REVIEWS

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B-Group Vitamins: Chemoprevention?

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Abstract

The importance of vitamins in the prevention of cancer has attracted the attention of consumers, nutritionists and scientists for decades. The mechanisms of carcinogenesis, extended in the context of the function of vitamins, i.e. regulation of and participation in metabolic processes in the cell, suggest a substantial impact of these compounds on the initial stages of carcinogenesis. One-carbon metabolism involving folic acid, vitamins B2, B6 and B12, and folate metabolism doesn't only generate methyl groups, thus determining epigenetic processes, modifications of the genome and carcinogenesis. It also provides the compounds involved in the DNA synthesis and repair processes, especially the synthesis of purines and pyrimidines and the conversion of dUMP (2-deoxyuridine monophosphate) to dTMP (2-deoxythymidine monophosphate). In light of these pathways, folate, together with vitamins B2, B6 and B12, became a subject of interest as compounds whose deficit or surplus can potentially have an impact on the processes of carcinogenesis. Literature reports, however, do not fully confirm that the influence on the synthesis of nucleotides is connected with the inhibition of carcinogenesis. The impact of individual vitamins involved in one-carbon metabolism on carcinogenesis and their role in the prevention of these conditions depend on the type of cancer and the dose administered. Nevertheless, the research conducted makes it possible to conclude a considerable and probably long-underestimated role of these compounds in the prevention of serious, difficult to treat or incurable diseases (**Adv Clin Exp Med 2016, 25, 3, 561–568**).

Key words: group-B vitamins, one-carbon metabolism, folic acid.

Medicinal products containing vitamins are not only vitamin and mineral conglomerates, which generally support the condition of the body in the autumn and winter, or during convalescence after debilitating diseases. They are also products of strictly therapeutic indications. We should mention both clinically-confirmed indications and those that remain in the realm of scientific reports or preclinical studies. And so, the issue of the importance of vitamins in the prevention of cancers has attracted the attention of consumers, nutritionists and scientists for decades. The mechanisms of carcinogenesis, extended in the context of the function of vitamins, i.e. regulation of and participation in metabolic processes in the cell, suggest a substantial impact of these compounds on the initial stages of carcinogenesis, among others, due to their antioxidant properties, i.e. by eliminating carcinogenic reactive oxygen species and because of their participation in one-carbon metabolism and thus, in the processes of DNA methylation – basic epigenetic processes for carcinogenesis, which involve folic acid, vitamins B2, B6 and B12. Scientific literature extensively discusses the importance of supplementation with antioxidants. Juggling with the qualitative composition of vitamin and mineral mixtures, which involves the flagship antioxidants, such as tocopherol, ascorbic acid, retinol and β-carotene, is the main theme of papers dealing with the relationship between vitamins and cancers. In the article under the intriguing title "Vitamins and cancer", van Poppel and van den Berg [1] dedicated only a very short section to the B-group by describing a representative of this group – folic acid. To justify this somewhat disturbed proportion in the interest of certain compounds important to the processes of carcinogenesis and tumor development, it should be noted that in 1997, when the above article was created, the panel of available research

methods with a focus on genetics was incomparably narrower than today. This explains the shifting of the center of gravity on carcinogens, such as free radicals, which can be determined, directly or indirectly. Discovering the subsequent processes responsible for damage to the genetic material has expanded the possibilities of their evaluation in terms of possible modulation or interference with the course of these processes. Therefore, this article aims to present the current state of knowledge in this regard, and is largely based on current reports and those from the past six years. The reports relate to the use of B-group vitamins in the prevention of cancers, not necessarily in terms of anti-free radical properties.

