INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Review Article

FOLIC ACID BIOAVAILABILITY: A REVIEW

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Article Received on: 01/03/20 Approved for publication: 03/04/20

DOI: 10.7897/2230-8407.110434

ABSTRACT

Folic acid is an essential vitamin belonging to the B complex family. Folic acid is essential in humans and is important in both cytoplasm and mitochondria of the cell. In Cytoplasm, it plays a vital role in amino acid and Purine synthesis. Humans do not have *de novo* synthetic pathways for folic acid; thus, regular intake is required. Folate deficiency is observed in people who either do not consume folate-rich foods or have pathological conditions that prevent folate availability. Deficiency of folate results in anaemia, neural tube defects, foetal anomalies in pregnant women and cognitive disorders in adults. Fortification of foods with folate plays a crucial role in populations who are folate- deficient. Mandatory fortification in several high-income countries has shown positive effects in the population. Due to the impact of fortification on reducing symptoms associated with folate - deficiency, it is worth considering folate fortification in low-income countries as a mandatory approach.

Keywords: Folic acid, tetra hydro folate, dihydrofolate, methyl folate, neural tube defects, supplementation, fortification

INTRODUCTION

Folic acid structure and functions

Folic acid is an essential vitamin belonging to the Vitamin B complex family. It is water-soluble. Folic acid consists of three distinct structural units which include 6-methyl pterin, para-amino benzoic acid and glutamic acid figure (1). The number of glutamate residues varies, giving rise to different forms of folic acid. Folic acid was first discovered in the 1930s as a factor found in microorganisms that could cure people of megaloblastic anaemia.

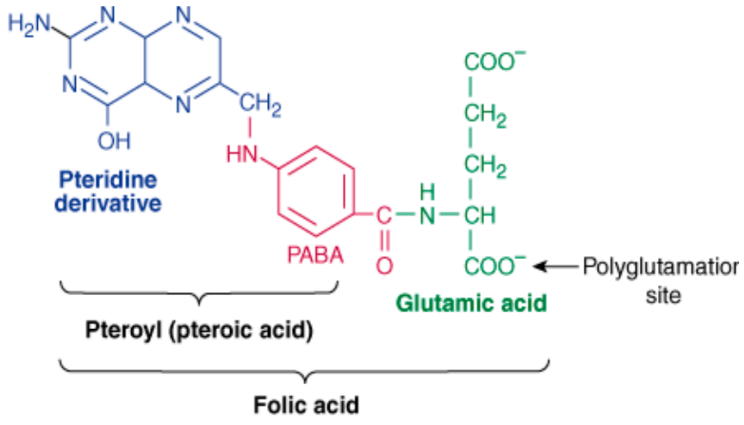

Figure 1: Folic acid structure [1]

Folic acid is abundantly found in green leafy vegetables. The naturally occurring form of folic acid is folate in foods. Folic acid is usually the synthetic form of the vitamin which is used in foods. For folic acid to be active in the cells, it is mainly converted to folate. Folate exists in different oxidative forms in the cells, which include tetrahydrofolate and dihydrofolate. Tetrahydrofolate is a critical cofactor in the synthesis of various purine nucleotides and thymine. Tetrahydrofolate is essential for the synthesis of amino acids such as Serine, Methionine, Glycine and Histidine. Dihydrofolate is essentially the product generated from tetrahydrofolate. Eventually, the dihydrofolate is regenerated to tetrahydrofolate for maintaining the folate levels in the cell. *De novo* purine synthesis requires the presence of 5,10-methylenetetrahydrofolate. The carbon 2 and carbon 8 of purine ring are derived from 5,10-THF respectively. *De novo* pyrimidine synthesis does not require THF. However, synthesis

of thymidine requires a methyl donor, and the methyl group is transferred from 5,10 THF. Thus, THF is essential for cell cycle activity and for repairing double-strand breaks in DNA. Tetrahydrofolate plays a vital role in single carbon transfer assisted reactions in biological systems.

