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REVIEWS: CURRENT TOPICS

Vitamin B_6-mediated suppression of colon tumorigenesis, cell proliferation, and angiogenesis (Review)

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Abstract

This review describes current research on the preventive effect of dietary vitamin B_6 against colon tumorigenesis and its possible mechanisms. Studies in cell culture have demonstrated that high levels of vitamin B_6 suppress growth of some cancer cells. From these studies it has been considered that supraphysiological doses of vitamin B_6 suppress tumor growth and metastasis. However, recent rodent study has indicated that azoxymethane-induced colon tumorigenesis in mice is suppressed by moderate doses of dietary vitamin B_6. Epidemiological studies also support an inverse relationship between vitamin B_6 intake and colon cancer risk. Potential mechanisms underlying the preventive effect of dietary vitamin B_6 have been suggested to include the suppression of cell proliferation, oxidative stress, nitric oxide (NO) synthesis, and angiogenesis.

Keywords: Vitamin B_6; colon cancer; cell proliferation; oxidative stress; nitric oxide; angiogenesis

1. Introduction

Vitamin B_6 has long been recognized as a cofactor for many enzymes, especially those involved in amino acid metabolism. Apart from its role as coenzyme, recent studies are unveiling a new role of vitamin B_6 as a chemopreventive agent. High levels of vitamin B_6 have been reported to suppress growth of animal or human cancer cells *in vitro* [1–4]. Animal research also indicated that a high dietary intake of vitamin B_6 suppresses herpes simplex virus type 2 transformed cell-induced tumor growth in BALB/c mice [5]. Thus, it has been considered that supraphysiological doses of vitamin B_6 may have potential use in antineoplastic therapy [6].

In 1997, Slattery et al. [7] reported the case-control studies indicating an inverse association between vitamin B_6 intake and colon cancer risk in the United States. This was confirmed by the case-control study in seven countries of Europe [8]. An inverse relationship between vitamin B_6 intake and prostate cancer incidence has been also reported [9]. Recent case-control study has further supported an inverse association between serum vitamin B_6 level and lung cancer risk [10]. Komatsu et al. [11] postulated that colorectal cancer risk might be reduced by moderate levels of dietary vitamin B_6 daily consumed by humans. To test this hypothesis, they have examined effect of dietary level of vitamin B_6 on the development of colon tumors in azoxymethane (AOM)-treated mice [11]. The results showed that colon tumorigenesis was significantly suppressed by moderate doses of dietary vitamin B_6 [11]. Thus, the preventive effect of vitamin B_6 against colon carcinogenesis has important dietary consequences. This review describes recent studies on the antitumor effect of vitamin B_6 and its mechanisms.

2. Colon tumorigenesis

Komatsu et al. [11] have studied the effect of dietary level of vitamin B_6 on AOM-induced colon tumorigenesis in mice. Mice were fed the diets containing 1, 7, 14 or 35 mg pyridoxine (PN) HCl/kg for 22 weeks, and given a weekly injection of AOM for the initial 10 weeks. One hour before termination, 5-bromo-2′-deoxyuridine (BrdU) was

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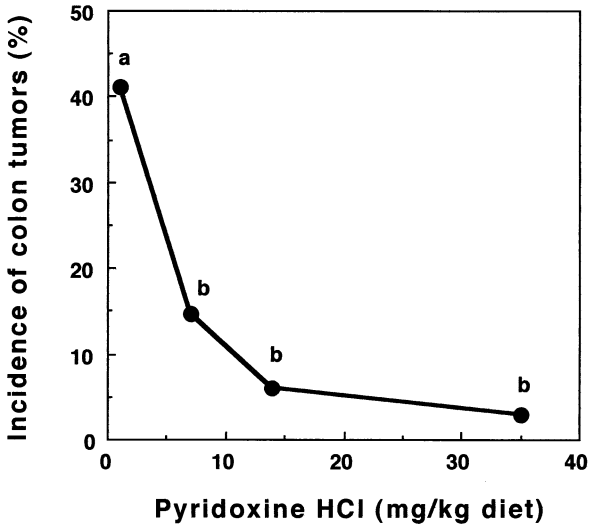

Fig. 1. Effect of dietary vitamin B_6 on the incidence of colon tumors in azoxymethane-treated mice. Mice were fed experimental diets containing various amounts of vitamin B_6 (1-35 mg pyridoxine HCl/kg diet), and given azoxymethane to induce colon tumors. Values show incidence of tumors (%) (n = 34). The incidence of the groups fed vitamin B_6 supplemented diets (7-35 mg pyridoxine HCl/kg diet) was significantly lower than that of the low vitamin B_6 diet group (1 mg pyridoxine HCl/kg diet). ($P < 0.05$) (J Nutr 2001;131:2204-7).