Molecular Basis of the Carcinogenic Process

Neoplasms arise as a result of the accumulation of genetic and epigenetic changes in the cell. The following groups of genes are involved in the process of carcinogenesis: 1) Protooncogenes are present in normal cells in an inactive form. Oncogenes are mutated protooncogenes. Protein products of the protooncogenes are involved in the regulation of growth, maturation and differentiation of cells involved in the transmission of transduction signals and function as transcription factors. So far, over 500 protooncogenes have been isolated and described, among which three groups were distinguished, i.e. genes encoding proteins that regulate the cell cycle, proteins involved in apoptosis and those having other important functions in a cell, such as the formation of ion channels; 2) Tumor suppressor genes, responsible for the control of replication and the genetic stability of cells, the so-called "guardians of the genome", encoding, among others, apoptotic proteins; 3) Mutator genes encoding DNA repair enzymes. Polymorphic variants of repair enzymes are one of the foundations of individual sensitivity to the action of mutagens and the risk of cancer development [2].

Epigenetic phenomena have become one of the most important areas of research in the pathogenesis of cancers (though not exclusive). The contemporary definition of epigenetics comprises a set of changes in the function of each gene inherited during mitosis and meiosis, but not resulting from changes in the primary DNA structure [3]. Epigenetic modifications are responsible for, among others, setting specific patterns of gene expression, which in turn are a key to the morphological and functional differentiation of cells within the human body. These modifications consist of: DNA methylation, chromatin protein modifica-

tions and, thus, the structure, and the functions of non-coding RNA [3]. Hypomethylation of the genome with the simultaneous hypermethylation of CpG islands in the promoter regions of the tumor most often involves suppressor and mutator genes and is associated with the suppression of their expression. DNA methylation changes occur at the very early stages of carcinogenesis [2]. Depending on the type of posttranslational modification of chromatin proteins, condensation or relaxation of the structure takes place. Then, DNA becomes more or less accessible to transcription factors and polymerases, resulting in changes in gene expression [2].

Genetic changes in normal cells occur under the influence of various factors, which include: physical (UV radiation, cosmetics), chemical (heavy metals, asbestos, substances contained in, e.g. automobile exhaust or cigarette smoke) and biological (viruses, bacterial toxins or metabolic intermediates, such as free radicals and hormones) [2].

Carcinogenesis, which is a continuous process, is composed of four stages: pre-initiation, i.e. the stage of exposure to carcinogens; initiation, i.e. the creation of the first mutation, which is irreversible and is passed on to daughter cells; promotion, i.e. the step when genetic and epigenetic changes are accumulated, and progression, i.e. the stage of development of the malignant tumor with all its characteristics and the ability to metastasize [2].

Folic Acid and...

The supply of methylating agents in the diet may significantly affect the level of DNA methylation and its changes in tumor cells. Such factors are contained in the compounds involved in one-carbon metabolism, i.e. de- and remethylation of methionine which provides methyl residues for modifying DNA [4]. The changes are initiated by dietary methionine and folate. Reduced forms of folate are metabolically active, and include dihydro- (DHF) and tetrahydro- (THF), among which THF is the most active form. Dihydrofolate reductase (DHFR) catalyzes reduction reactions [4, 5]. The transfer of one-carbon active groups within the THF molecule, such as methyl-, methylene, methenyl-, formyl- and formimino-, determines the formation of coenzymes involved in purine and pyrimidine synthesis and metabolism of certain amino acids [4, 6].

N^5, N^{10}-methylenetetrahydrofolate, one of the forms of folate serving as coenzymes, is formed by the conversion of serine to glycine with the participation of serine hydroxymethyltransferase and

pyridoxal phosphate (vitamin B6). The reduced compound N^5-methylenetetrahydrofolate, whose formation involves methylenetetrahydrofolate reductase (MTHFR) and vitamin B2 (riboflavin), is a donor of the methyl group in the remethylation of homocysteine to previously demethylated methionine, formed in the creation of homocysteine with concomitant regeneration of THF. This reaction is catalyzed by methionine synthetase and vitamin B12 in the form of methylcobalamin as a coenzyme. The supply of methionine accumulated in this way provides a substrate for the synthesis of the main donor of active methyl groups – S-adenosyl methionine (SAM) [4, 6].

