



Folic acid supplementation: some practical aspects

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Abstract

Since 1956, when Harman first postulated the free radical theory of aging, numerous studies have been carried out to test the protective action of antioxidants. One of these protective compounds used in antioxidant therapy is folic acid (FA). Folate deficiency can lead to several pathologies and its protective role is very well known. Because the negative effects of the synthetic form on the metabolism of folates and the controversy about the role of folic acid in cancer, the question is whether or not folic acid is good for everyone. In this paper we summarize some aspects of the biochemistry of folic acid and we show some precautions that should be taken into consideration when supplementing with this compound.

Introduction

The biological aging can be defined as the progressive loss of the optimal functions as a function of the age of an organism because of intrinsic factors [1,2]. Although several hypotheses exist that try to explain the causes of the aging, only a few are widely accepted, which does not mean that they are the correct ones. Among these hypotheses, the theory of Harman [3], which is more than 50 years old and continuously reviewed [4], postulates that the macromolecular damage

induced by the reactive oxygen species (ROS) is the main causal factor of the aging process. The ROS are produced during the course of the normal metabolism, mainly in the reactions of detoxification by the microsomal cytochrome P-450 system [5,6] and in the electron transfer in mitochondria [7]. Within this context, the oxidative damage of specific protein has been considered like one of the mechanisms by which oxidative stress is related to the loss of physiological functions that takes place in the aging and neurodegenerative diseases. In this sense, it is necessary to mention that all the proteins are not equally susceptible to the oxidative damage. Also, the effects of ROS are not either general or indiscriminate.

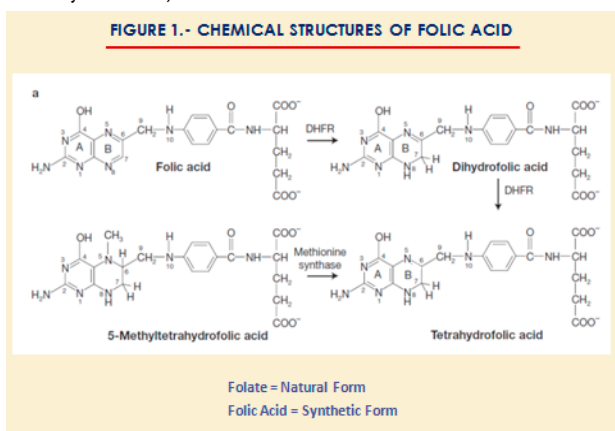
According to the free radical theory, it is reasonable to think that the reduction of oxidative stress by antioxidants can prevent the situation caused by ROS and, therefore, antioxidants should have “anti-aging” effects. This is the reason why people use antioxidants along with the fact that it is generally accepted that antioxidants vitamins are good for the health and that epidemiologic studies relate fruit intake with less incidence of diseases. Unfortunately, nowadays, none of the antioxidant formula allows us to “escape” from getting old and the clinical utility and health benefits of antioxidants are even in doubt.



Besides this fact, it is true that several pathologies have been associated with the oxidative damage produced by ROS and many studies have demonstrated the beneficial effects of different protective, antioxidant compounds. One of these protective compounds used in antioxidant therapy is folic acid (FA), which has been extensively by our research group.

Structure, digestion and absorption of folates/folic acid

Folate/folic acid is a generic term for a family of compounds, all of them have a common structure (Fig. 1) containing the pteridine ring, p-amino benzoic acid, and glutamic acid. The pteridine-benzoic acid skeleton can be conjugated with one or more L-glutamic acid residues. The pteridine ring can be reduced either with hydrogen at the 5,6,7, and 8 positions (tetrahydrofolate-THF) or with hydrogen at the 7 and 8 positions (dihydrofolate-DHF). These reduced derivatives are the active forms and can accept one carbon unit at the 5 and 10 positions (Methyl-THF or Methyl-DHF).



Folic acid is synthetic form used in dietary supplements and in fortified foods. It contains a single glutamic residue, it is totally oxidized and, therefore, it is very stable. Folate is the natural