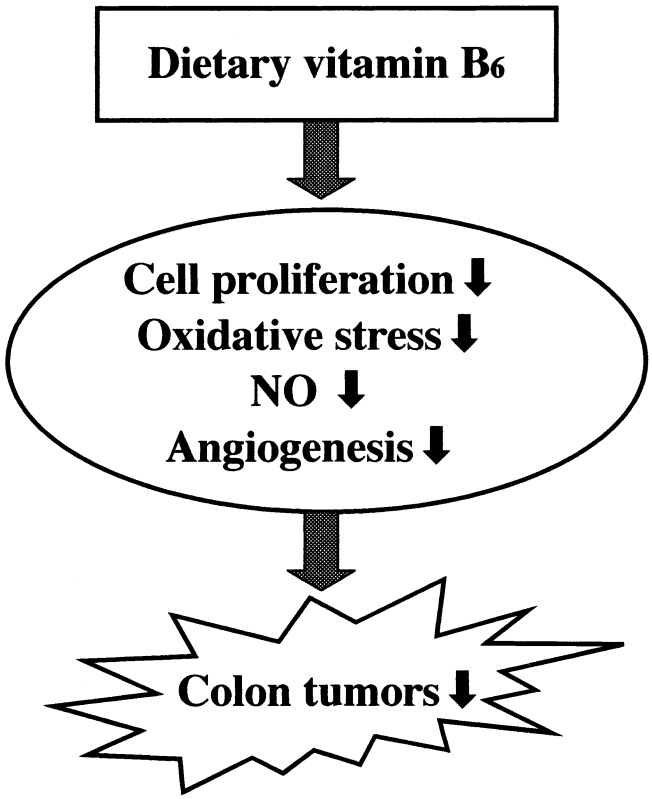

Fig. 2. Possible mechanisms by which vitamin B_6 exerts its preventive effect on colon tumorigenesis.

given by injection for immunohistochemical analysis of cell proliferation. A 1 mg PN HCl/kg diet has been reported to be the minimum level required for preventing growth depression caused by vitamin B_6 deficiency [12]. Supplementation of vitamin B_6 caused no influence on the food intake and growth. Supplemental dietary vitamin B_6 markedly suppressed the incidence of colon tumors (Fig. 1) and the number of colon tumors compared with the 1 mg PN HCl/kg diet group. Compared with the 1 mg PN HCl/kg diet, the minimum level for preventing colon tumorigenesis examined here was 7 mg PN HCl/kg diet. In general, the greatest suppression of tumorigenesis by dietary vitamin B_6 was observed in the mice fed 14 and 35 mg PN HCl/kg diets. The recommended level in the AIN-93 diet is 7 mg/kg diet [13]. The optimum requirement of PN HCl appears to be 14-35 mg/kg for suppression of colon tumorigenesis. Supraphysiological dose of vitamin B_6 has been believed to be potentially useful for antineoplastic therapy [6]. The result, however, implies that colon carcinogenesis can be significantly reduced by moderate levels of dietary vitamin B_6. In the mice fed the 35 mg PN HCl/kg diet, no toxicity symptoms were observed throughout the entire feeding period.

3. Cell proliferation

Cell hyperproliferation is involved in the mechanism of carcinogenesis. Komatsu et al. [11] have found that supplemental vitamin B_6 reduced the BrdU-labeling index (index of cell proliferation) in the colon of mice, whereas vitamin B_6 had no significant effect on colonic epithelium apoptosis. The labeling index in all of the colon epithelium of the 14 and 35 mg PN HCl/kg diet groups was significantly lower than that of the 7 mg PN HCl/kg diet group. The labeling index of proliferating cells in all of the colon epithelium significantly correlated with the number of tumors. The labeling indices of c-*myc* and c-*fos* proteins (oncogene products relating to cell proliferation) in the colonic crypts were also significantly reduced by supplemental vitamin B_6. The results imply that lowered cell proliferation by dietary vitamin B_6 leads to its antitumor effect (Fig. 2).