These briefly presented stages of one-carbon and folate metabolism generate not only methyl groups, thus determining epigenetic processes, modifications of the genome and carcinogenesis. They also provide the compounds involved in DNA synthesis and repair processes, especially the synthesis of purines and pyrimidines and the conversion of dUMP to dTMP, which occur with the participation of thymidylate synthetase and N^5, N^{10}-methylenetetrahydrofolate generated through glycine formation, as it was mentioned [4]. In light of these pathways, folate, together with vitamins B2, B6 and B12, became a subject of interest as compounds, whose deficit or surplus can potentially have an impact on the processes of carcinogenesis.

Vitamin Chemoprevention?

It is difficult to divide a discussion on the importance of one-carbon mechanism cofactors in the processes of carcinogenesis and chemoprevention into individual compounds; however, based on the current knowledge, relatively more reports refer to folate. In the context of cancers, the superiority of these compounds over the other vitamins, whose function is mainly co-enzymatic, may be due to a direct effect of folate as a donor of methyl groups on the process of DNA strand damage and repair. In mammalian cells, one-carbon residues coming from folate are essential for the synthesis of dUMP, dTMP and purine [4, 7]. Folate deficit induces carcinogenic breaks in the DNA strand by several mechanisms, including the impaired synthesis of dTMP from dUMP. As a result of dTMP deficiency, DNA polymerases, having relatively low specificity, incorporate uracil instead of thymine into the chain, which in turn is cut by corrective glycosylase with the resultant cracks in single or double stranded DNA, and the consequent genetic instability [7]. According to Choi and Mason [7], cells growing in media, which are deficient in folate, exhibit chromosomal aberrations. Rat colonocytes,

supplemented after a period of deficit with precursors of purine and pyrimidine, showed a partial repair of damage resulting from the incorporation of uracil into the DNA. The reduced ability of normal DNA repair, as a consequence of a deficit of folate, may also be associated with damage to the gene *p53*. The protein product of this gene is an important regulator of repair processes, as for instance the product of *hMLH1* gene, one of the major mismatch repair genes, wherein the hypomethylation of CpG islands impairs the function of post-translational repair due to low levels of folate [7].

Discussing the undoubted importance of folate to the body in the context of cancer processes, their initiation and inhibition, it should be noted that this influence is not only ambiguous, but, above all, not unconditional. This is perfectly illustrated by the title of the article by Joel B. Mason in the American Journal of Clinical Nutrition from 2011: "Folate consumption and cancer risk: A confirmation and some reassurance, but we're not out of the woods quite yet" [8], or this written by David Smith in 2008: "Is folic acid good for everyone?" [9]. The authors confirm the importance of these protective compounds, which support genetic stability and silence oncogenes, however, they also emphasize the importance of the dose. Excessive availability of folate compounds, which are essential to nucleotide synthesis, accelerates the proliferation of rapidly dividing cells, leading to the proliferation of tumor cells. Therefore, the chemopreventive properties of folate refer only to healthy people. The beneficial effects of these compounds and their supply to patients already burdened with cancer require careful consideration, especially that the results of the research conducted so far have not been conclusive.

Numerous reports support the hypothesis about the dual role of folate in anticancer therapy. Tomaszewski et al. [10] have demonstrated a direct relationship between the level of folate in serum and the proliferation of prostate cancer cells, wherein an increased level was not a result of the supplementation with folic acid, but the metabolic disorders of folate and/or solid diet fortified with this compound. Moreover, higher levels of folate in serum were observed in patients with the prostate cancer burden (62.6 nM). For comparison, in the control group, the level was 17.4 nM. The stimulatory effect of folate on cell proliferation is also due to their ability to up-regulate membrane transporters of folate and the increased transport of these compounds from the serum by cells.

