carotenoids, retinol and α -tocopherol are decreased in men with laryngeal cancers. Following enteral formula feeding in such patients, carotenoid levels are decreased

whereas retinol and tocopherol levels are increased. Thus, monitoring of serum retinol and carotenoid levels in the patients with laryngeal cancer and addition of carotenoids to enteral formulas may prevent oxidative damage (25).

Higher intake of vitamin C has been found to be associated with lower risk of salivary gland cancer (26). Some protection by vitamin C and vitamin E against thyroid cancer is also suggested. However, β -carotene has been shown to have an inverse association with thyroid cancer (27).

Several phenolics of the chewed plant products can generate free radicals deleterious to the oral mucosa (28). Serum vitamin A, β -carotene and vitamin C are significantly reduced in oral leukoplakia (an oral precancerous condition) (29). Further, β -carotene and several retinoids can induce their regression (28). A beneficial clinical response has also been observed in subjects with oral submucous fibrosis (another oral premalignant lesion) to multiple micronutrient interventions including the antioxidant vitamins (30).

Gastric cancer: Higher intakes of β -carotene, vitamin C and vitamin E have been found to lower the incidence as well as mortality of stomach cancer. Mixtures of retinol / zinc and α -carotene / α -tocopherol / selenium have also been shown to be protective (31). It has been suggested that in advanced gastric cancer, antioxidant vitamins along with the standard chemotherapeutic regimes should be encouraged as they have been shown to be well tolerated and also prolong patients' survival (32).

Colorectal cancer: It is reported that some types of colorectal cancer do reflect mutations in genes involved in DNA mismatch repair and ROS / RNS may accelerate such changes (1). It has been demonstrated that colorectal epithelial cell proliferation rates are lower in patients with higher intakes of vegetables and fruits and in those who take vitamin /mineral supplements (33). For the structural integrity and functioning of the epithelium, tocopherols, retinol and carotenoids may be essential. Colon cancer patients undergo a significant decrease in their antioxidant reserves as shown by decreased tissue carotenoid levels in sigmoid colon cancer subjects compared to healthy subjects or subjects with polyps (34). Following β -carotene supplementation, significant increases in retinol, α -carotene and β -carotene levels in plasma have been reported. Further, β -carotene has been shown to accumulate in human colonic neoplastic tissue and may be utilized to augment cytotoxicity of chemotherapeutics or to prevent malignant transformation of cells (35). Antioxidant vitamins may protect against colon cancer by inducing specific detoxifying enzymes like NAD(P)H: quinone reductase, which may also serve as the suitable biomarkers (36).

No effect on the growth of adenomatous polyps has been shown by antioxidant vitamin supplementation although β -carotene may provide some protection (37). Antioxidant vitamins may, however, have a protective role on new adenoma formation and on the recurrence rate of adenomatous polyps (37, 38).

Breast cancer: Studies suggest that low plasma β -carotene levels and lower breast adipose concentrations of retinoids and some carotenoids

are associated with increased risk of breast cancer (39). Mean plasma vitamin E levels have also been found to be reduced in breast cancer, the decrease being directly related with the TNM stage of the cancer (40). Dorgan and associates (41) have reported that a few non- β -carotene carotenoids may be protective. Others, however, deny the protective role of any antioxidant vitamin against breast cancer (42). The conflicting results in relation to antioxidant vitamins and breast cancer could be due to the reason that breast cancer is known to be more closely associated with dietary fat intake (43).

Cervical cancer: Maintaining an adequate plasma and tissue concentration of β -carotene may be necessary for the prevention of cervical cancer and precancer. It has been stated that not all plasma micronutrient levels reflect their concentration in cervical tissue and it may be necessary in some cases to measure their tissue concentrations to define their role in cervical carcinogenesis (44).

Studies have shown significantly increased risk of cervical dysplasia to be associated with low dietary intakes and / or low blood levels of β -carotene and vitamin C (12). Mean plasma β -carotene levels are raised by β -carotene supplementation in non-smoker cervical dysplasia patients (45).

Liver cancer: Studies have suggested that β -carotene and α -tocopherol may be protective against hepatic carcinogenesis (46, 47). Liver and tumor tissue of patients with malignant hepatic neoplasms contain lower levels of SOD and catalase and higher levels of MDA. Preoperative treatment with α -tocopherol, retinol and ascorbic acid has been shown to significantly reduce hepatic MDA levels and increase catalase activity. Further, purulent and septic complications were less frequent than in controls. Thus, such antioxidant treatment may improve the effectiveness of liver cancer therapy (48).

Cancer in children: It has been found that age and sex-adjusted serum levels of retinol, β -carotene and α -tocopherol are inversely associated with childhood malignancies (49). Following treatment for leukemia, retinol and selenium levels are increased. Recently, it has been demonstrated that taking prenatal antioxidant vitamin supplements as well as folate throughout pregnancy may prevent brain tumors in offsprings (50).