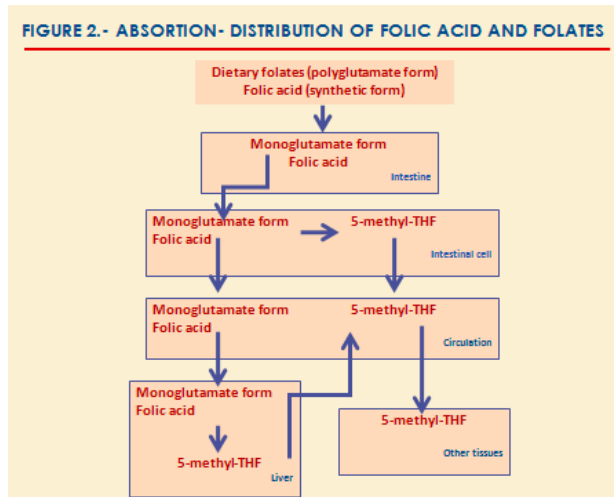
form of the vitamin found in foods, and contains several glutamic residues. Leafy green vegetables (like spinach-140 µg/100 g), fruits (like citrus fruits and juices-20-40 µg/100 g), dried beans (180 µg/100 g) and liver (182 µg/100 g) are all natural sources of folate. Also, fortified cereals have become a very important contributor of folic acid to the diet (50-200 µg/100 g). As folic acid is a water-soluble vitamin it is lost (up to 90 %) during cooking. This can be reduced by avoiding over-cooking, and steaming or microwaving vegetables instead of boiling.

Polyglutamate form must be digested to the monoglutamate form for absorption (Fig. 2). The hydrolysis is performed by the enzyme pteroylpolyglutamate hydrolases, which is zinc-dependent and, therefore, zinc deficiency impairs the digestion and absorption of folate. Folic acid does not need to undergo digestion because it is already present in the monoglutamate form. Therefore, it has a higher bioavailability (> 90 %) than natural folates (~ 50 %), being rapidly absorbed into intestinal cells. Chronic alcohol ingestion diminishes the absorption of this vitamin.

Inside the intestinal cell, the mono-glutamic molecules (coming from the natural and synthetic forms) are reduced and methylated to form 5-methyl-THF by the participation of the enzyme NADPH dependent dihydrofolate reductase, whose activity is really low in humans.

Folate is absorbed by both saturable and unsaturable mechanisms. The saturable processes are specific and tend to occur in the upper small intestine. These processes mediate the absorption of a variety of folates, usually after the polyglutamated forms have been hydrolyzed to monoglutamates. After entering the intestinal cell, folates are usually

converted to 5-methyl-THF. The entire process of specific absorption takes approximately 1 h from the time of ingestion.



Non specific, unsaturable absorption predominates in the ileum and allows nearly all folate reaching that site to be absorbed in linear proportion to the amount presented. This mechanism assumes importance whenever ingested folate exceeds the limited capacity of specific jejune absorption (200 µg). This is particularly relevant for the synthetic form (folic acid) since this process can deliver large amounts of unreduced folic acid from supplements that can reach the liver directly. In fact it has been postulated that liver instead of intestinal cells is the main tissue for the synthetic form (folic acid). This process can deliver large amounts of unreduced folic acid from supplements into the bloodstream without modification producing a chronic presence of non metabolized folic acid in blood. It has been found that non metabolized folic acids can be found after intake of 260–280 µg.

The enzyme methyl reductase is also present in liver, so that both monoglutamate forms and folic acid can be transformed to 5-methyl-THF, which in turn goes into the bloodstream. As can be seen, 5-methylTHF is the main form of folate found in

plasma. However, when supplementing with folic acid, and due to the low activity of both intestinal and hepatic reductase, it can be found a significant proportion of non metabolized folic acid in the bloodstream.

Most folate circulates in the blood attached to albumin and other proteins, although one third circulates unattached. Then, folates are distributed to tissues, mainly to those of high cellular turnover such a bone marrow, intestinal mucosa, because these tissues need folic acid for DNA synthesis.

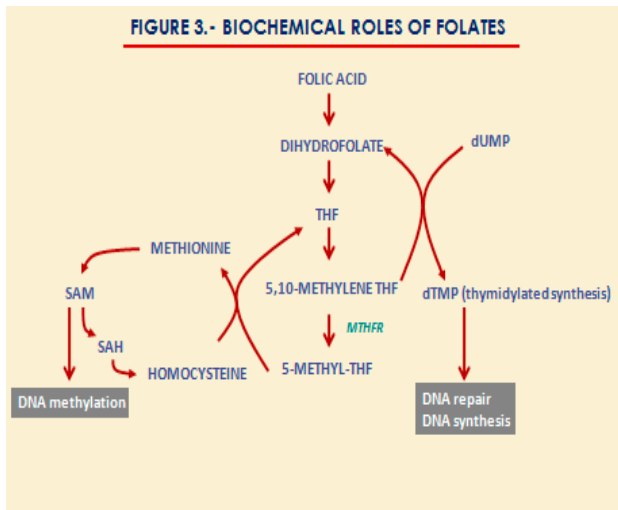
The presence of unmetabolized folic acid is an important issue because this form elicits and antifolate effect via competitive interaction with carriers, receptors and enzyme within the body. Thus, it has been described that folic acid can compete with the entrance of 5-MTHF into the brain [8]. Also, the excess of folic acid in the bloodstream has been inversely correlated with the activity of natural killer lymphocytes, which are one of the first lines of defense against cancer. In addition, unmetabolized form can diminish the levels of cellular methionine. In summary, large increases in the concentrations of FA can inhibit several folate-dependent enzymes.