PLP has been found to be effective inhibitor of many enzymes that have binding sites for phosphate-containing substrates or effectors, including RNA polymerase [14,15], reverse transcriptase [16], and DNA polymerase [17,18]. Oka et al. [19,20] reported that vitamin B_6 deficiency generally enhances gene expression in rat liver, including that of glycogen phosphorylase. Recently, they have reported that PLP first binds to Lys197 of HNF1 protein, a tissue-specific transcription factor, and modulates DNA binding activity, which in turn suppresses expression of the albumin gene in rat liver [21]. The possibility that the inhibitory effect of dietary vitamin B_6 on colon cell proliferation is mediated through alternations of gene expression remains to be examined.

4. Oxidative stress

It has been reported that c-*myc* and c-*fos* expression can be induced by oxidative stress [22,23]. *In vitro* study has indicated that vitamin B_6 has a strong antioxidative effect [24]. Some animal research demonstrated that vitamin B_6 deficiency leads to greater lipid peroxidation in plasma and liver when the animal consumes a high fat diet [25]. Thus, Komatsu et al. [26] postulated that the suppression effect of vitamin B_6 on the cell proliferation might be mediated through reduced oxidative stress. As oxidative stress markers, colonic 8-hydroxyguanosine (8-OHdG) and 4-hydroxy-2-nonenal (4-HNE) were examined in the AOM-treated mice fed graded levels of vitamin B_6. The immunohistochemical analysis showed that supplementation of vitamin B_6 to low vitamin B_6 diet (1 mg/kg diet) significantly suppressed the levels of 8-OHdG and 4-HNE [26]. The alteration of these oxidative stress markers significantly correlated with the expression of c-*myc* and c-*fos* proteins. The concentration of serum lipid peroxide was also higher in the 1 mg pyridoxine HCl/kg diet group than in the other groups [26]. Thus, reduced oxidative stress by vitamin B_6 supplementation might be in part involved in the mechanism of its preventive effect on tumorigenesis (Fig. 2).

5. Nitric oxide

Nitric oxide (NO), one of the oxygen free radicals, has been considered to play an important role in the colon carcinogenesis by elevating cyclooxygenase-2 and angiogenesis [27,87]. The expression and activity of inducible NO synthase (iNOS) are higher in human adenomas [29]. Some studies have also demonstrated that AOM-induced colon tumors have increased an expression and/or activity of iNOS when compared to levels in adjacent colonic tissue [30]. The production of NO and the expression of iNOS mRNA is elevated by oxidative stress, which is induced by superoxide [31]. Komatsu et al. [26] have further examined the effect of dietary vitamin B_6 on colonic expression of iNOS protein in AOM-treated mice. The result showed a significant depression in the colonic iNOS protein by elevating dietary vitamin B_6. The expression of iNOS protein was associated with the levels of 8-OHdG and 4-HNE. Thus, the lower NO production by vitamin B_6 might also partially lead to reduced colon tumorigenesis (Fig. 2).

6. Angiogenesis

Some tumor cells recruit new capillary blood vessels, called angiogenesis, that support the growth [32]. Metastases are also dependent on angiogenesis to be shed from a primary tumor and to grow at their target organ. Therefore, inhibition of angiogenesis induced by tumor and metastasis cells is a promising therapeutic strategy for cancer [32].