On the basis of their studies Ly et al. [11] suggest that the high intake of folic acid during pregnancy and lactation increases the risk of breast cancer in the offspring, which is associated with

the participation of folic acid in the generation of methyl residues used in epigenetic DNA processes and the potential activation of oncogenes and/or silencing of tumor suppressor genes. In order to support the results that indicate the possibility of the carcinogenic activity of folic acid and its derivatives in breast and prostate cancers, Kotsopoulos et al. [12] have noted that folate deficiency in the diet of rats, during the promotion of type adenocarcinoma tumors, resulted in a significant reduction of the incidence and extent of tumors. Interestingly, neither a deficit nor supplementation with these compounds had significance for the development of cancers, if they were at the stage of initiation of the process of carcinogenesis. Rats with liver tumors induced by diethylnitrosamine manifested, among others, decreased cell proliferation and an increase in the level of glutathione transferase, comprising the protective effect of folic acid at a dose of 160 μg, and in the absence of such effects at a dose of 80 μg [13].

According to Sauer et al. [14], demonstration of a causal link between high levels of folate and carcinogenesis should be the subject of further research, just as the evaluation of the effects of chronic exposure to high doses of folate and the importance of un-metabolized folic acid.

As a counterbalance to the described results, Crott et al. [15] report that high doses of folate reduce the risk of colon cancer by 30–40% compared to the subjects receiving a small amount of this vitamin. These results were supported by determining a simple relationship between the incidence of this tumor type and the age of the subjects, because with age, bioavailability of folate decreases and intestines become more sensitive to the deficit of these compounds. Hence, the risk of developing colon cancer increases from 0.07 to 4.0% between the ages of 0 and 79 years, respectively.

The importance of using appropriate doses of folate to obtain the effect is due to their metabolic paths, and indirectly because of the type of supplementation, i.e. natural diet rich in folate and vitamin supplements. In the latter case, folic acid is the supplemented active substance, which needs the aforementioned DHFR enzyme to achieve a naturally occurring reduced form [16]. Polymorphism of the *DHFR*-19-bp gene by generating its overexpression disrupts folate metabolic processes, and thus also disturbs the level of SAM, DNA methylation processes and finally, modifies the transcription factor binding sites. In this light, it becomes important to find a positive correlation between the *DHFR* genotype and the risk of breast cancer in women with folic acid supplementation. The cohort study the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial demonstrated an approx. 20% increase in the risk of breast cancer in postmenopausal women supplemented with a daily dose of folic acid above 400 μg [16]. Figueiredo et al. [5] reported that the amount of folic acid exceeding 400 μg saturates the DHFR enzyme. As a result, unreduced folic acid may reduce the effectiveness of cytotoxic NK cells, which constitute the first line of defense against pathogens and carcinogenic cells. In the same paper, the authors describe the Aspirin Folate Polyp Prevention Study indicating an inverse relationship between the risk of rectal cancer and the intake of dietary folate, however, the relationship was observed only in patients not taking multivitamin supplements. According to Smith et al. [9], free folic acid, in addition to the interactions with NK cells, can compete with natural reduced folate for binding sites on enzymes, transport proteins or binding proteins as well as disturb cellular transport.

Inoue-Choi et al. [17] also confirmed the relationship between a dose of these compounds and their action. They demonstrated a significantly increased risk of breast cancer among women taking 400 μg of folate per day in the form of dietary supplements, but they were also additionally exposed to a high nitrate supply contained in the drinking water, which provided carcinogenic nitroso compounds. It is worth noting that such a relationship was not observed in the group with low folate intake.

Sex and *MTHFR* gene polymorphism also define the role of folate in chemoprevention. Curtin et al. [18] published results indicating the influence of sex, *MTHFR* polymorphs and hormonal levels, but also the source of folate, on the increased risk of rectal cancers with the phenotype characterized by methylated CpG islands. As shown, the increased risk related to men being on a diet fortified with folic acid and having genotype *MTHFR 1298AA*. As usual in the case of such studies, it is difficult to obtain the same tendencies in the results. Both Collin et al. [19] and Eussen et al. [20] conducted meta-analyzes which showed no correlation between *MTHFR* gene polymorphism and the risk of cancer of the prostate, or rectum, respectively.