Biochemical functions and biological activity

In the circulation, folate is mainly present as 5-MeTHF, which is taken by the cells via specific carriers or receptors. In the cell, folate donates one-carbon units for use in methylation reactions and in purine and thymidine synthesis. The utilization of 5-MeTHF starts with the transfer of a methyl group to homocysteine, which is converted in methionine. Then, the THF formed gets a methyl group from serine, before the participation in several biochemical interconversions involved in purine and thymidine synthesis (Fig. 3)



FIGURE 3.- BIOCHEMICAL ROLES OF FOLATES



Deficiency of folates

Folate deficiencies are observed in some population groups under some special circumstances. For instance, in the elderly caused by inadequate intake; in pregnancy as a consequence of an increase of the requirements; intestinal diseases, where folate absorption is diminished; in chronic alcoholism, because alcohol affects folate absorption; in spite of the fortification policy, typical folate intakes are found to be sub-optimal in the diet of many people [9]. This widespread under-provision of folate is generally attributed to the poor bioavailability of natural food folates.

Folic acid and health

As we have seen, folate plays an important role in the biosynthesis of thymidine and de novo generation of methionine for genomic and non-genomic methylation reactions. They are also required for purine synthesis as well as serine-glycine interconversion. This means that folate is important for the structural integrity of DNA. Folate deficiency may cause accelerated telomere shortening [10]. Folate is also critical for maintaining homocysteine levels within a non-pathological range. High blood plasma levels of

homocysteine have been linked to many major disease conditions such as cardiovascular disease, Alzheimer, dementia and certain cancers [11,12]. These effects can be due to either a neurotoxic effect of homocysteine either to a lower availability of s-adenosylmethionine, which can lead to lower methylation levels of brain tissues. The levels of homocysteine can be elevated in the case of methylenetetrahydrofolate reductase (MTHFR) polymorphisms.

There is a big list of studies that implicate folate status with a range of chronic developmental diseases [8]. The most affected tissues are those with a high cellular turnover such as hematopoietic precursors, epithelial cells, etc. This is the reason why the clinical symptoms of acute effect of antifolate compound such a methotrexate are digestive, epithelial and hematologic problems. In addition, folate has a well established protective role against both occurrence and recurrence of neural tube defects (NTD). More recently, it has been described that low folate status is associated with poor cognitive function and dementia in the elderly [13]. On the contrary, the supplementation with folic acid over a 3-year period appears to reduce the rate of cognitive decline in older adults [14].

Other protective aspects of folic acid base are less known. Our groups have been working on the antioxidant properties of folates [15-17]. Some results from these studies are: folic acid supplementation improves the levels of oxidative stress markers in individuals with hypertension, overall in those patients whose initial parameters values were highest; Folic acid is useful in the prevention of damage and health problems of individuals born from mothers that have alcohol during pregnancy and lactation; the antioxidant



capacity of folic acid is similar to vitamin E, so that folates can be useful in protecting the aqueous part of the cells; folic acid protects very important pathways from oxidation such as rho guanine nucleotide dissociation inhibitor -RhoGDI-1- (an important protein involved in signal transduction), protease ER60 (involved in assembly of major complex histocompatibility and chaperone function) and gelsolin (involved in the response to stress and cellular differentiation).

In addition, it is worthy to note the dual role of folate on cancer. Folate plays a key role in cellular differentiation and proliferation as well as in DNA repair, so that it is necessary for tissue maintenance and regeneration [18]. Low folate status is associated with DNA strand breaks, and impaired DNA repair, increased mutations and aberrant methylations. Folate is critically required for cell division and growth because it is a cofactor in the *de novo* synthesis of purines and thymidylate and thus in nucleic acid synthesis. However, in cancer cells, where DNA replication and cell division occur at a rapid rate, removal of folate or a blockade of its metabolism causes inhibition of tumor growth. This is the basis of the use of antifolate drugs in cancer chemotherapy (methotrexate).

Consequently, the timing and dose of folate intervention are critical: if folate supplementation is started before the establishment of neoplastic process, the development and progression of the tumor is suppressed. On the contrary, if supplementation is started once the tumor is present, it can enhance its growth and progression [18,19]

In summary, it is very well known that folic acid protects important biological processes and prevents some important diseases. However, it is necessary to avoid daily doses higher than 200 µg

of synthetic form for a long time and, ideally, it is recommendable to be sure that a neoplastic process has not started before supplementation.

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