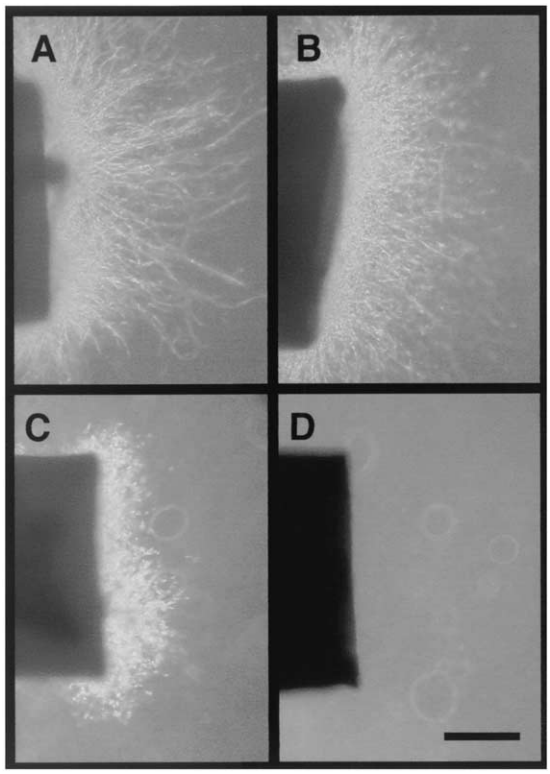

Fig. 3. Antiangiogenic effect of pyridoxal 5′-phosphate. Aortic segment in collagen gel on 7 day of culture in the absence of pyridoxal 5′-phosphate (PLP) (A), and in the presence of PLP 50 μmol/L (B), 500 μmol/L (C) and 5 mmol/L (D). Microvessel outgrowth was inhibited in a dose-dependent manner. Bar equals 500 μm (Int J Mol Med 2001;8:505-8).

Matsubara et al. [33] have studied the effect of vitamin B_6 on angiogenesis using an *in vitro* model, in which rat aortic ring was cultured in collagen gel [34,35]. This model is used and evaluated as a good model to investigate angiogenesis phenomenon and to find antiangiogenic agents [36,37]. Microvessels appeared from the edge of aortic ring and elongated in the absence of vitamin B_6 (Fig. 3A). The elongation of microvessels was strongly inhibited in the presence of PN and PLP (Fig. 3B–D). PLP showed stronger inhibitory effect than PN. PLP suppressed the elongation of microvessels in a dose-dependent manner. They further studied the effect on the growth of human umbilical vein endothelial cells, being considered to be associated with angiogenesis. The results showed that PLP suppressed the proliferation of endothelial cells, but did not affect the tube formation on reconstituted membrane (Matsubara et al. unpublished results). The antiangiogenic effect appears to be ascribed to antiproliferation activity for endothelium cells. Further *in vivo* study is necessary to test whether dietary vitamin B_6 suppresses colon carcinogenesis by reducing angiogenesis.

7. Other mechanisms

In vitamin B_6 deficiency, antibody production may be indirectly impaired [38]. Grindley et al. [4] showed that

high dietary intake of vitamin B_6 (74.3 mg PN/kg diet) suppresses herpes simplex virus type 2 transformed cell-induced tumor growth and enhances immune status compared with a 1.2 mg PN/kg diet in BALB/c mice. The preventive effect of vitamin B_6 against colon tumorigenesis might be in part mediated through alteration in immune function.

Vitamin B_6 plays an important role in homocysteine metabolism serving a critical role in homocysteine catabolism. In the process of homocysteine catabolism, cysteine, a main component for the synthesis of glutathione, is generated. Gutathione serves as an important cofactor of the glutathione S-transferase and glutathione peroxidase, which function in the detoxification of many toxic or carcinogenic compounds. Vitamin B_6 also facilitates the transfer of a methyl group to tetrahydrofolate, yeilding 5, 10-methylenetetrahydrofolate needed for reactions generating thymidylate and purines. Thus, disruption of these reactions may lead to imbalances in methyl groups required for methylation process, including DNA methylation. Altered DNA methylation has been observed in tumors at several sites [39,40]. The alterations of these metabolisms by dietary vitamin B_6 might lead to the antitumor effect of vitamin B_6.

8. Conclusions

This review focused on the preventive effect of dietary vitamin B_6 against colon tumorigenesis. Its antitumor effect in the mice received AOM appeared to be evident by moderate dietary levels of vitamin B_6 being close to the recommended level in the diet. Recent epidemiological studies also support an inverse association between vitamin B_6 intake and colon cancer risk. The preventive effect of vitamin B_6 against colon tumorigenesis in mice appears to be mediated through suppressing cell hyperproliferation, oxidative stress, and NO synthesis in the colon. *In vitro* studies have further provided evidence for antiangiogenic effect of vitamin B_6. These studies emphasize the importance of vitamin B_6 as an antitumor factor [28].

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