In certain cases, the type of tumor and its stage of development is a condition determining the chemopreventive effect of folic acid. In the cohort study the Vitamins And Lifestyle (VITAL), Maruti et al. [21] recorded a 22% lower risk of the breast cancer incidence in women receiving more than 1272 DFE daily (equivalent to the consumption of folate = synthetic folic acid in μg × 1.7 + folate from food) for approx. 10 years compared to women taking daily ≤ 345 DFE; the protective effect of folate positively correlated with the dura-

tion of taking the increased amount. The time that elapsed from starting the supplementation to a diagnosis of cancer less than 10 years had no effect on the risk of incidence of this tumor. Interestingly, the chemopreventive effect of folate was observed in cancers with the lack of estrogen receptors – ER (–). In the Netherlands Cohort Study, De Vogel et al. [22] showed that high folate intake positively correlates with the risk of colon cancer in men with a mutation in the suppressor gene – APC+, while negatively in men without the mutation – APC-. This type of relationship has not been observed in women, which indicates the importance of sex in terms of folate chemoprevention.

Kim [23] formulated a very important consideration regarding the importance of folic acid supplementation to the risk of developing cancer. The author emphasizes that the effects of folic acid deficit cannot be seen directly, but rather indirectly, as a result of this deficit, e.g. one-carbon metabolism disorders. Considering these changes as a source of methyl groups, the obvious suggestion is raised that vitamins B2, B6 and B12, which also participate here, indirectly determine epigenetic processes, i.e. methylation and demethylation of CpG islands by affecting the profile of oncogenes and suppressor genes and they are involved in the synthesis of purines and pyrimidines. In an article dealing with the duality of folate nature, with the significant title: "Folate, cancer risk, and the Greek god, Proteus: A tale of two chameleons" Mason [24] emphasizes that the mentioned vitamins also determine normal one-carbon transformations, which are relevant to the control of carcinogenesis and their deficiencies insidiously, because in the absence of visible symptoms of the deficit, regardless of the supply of folate, result in disturbances of the DNA structure. According to the author, mathematical modeling of the kinetics of enzymes involved in one-carbon metabolism revealed that the critical function of these transformations, understood as an initial step in the synthesis of nucleotides, is a comprehensive action of folic acid, vitamins B2, B6 and B12. According to this line of thought, the idea is suggested that all the vitamins involved in one-carbon metabolism may have a potential importance to the development of carcinogenesis. Vitamin B6, in addition to participating in one-carbon metabolism, is also involved in approx. 100 enzymatic reactions by reducing oxidative stress, but also cell proliferation and angiogenesis, as it was shown ex vivo in the case of pyridoxal phosphate and pyridoxal in the concentration range: 0.05–0.25 mM, using human umbilical vein endothelial cells-HUVEC [5, 25]. It participates in the catabolism of homocysteine to glutathione, thus affecting the antioxidant pro-

tection and detoxification of carcinogens [26, 27]. Vitamin B2 plays a role in modifying the binding of carcinogens to DNA. Pangrekar et al. [28] demonstrated this effect in male Wistar rats exposed to benzo [a] pyrene. Supplementation with vitamin B2 reduced the levels of the tritium labeled compound, attached to DNA in almost all animal tissues, and at the highest degree in the lungs.

Studying the effect of folic acid and vitamins B2, B6 and B12 on the expression of the gene encoding p53 protein in mice, Liu et al. [29] found that a 10-week deficit of all of these compounds resulted in a much greater effect compared to a deficit of only folic acid in terms of the induction of DNA damage in the p53 gene region vulnerable to mutations, hypomethylation and a significant reduction of its expression and the expression of its regulator MDM2. The same author, in a later work [30] on carcinogenesis in mice BAT-LacZxApc1638N showed that the deficit of all these B vitamins in animals induced the activation of the major signaling Wnt pathway in rectal carcinogenesis as well as mutations in the suppressor gene Apc, together with an increase in the expression of Jun and Pitx2 genes involved in the processes relevant to the development of tumors, i.e. cell proliferation, transformation and apoptosis. The participation of vitamins B2 and B6 in the metabolism of homocysteine, which is involved in the etiology of colorectal cancer, justifies the recognized inverse correlation between the doses of these vitamins consumed during the day and a decrease of the risk of this cancer, as demonstrated in the Women's Health Initiative Observational Study conducted in the USA [27].