The role of antioxidant vitamins in the chemoprevention of other cancers such as prostate (31), skin (51), bladder (31), ovary (52) etc. though has been studied, not enough information is available to draw definite conclusions.

REFERENCES

1. Wiseman, H. and Halliwell, B. (1996) Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem. J.* 313, 17-29.
2. Singh, V.N. and Gaby, S.K. (1991) Premalignant lesions: role of antioxidant vitamins and beta-carotene in risk reduction and prevention of malignant transformation. *Am. J. Clin. Nutr.* 53 (Suppl), 386S-390S.

3. Niki, E., Noguchi, N., Tsuchihashi, H. and Gotoh, N. (1995) Interaction among vitamin C, vitamin E and beta-carotene. *Am. J. Clin. Nutr.* 62 (Suppl), 1322S-1326S.
4. Malone, W.F. (1991) Studies evaluating antioxidants and beta-carotene as chemopreventives. *Am. J. Clin. Nutr.* 53 (Suppl), 305S-313S.
5. Burton, G.W., Traber, M.G., Acuff, R.V., Walters, D.N., Kayden, H., Hughes, L., and Ingold, K.U. (1998) Human plasma and tissue alpha-tocopherol concentrations in response to supplementation with deuterated natural and synthetic vitamin E. *Am. J. Clin. Nutr.* 67, 669-684.
6. Kucuk, O., Pung, A., Franke, A.A., Custer, L.J., Welkens, L.R., Le Marchand, L., Higuchi, C.M., Coonev, R.V. and Hsu, T.C. (1995). Correlations between mutagen sensitivity and plasma nutrient levels of healthy individuals. *Cancer Epidemiol. Biomarkers Prev.* 4, 217-221.
7. Wang, W., Kucuk, O., Franke, A.A., Liv, L.Q., Custer, L. J. and Higuchi, C.M. (1996) Reproducibility of erythrocyte polyamine measurements and correlation with plasma micronutrients in an antioxidant vitamin intervention study. *J. Cell. Biochem.* 62, 19-26.
8. Odin, A.P. (1997) Vitamins as antimutagens: advantages and some possible mechanisms of antimutagenic action. *Mutat. Res.* 386, 39-67.
9. Slattery, M.L., Schaffer, D., Edwards, S.L., Ma, K.N. and Potter, J.D. (1997) Are dietary factors involved in DNA methylation associated with colon cancer? *Nutr. Cancer* 28, 52-62.
10. Slattery, M.L., Potter, J.D., Coates, A., Ma, K.N., Berry, T.D., Duncan, D.M. and Caan, B.J. (1997) Plant foods and colon cancer: an assessment of specific foods and their related nutrients (United States). *Cancer Causes Control* 8, 575-590.
11. Sies, H. and Stahl, W. (1995) Effects of carotenoids in cellular and animal systems. *Am. J. Clin. Nutr.* 62 (Suppl), 1315S-1321S.
12. Krinsky, N.I. (1991) Effects of carotenoids in cellular and animal systems. *Am. J. Clin. Nutr.* 53 (Suppl), 238S-246S.
13. Frei, B. (1994) Reactive oxygen species and antioxidant vitamins: mechanisms of action. *Am. J. Med.* 97 (Suppl), 5S-13S.
14. Albanes, D., Virtamo, J., Taylor, P.R., Ruatalahti, Pietinen, P. and Heinonen, O.P. (1997) Effects of supplemental beta-carotene, cigarette smoking and alcohol consumption on serum carotenoids in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Am. J. Clin. Nutr.* 66, 366-372.
15. Kaiser, S., Di Mascio, P., Murphy, M.E. and Sies, H. (1990) Physical and chemical scavenging of singlet molecular oxygen by tocopherols. *Arch. Biochem. Biophys.* 227, 101-108.
16. Chan, A. C. (1993) Partners in defense, vitamin E and vitamin C. *Can. J. Physiol. Pharmacol.* 71, 725-731.