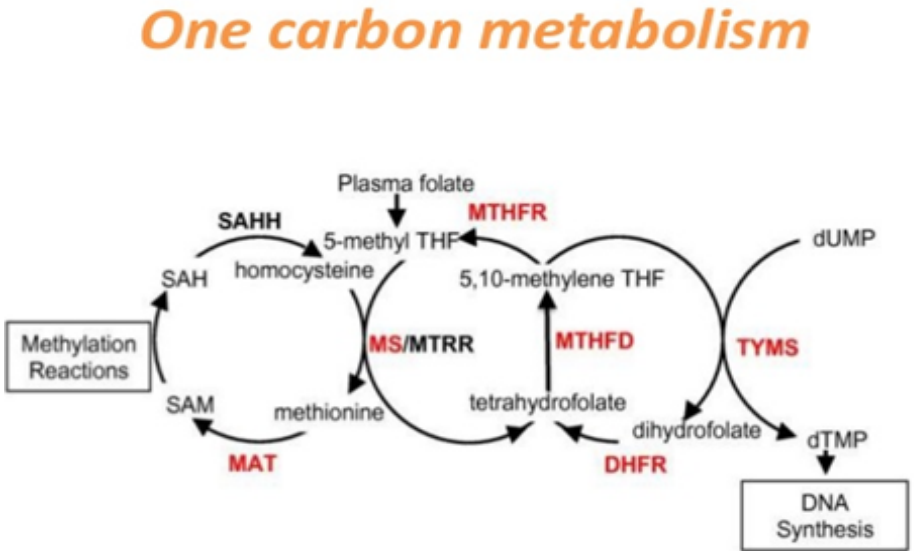

Figure 2: One-carbon metabolism [2]

In the cytosol, the conversion of Serine to Glycine is mediated by serine hydroxy methyltransferase. It involves a one-carbon transfer in the form of formaldehyde from serine resulting in the formation of glycine. Tetrahydrofolate is a regulator of this enzyme. Folate plays a vital role in mitochondrial one-carbon metabolism. In mitochondria, dimethylglycine is converted to sarcosine and then glycine, which involves one-carbon transfer. Tetrahydrofolate serves as a regulator of this enzymatic process [3].

Humans do not have enzymes for *de novo* biosynthesis of folate. Also, humans cannot store folate for a long time and hence it is a primary requirement that needs to be incorporated very frequently in the diet. Folic acid is absorbed as folate by both passive and active means. Passive transport is very ineffective, as only small quantities can be absorbed by this method. Active transport is the major route for absorption. Reduced folate carrier is a protein found on the surface of the lumen, which plays an essential role in the transport of dietary folate. Folyl conjugase enzymes are also present in the intestine, which helps in the uptake of folate. Specific transport proteins mainly mediate folate distribution to various organs. The absorption of folate in human is mainly affected by age, health conditions and genetic disorders. In pregnant women, folate binding protein in placenta helps in supply of folate to the foetus. The liver is the main organ controlling the transport of folate to different tissues [4].

Folate metabolism is not very clearly established in humans. This is mainly due to the lack of animal models that could be used for understanding human folate metabolism. Due to its essential function in the cell, they have developed efficient methods for transport of folate. Reduced folate carrier, anion exchanger and folate receptor are the main transporters found on the surface of different cells which are involved in folate transport. The folate receptor is a transmembrane protein that transfers folate directly into the interior of the cell. In the cell, folate is critical in cytoplasm and mitochondria. In the cytoplasm, it is involved in the amino acid synthesis and purine synthesis. In mitochondria, it is included in glycine and serine synthesis. Polyglutamylation of folate affects the enzymatic activity of a few enzymes where folate is a cofactor [5].

Biological sources for folic acid

Folate is present in many of the foods that are consumed. The most common ones include eggs, liver, yeast, green leafy vegetables, fruits, oranges, beans and eggs, respectively. Many of the foods that contain folate are also rich in ascorbic acid, which is an antioxidant that protects folate. Folate insufficiency or deficiency are associated with low consumption of fresh fruits, vegetables and liver. Studies done in USA have shown that the average consumption of folate is less than 0.2mg per day. Thus, folate insufficiency. Folate status is reflected immediately in serum and diminishes with time. Folate in RBC is present for the entire life span and levels are indicative of the extent of folate present. [6].