In the case of riboflavin derived from food, and natural and synthetic folate, an inverse dose-dependent correlation was demonstrated with the occurrence of precancerous changes in the endothelium of the cervix in 214 women tested at the clinic in Oahu, Hawaii in the years 1992––1996 [31]. The serum concentrations of estrogen-dependent RCP protein, a riboflavin transporter, above or equal to 1.0 ng/mL proved to be a biomarker of breast adenocarcinomas: 88% at stage I and II, and 100% at stage III and IV [33]. Population studies, testing a total of approx. 1,900 women in Mexico City, revealed that supplementation with folate in the range of 224–454 µg/day and vitamin B12 in the range 2.61–7.46 µg/day independently correlated with a decreased risk of breast cancer in women, especially at postmenopausal age. There was no such correlation for vitamin B6 [33]. On the other hand, Weiwei et al. [34], in control studies which included 655 women, found that the daily intake of folate in the amount of less than 450 µg and vitamin B6 in the amount of less

than 0.84 mg significantly raised the risk of breast cancer and *MTHFR* gene polymorphism.

Literature reports, however, do not fully confirm that the influence on the synthesis of nucleotides is connected with the inhibition of carcinogenesis, as it was demonstrated in the studies described above. Similarly, as in the case of folic acid, the type of tumor and the dose of the compounds were decisive factors.

Exposing nude mice to 712-dimethyl-[α] anthracene and UVB, Lu et al. [35] showed the exaggerated tumorigenesis in the skin of animals in the case of pyridoxine hydrochloride at doses of 1, 7 and 35 mg/kg of food, wherein a significant difference in the severity of this effect was observed as well as the pyridoxal phosphate levels in plasma between doses of 1 to 7 and 35 mg/kg. Shimada et al. [36] have confirmed the relationship in the effect of pyridoxine on the prevention of cancers depending on the nature of the tumors. In female Sprague-Dawley rats, pyridoxine at a dose of 7 and 35 mg/kg of food reduced the dose-dependent incidence of breast adenocarcinoma after 98, 104 and 111 days of exposure to dimethyl-[α]anthracene. Collin et al. [37] in turn, observed a positive correlation between the serum levels of vitamin B12 and an increased risk of prostate cancer, which they associated with increased biosynthesis of polyamines in tumor cells and, therefore, the raised demand for methionine synthetase, for which vitamin B12 is a cofactor. A similar relationship, but in relation to mainly hematological malignancies, has been noted by Arendt et al. [38] in Danish cohort studies involving more than 300,000 people.

Summary

The increasing research opportunities and advancing knowledge about the properties of vitamins, displayed at the subcellular level, create a belief that their supplementation will protect us from modern diseases such as cancers. First of all, vitamins having antioxidant properties, such as C, E and beta-carotene cross our minds. In the fight against cancer, scientific interest is also directed toward folic acid, which was mentioned in the article, due to its relationship with the processes of DNA methylation, or a new trend in recent years – vitamin D3, which interacts with exceptionally many areas of the cellular biochemical and genetic machinery. However, the results described in this paper, which aim to confirm an inverse relationship between vitamin supplementation and the risk of cancer, also do not always meet the needs of researchers, indicating a variety of responses depending on the type of cancer, dose, and vitamin origin. The superiority of vitamins in a natural form [39] is a commonly-known fact which is often confirmed in this kind of study.

However, the studies conducted on the effects of vitamins on the prevention of chronic diseases make it possible to draw some conclusions about the noteworthy and probably long-underestimated role of these compounds in the prevention of serious, difficult-to-treat or incurable diseases. In the light of more and more reports which prove their importance, we can hopefully expect the emergence of new and surprising properties which still remain undiscovered.

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