17. Van Poppel, G. and van den Berg, H. (1997) Vitamins and cancer. *Cancer Lett.* 114, 195-202.
18. Krajcovicova-Kudlackova, M., Simoncic, R., Babinska, K., Bederova, A., Brtkova, A., Magalova, T. and Grancicova, E. (1995). Selected vitamins and trace elements in blood of vegetarians. *Ann. Nutr. Metab.* 39. 334-339.
19. Yong, L.C., Brown, C.C., Schatzkin, A., Dresser, C.M., Slesinski, M.J., Cox, C.S. and Taylor, P.R. (1997) Intake of vitamins E, C, and A and risk of lung cancer. The NHANESI epidemiologic follow up study. First National Health and Nutrition Examination Survey. *Am. J. Epidemiol.* 146, 231-243.
20. Ziegler, R.G. (1991) Vegetables, fruits, and carotenoids and the risk of cancer. *Am. J. Clin. Nutr.* 53 (Suppl), 251S-259S.
21. Pool-Zobel, B.L., Bub, A., Mullen, H., Wollowski, I. and Rechkemmer, G. (1997) Consumption of vegetables reduces genetic damage in humans: first results of a human intervention trial with carotenoid-rich foods. *Carcinogenesis* 18, 1847-1850.
22. Lal, H. (1994) Biochemical studies in head and neck cancer. *Clin. Biochem*, 27, 235-243.
23. Lal, G., Yadav, S.P.S., Aggarwal, S.K., Bansal, S., Chugh, K. and Lal, H. (1996) Plasma vitamin E status in head and neck cancers. *Ind. J. Clin. Biochem.* 11, 46-48.
24. Bairati, I., Brochet, F., Roy, J., Gelinas, M., Nabid, A., Tetu, B., Masse, B. and Meyer, F. (1996) Prevention of second primary cancer with vitamin supplementation in patients treated for head and neck cancers. *Bull. Cancer Radiother.* 83, 12-16.
25. Olmedilla, B., Granado, F., Blanco, I. and Rojas-Hidalgo, E. (1996) Evaluation of retinol, alpha-tocopherol, and carotenoids in serum of men with cancer of the larynx before and after commercial enteral formula feeding. *J. Parenter. Enteral Nutr.* 20, 145-149.
26. Horn-Ross, P.L., Morrow, M. and Ljung B.M. (1997) Diet and the risk of salivary gland cancer. *Am. J. Epidemiol.* 146, 171-176.
27. D'Avanzo, B., Ron, E., LaVecchia, C., Franceschi, S., Negri, E. and Ziegler, R. (1997) Selected micronutrient intake and thyroid carcinoma risk. *Cancer* 79, 2186-2192.
28. Stich, H.F., Mathew, B., Sankaranarayanan, R. and Nair, M.K. (1991) Remission of precancerous lesions in the oral cavity of tobacco chewers and maintenance of the protective effect of beta-carotene or vitamin A. *Am. J. Clin. Nutr.* 53 (Suppl), 298S-304S.
29. Ramaswamy, G., Rao, V.R., Kumaraswamy, S.V. and Anantha. N. (1996) Serum vitamins' status in oral leucoplakias- a preliminary study. *Eur. J. Cancer B. Oral Oncol.* 32, 102-122.
30. Maher, R., Aga, P., Johnson, N.W., Sankaranarayanan, R. and Warnakulasuriya, S. (1997) Evaluation of multiple micronutrient supplementation in the management of oral submucous fibrosis in Karachi, Pakistan. *Nutr. Cancer* 27, 41-47.