Folic acid is synthesized in bacteria from GTP (guanosine-5 – triphosphate). GTP is converted to pterin by GTP cyclohydrolase I and is the committed step in the whole process. The pterin ring undergoes a series of reactions that result in the formation of dihydropterin pyrophosphate. The dihydropterin pyrophosphate is conjugated to para-aminobenzoic acid by dihydropteroate synthase. The conjugate is complexed with glutamate to form dihydrofolate. Dihydrofolate reductase (DHFR) catalyses the conversion of dihydrofolate to tetrahydrofolate. Folic acid synthesis has been extensively studied to understand the various inhibitors that could be developed to inhibit the pathway. These act as potent anti-microbial molecules. One of the earliest to be discovered was sulphonamides which act as an inhibitor of dihydropterotate synthase as they act as analogues of para-aminobenzoic acid. Dihydrofolate reductase is inhibited by diaminopyrimidine class of antibiotics such as trimethoprim. A combination of sulphonamides and diaminopyrimidines have been used for treating bacterial infections successfully [7]. Methotrexate or amethopterin is another drug that is widely used in inhibiting DHFR. Methotrexate is a structural analogue of folate that competitively binds to DHFR. It is one of the prime drugs used in cancer and autoimmune disorders [8].

Different types of folic acid

Additional glutamate residues are added to folic acid by folyl polyglutamate synthetase (FPGS). FPGS are seen in different organisms and play an important role in the generation of polyglutamylated folate. Polyglutamated species of folate have a very weak affinity for folate transporters. They are restricted to intracellular compartments and cannot be transported easily. Polyglutamylation affects folate availability in various compartments. The presence of polyglutamylation results in the association of such derivatives with enzymes utilising folate cofactors. Plant methionine synthases, serine hydroxymethyl transferase and glycine decarboxylase complex show a very high affinity for polyglutamylated folate over mono folate derivatives [9]. In the budding yeast *Saccharomyces cerevisiae*, the FPGS is very important in maintaining the mitochondrial genome and in methionine synthesis. Mutations in this gene result in folic acid auxotrophy in *S.cerevisae* [10]. Folate in cells exists in different forms based on the modification of the pteridine ring system. The unmodified form includes folic acid. The various modified pterdine ring-based folates include tetrahydrofolate, dihydrofolate, 5,10-methylenetetrahydrofolate, 10-formyl tetrahydrofolate and 5-methyl tetrahydrofolate respectively.

Folic acid deficiency and its effect

Folate deficiency is often associated with the increased nutritional requirement of folate or due to a lack of folate-rich food. Several conditions such as alcoholism, use of antifolate drugs or any other pathological conditions could affect folate availability resulting in its deficiency. Folate deficiency has multiple effects as it is involved in a large number of cellular processes which are basic for the functioning of the cell. Increased folate is seen during pregnancy. Folate is essential for neural tube development and folate insufficiency during foetus growth can have serious effects on the development of the baby. During pregnancy, due to a large number of cell division and replication events, the amount of folate required is very high. If folate is not taken in sufficient amounts, it will affect the child and the mother. The foetus develops complications in the absence of folate, which include neural tube defects, congenital heart defects, the low weight of the baby and neonatal folate deficiency [11].

Low folate levels are associated with many of the cancers such as leukaemia, colorectal, breast, prostate and lymphomas. Due to low folate status in the body, there is a large number of DNA strand breaks in the cells which are not effectively repaired, resulting in induction of cancerous growth in the system. Low folate is associated with hypomethylation of DNA, which results in overexpression of proto-oncogenes which causes cancer [12].