31. Patterson, R.E., White, E., Kristal, A.R., Neuhouser, M.L. and Potter, J.D. (1997) Vitamin supplements and cancer risk: the epidemiologic evidence. *Cancer Causes Control* 8, 786-802.
32. Pyrhonen, S., Kuitunen, T., Nyandoto, P. and Kouri, M. (1995) Randomised comparison of fluorouracil, epidoxorubicin and methotrexate (FEMTX) plus supportive care with supportive care alone in patients with non-resectable gastric cancer. *Br. J. Cancer* 71, 587-591.
33. Bostick, R.M., Fosdick, L., Grandits, B.A, Lillemoe, T.J., Wood J.R., Grambsch, P., Louis, T.A. and Potter, J.D. (1997) Colorectal epithelial cell proliferative kinetics and risk factors for colon cancer in sporadic adenoma patients. *Cancer Epidemiol. Biomarkers Prev.* 6, 1011-1019.
34. Pappalardo, G., Maiani, G., Mobarhan, S., Guadalaxara, A., Azzini, E., Raguzzini, A., Salucci, M., Serafini, M., Trifero, M., Illomei, G. and Ferro-Luzzi, A. (1997) Plasma (carotenoids, retinol, alphanatocopherol) and tissue (carotenoids) levels after supplementation with beta-carotene in subjects with precancerous and cancerous lesions of sigmoid colon. *Eur. J. Clin. Nutr.* 51, 661-666.
35. Maini, G., Pappalardo, G., Ferro-Luzzi, A., Raguzzini, A., Azzini, E., Guadalaxara, A., Trifero, M., Frommel, T. and Mobarhan, S. (1995) Accumulation of beta-carotene in normal colorectal mucosa and colonic neoplastic lesions in humans. *Nutr. Cancer* 24, 23-31.
36. Wang, W. and Higuchi, C.M. (1995) Induction of NAD (P) H: quinone reductase by vitamins A, E and C in Colo 205 colon cancer cells. *Cancer Lett.* 98, 63-69.
37. Hofstad, B., Almendingen, K., Vatn, M., Andersen, S.N., Owen, R.W., Larsen, S. and Osnes, M. (1998) Growth and recurrence of colorectal polyps: a double-blind 3-year intervention with calcium and antioxidants. *Digestion* 59, 148-156.
38. Ponz de Leon, M. and Roncucci, L. (1997) Chemoprevention of colorectal tumors: role of lactulose and of other agents. *Scand. J. Gastroenterol.* 222 (Suppl), 72-75.
39. Zhang, S., Tang, G., Russell, R.M., Mayzel, K.A., Stampfer M.J., Willett, W.C. and Hunter, D.J. (1997) Measurement of retinoids and carotenoids in breast adipose tissue and a comparison of concentrations in breast cancer cases and control subjects. *Am. J. Clin. Nutr.* 66, 626-632.
40. Chitkara, N., Dadoo, R.C., Bansal, S., Chugh, K., Aggarwal, S.K., and Lal, H. (1996) Plasma vitamin E levels in carcinoma breast. *Ind. J. Clin. Biochem.* 11, 162-164.
41. Dorgan, J.F., Sowell, A., Swanson, C.A., Potischman, N., Miller, R., Schussler, N. and Stephenson, H.E. Jr. (1998) Relationships of serum carotenoid, retinol, alpha-tocopherol, and selenium with breast cancer risk: results from a prospective study in Columbia, Missouri (United States). *Cancer Causes Control* 9, 89-97.
42. Verhoeven, D.T., Assen, N., Goldbohm, R.A., Dorant, E., van't Veer, P., Sturmans, F., Hermus, R.J. and Brandt, P.A. (1997) Vitamins C and E, retinol, beta-carotene and dietary fibre in relation to breast cancer risk: a prospective cohort study. *Br. J. Cancer* 75, 149-155.

43. Statland, B.E. (1992) Nutrition and cancer. *Clin. Chem.* 38, 1587-1594.
44. Peng, Y.M., Peng, Y.S., Childers, J.M., Hatch K.D., Roe, D.J., Lin, Y. and Lin, P. (1998) Concentrations of carotenoids, tocopherols, and retinol in paired plasma and cervical tissue of patients with cervical cancer, precancer, and noncancerous diseases. *Cancer Epidemiol. Biomarkers Prev.* 7, 347-350.
45. Palan, P.R., Chang, C.J., Mikhail M.S., Ho, G.Y., Basu, J. and Romney, S.L. (1998) Plasma concentrations of micronutrients during a nine-months clinical trial of beta-carotene in women with precursor cervical cancer lesions. *Nutr. Cancer* 30, 46-52.
46. Sarkar, A., Basak, R., Bishayee, A., Basak, J. and Chatterjee, M. (1997) Beta-carotene inhibits rat liver chromosomal aberrations and DNA chain break after a single injection of diethylnitrosamine. *Br. J. Cancer* 76, 855-861.
47. Wu, C.G., Hoek, F.J., Groenink, M., Reitsma, P.H., van Deventer, S.J. and Chamuleau, R.A. (1997) Correlation of repressed transcription of alpha-tocopherol transfer protein with serum alpha-tocopherol during hepatocarcinogenesis. *Int. J. Cancer* 71, 686-690.
48. Gorozhanskaia, E.G., Patiutko, I.I., Sagaidak, I.V. (1995) The role of alpha-tocopherol and retinol in correcting disorders of lipid peroxidation in patients with malignant liver neoplasms. *Vopr. Onkol.* 41, 47-51.
49. Malvy, D.J., Arnaud, J., Burtschy, B., Sommelet, D., Leverger, G., Dostalova, L., and Am ed ee-Manesme, O. (1997) Antioxidant micronutrients and childhood malignancy during oncological treatment. *Med. Pediatr. Oncol.* 29, 213-217.
50. Preston-Martin, S. (1977) Antioxidants and brain tumor prevention in children. *Family Practice News (U.S.)* Dec 1.
51. Karagas, M.R., Greenberg, E.R., Nierenberg, D., Stukel, T.A., Morris, J.S., Stevens, M.M. and Baron, J.A. (1997) Risk of squamous cell carcinoma of the skin in relation to plasma selenium, alpha-tocopherol, beta-carotene, and retinol: a nested case-control study. *Cancer Epidemiol. Biomarkers Prev.* 6, 25-29.
52. Helzlsouer, K.J., Alberg, A. J., Norkus, E. P., Morris, J.S., Hoffman, S.C. and Comstock, G.W. (1996) Prospective study of serum micronutrients and ovarian cancer. *J. Natl. Cancer Inst.* 88, 32-37.