Folate is involved in the synthesis of homocysteine. Alteration in the homocysteine pathway results in lowering the uptake of folate. This leads to hypertension and the effect on heart functioning. Folate deficiency is associated with megaloblastic anaemia. The earliest symptoms being a decrease in serum and RBC folate. Changes in the bone marrow and other dividing tissues become prominent. Megaloblasts begin to appear, and blood cells look highly abnormal with the fragmentation of nuclei. Erythroblast replication is inhibited, which results in lack of RBC synthesis, and hence the oxygen-carrying capacity of blood is reduced [13].

The global scenario of folic acid deficiency or insufficiency

Folate deficiency has been studied worldwide in order to understand the magnitude of the problem. A detailed study in various countries has shown that it is, in fact, a major public health problem. Many of the countries analysed have folate deficiency which occurs at different age and biological groups. Preschool children are maximum affected amongst Venezuelans (33.8%). The highest deficiency was seen in pregnant women of Costa Rica (43.8%) and Venezuela (25.5%). Up to 15% of the elderly population in the UK is affected by this deficiency. In USA before fortification of foods, 2.3% of school children, 24.5% of adults and 10.8% of elderly were found to be deficient in folate. However, a complete picture of folate deficiency is difficult to be obtained since many countries do not conduct regional screening and some of them do not even screen for folate deficiency [14].

Inadequate folate in women of reproductive age (WRA) can result in megaloblastic anaemia in pregnant women and neural tube defects in the fetus. A systematic survey was carried out to understand the folate status in countries with WRA. As per the review conducted the prevalence of folate deficiency was greater than 20% in lower-income countries. It was less than 5% of higher-income countries. However, the folate deficiency was assessed by different methods in different countries which could show differences in the overall outcome. Afghanistan, Cambodia, Sierra Leone and Ethiopia reported folate deficiency. Belize and Jordan reported folate insufficiency as a factor for neural tube defects [15].

Overview on supplementation of folic acid

Folic acid as a drug in the market

Folic acid is a significant nutraceutical with very high demand in the market. It is mainly used in the food industry and during the periconception period. The global market value of folic acid is 665.2 million in 2015. It is expected to grow at a rate of 4.1% per year from 2019 to 2026. The better utilisation of folic acid is for nutraceuticals followed by food beverages and pharmaceuticals. Folic acid is sold under brand name as Folvite, Focalgin B, Ortho D, Bonnisara and Restora Rx. All of them have folic acid along with supplements to enhance the absorption of folic acid in the digestive system (Folic acid brand name list).

Supplements of folic acid (Active and oxidized form)

The synthetic form of folate is folic acid. This is the most common form found in tablets and used in fortification of foods. The dietary form of folate is dihydrofolate. Dihydrofolate is found in green leafy vegetables, eggs and whole grains. Both folic acid and folate are inactive substrates. The biologically active form of folate is L-methyl folate. It crosses the blood-brain barrier and is vital for neural development. It plays an important role in the synthesis of neurotransmitters such as serotonin, dopamine and nor epinephrine. L-Methyl folate is synthesized from inactive folic acid. Folic acid is converted to tetrahydrofolate in series of reactions catalysed by DHFR. The Dihydrofolate is converted to 5,10 methylene tetrahydrofolates by serine hydroxymethyltransferase. The final conversion of methylenetetrahydrofolate is done by methylenetetrahydrofolate reductase to generate the biologically active methyl folate. Methyl folate supplementation in the form of capsules is also prescribed for curing folic acid-induced anaemia and for neural tube defects[16].

Fortification of foods with folic acid

Food fortification is defined as enhancing the micronutrient supply by adding micronutrients to the foods for enhancing uptake by the population. This results in improving the

nutritional quality of food and subsequently helping the population to overcome the micronutrient deficiency. Supplementation of foods with micronutrients has been done for a very long time. Iodine was the first element to be supplemented in salts. Following the success of iodine fortification vitamins such as niacin, thiamine and riboflavin were used for food supplementation to increase the availability. The fortification of food with micronutrients is a feasible option in low-income countries for reaching a large population and thus helping in overcoming the deficiency. USA was the first country to introduce wheat flour fortification with folic acid. The introduction of fortified flour resulted in a decrease in neural tube defects and enhanced folate status in blood. Based on these observations, Canada and Chile also introduced fortified wheat flours to overcome folic acid insufficiency. Fortification has several advantages which include maintaining the nutrient level in the body, enhancing the nutritional uptake, cost-effectiveness and minimal toxic effects [17].

There are different types of fortification which include mass fortification, target driven and market-driven fortification. Mass fortification is usually achieved where the deficiency targets bulk of the population. Target driven is for a selective group of population which suffer from a specific deficiency. This group usually includes school children and infants who are susceptible to deficiency. Market-driven fortification includes the manufacturer fortifying the food to help in maintaining the nutrient levels for public health.

Folic acid is stable and can be added to foods relatively easily. It is moderately heat stable and is susceptible to oxidation or reduction. It is light yellow and is added to foods ranging from 1.5 to 2.4ppm. A maximum loss of 20% is seen during the fortification of biscuits and pasta. However, most of it is available for consumption. There are no much negative effects reported for folic acid fortified foods since the amount in foods typically low and is not overdosed [17].

Global review on the impact of fortification

Several of the studies have been done, which prove that fortification has a positive impact on folate availability and uptake in the general population. Studies were done in USA and Canada wherein fortified flours were used to show a 25 to 50% reduction in haemolytic anaemia and neural tube defects. Large scale studies done in China in both high risk and low-risk areas showed that 400 micrograms of folic acid could prevent the incidence of anaemia significantly. In pregnant women, the incidence of foetal abnormalities was significantly reduced upon fortification with folic acid. Studies observed that folic acid fortification reduces the cognitive problems associated with patients. Up to 1000 micrograms per day of folic acid have been tested in fortification with no adverse effects reported [18].

Mandatory fortification of wheat flour and cereal grains with folic acid has been applied in 53 countries. In USA wheat flour fortification was implemented in 1996. 140 µg of folic acid per 100 g of enriched cereal grain product is effective in preventing the folate deficiency symptoms. In Canada, a mandatory fortification was introduced in 1998, and 150 µg of folic acid per 100 g of cereal was found to be sufficient to avoid the various defects. Apart from these two countries Chile, South Africa and Costa Rica, have all used mandatory fortification. In all the countries where mandatory fortification was done, the neural tube defect incidence reduced anywhere from 19-40%. In all these countries, the subjects have shown enhanced serum and RBC folate, which is associated with reduced neural tube defects [19].

Amarin and Obeidat (2010) studied the effect of folic acid fortification in the prevention of neural tube defects in Jordan. The main objective was to find a positive correlation between fortified foods intake and the incidence of neural tube defects in pregnant women. The study observed that flour fortification reduced the incidence of neural tube defects from 1.85% to 1.05% per 1000 births [20].

DISCUSSION

Folic acid is an essential micronutrient. However, global status on the extent of deficiency is not exactly clear. The various studies related to folate deficiency have been studied in high-income countries, and hence there is a lot of data available. However, in low-income countries, the picture is not very clear. So, it is essential to analyse the folate status in the general population to understand the extent of deficiency in the given population. Based on the analysis supplementation of folic acid needs to be tried out. Fortification based on literature is the best idea to address this issue. A systematic study using fortified foods needs to be carried out in order to understand the impact of fortification in low - income countries.

ACKNOWLEDGMENT

The authors would like to thank the Faculty of Pharmacy and Medical Sciences –University of Petra and Faculty of Agriculture -Jordan University, for supports on finishing this research work.

CONCLUSION

WHO has already developed guidelines for the fortification of foods with folic acid. There is extensive data that prove that fortification helps in reducing the symptoms associated with folate deficiency. Hence, it is important to consider fortification as a primary requirement to eliminate folate deficiency. Mandatory fortification needs to be achieved in order to address this issue. The role of flour manufacturing companies in mandatory fortification of foods will be a key point for consideration.

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Cite this article as:

Zainab Z. Zakareia *et al*. Folic acid bioavailability: A review. Int. Res. J. Pharm. 2020;11(4):8-12 http://dx.doi.org/10.7897/2230-8407.110434

Source of support: Nil, Conflict of interest: None Declared