Folic Acid Effect on Arginase Activity in Human Colostrum and Mature Milk

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Abstract

Folic acid, or folates are available from a large variety of sources, such as green leafy vegetables (spinach, beans) and liver. The importance of folates is based on their requirement as coenzymes in the transfer and utilization of one-carbon units in a variety of biosynthetic reactions. Folate is an essential cofactor for the de novo biosynthesis of purine nucleotides and thymine, thus influencing RNA and DNA synthesis. Therefore, during periods of rapid cell regeneration and growth, such as pregnancy and infancy, increased amounts of folate are required. Folate has the roles in the prevention of neural tube defects, an important cause of infant mortality and disability. The periconceptional folic acid intake reduces the risk of neural tube defects.

Arginase, as part of the urea cycle, detoxifies human body from ammonia. Its activity is important in the production of ornithine, subsequently leading to the production of polyamines (spermine and spermidine), glutamate or proline, and nitric oxide. Arginase is normally present in mother’s milk.

In the present study, the effect of folic acid supplementation during pregnancy on the dynamics of arginase activity and malondialdehyde concentration in human colostrum and mature milk was studied. The obtained results suggest that in breastfeeding mothers’ colostrum and mature human milk samples, supplemented with folic acid during pregnancy (400 µg/daily periconceptionaly), the arginase activity increases compared with the enzyme activity in milk samples taken from mothers who did not take folic acid. At the same time, the supplementation with folic acid causes malondialdehyde decrease in colostrum and mature milk.

Key words: arginase activity, malondialdehyde, folic acid, colostrum, mature human milk

Introduction

Human milk represents the main food for the newborns. Folic acid, folacin or pteroil glutamic acid belongs to the group of water soluble vitamins. Fresh leafy green vegetables (cauliflower), kidney and liver are rich sources of folic acid. Although, according to chemical nomenclature, the difference between folate and folic acid is just one proton, in general the term folic acid is applied to the synthetic form of B9 vitamin. Folic acid is the more stable of the two forms and so is more suitable for use in tablets and fortified foods. Folate is one of many compounds of fruits and vegetables that could be acting as a cytoprotective agent. Liver tissue serves as storage for folic acid (1,2).

According to its structure, folic acid belongs to conjugated pteridines, consisting of a pteridine ring, paraaminobenzoic acid and glutamic acid, monoglutamate or polyglutamate.

Only the monoglutamate form may be absorbed in intestinal wall. Folic acid is then reduced within cells to dihydrofolate (DHF) and, further to tetrahydrofolate (THF), by the action of dihydrofolate reductase (DHFR). The basic function of folic acid, as of THF derivatives, is to carry and transfer one carbon units, such as methyl-, methylene-, methenyl-, formyl or formimino-groups to various substrates in a variety of enzymatic reactions that are intimately related in synthesis of RNA and DNA, stability, integrity, and repair of DNA (Scheme 1).

Folate plays an essential role in remethylation of homocysteine to methionine, which is a precursor of
S-adenosylmethionine, the primary methyl group donor for the most biological methylations, and for phospholipid (lecithine) synthesis. DNA methylation is an important epigenetic determinant in gene expression, in the maintenance of DNA integrity and stability, in chromosomal modifications, and in development of mutations (2-5), which can regulate tissue-specific expression of certain genes that is especially important during embryogenesis (6).

In folate deficiency, all forms of folate are reduced within cells, impairing the growth and maturation of rapidly growing tissues and cells (7-10). Folate deficiency enhances DNA hypomethylation. Genomic and site-specific DNA hypomethylation has previously been considered as a leading mechanism by which folate depletion enhances mutagenesis, so folate supplementation can correct some of these defects (10-12).

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The objective of this study was to determine the effect of folic acid supplementation to pregnant women on arginase activity and concentrations of oxidative stress indicator malondialdehyde (MDA) in colostrum and mature human milk samples during the first month of lactation.

Materials and Methods

The study included 45 women, 18-39 years old, who have given birth at the Clinic for Obstetric and Gynecology in Clinical Center, Faculty of Medicine, University of Nis, Serbia, with normal delivery, without the existence of complications such as diabetes, hypertension or eclampsia. 27 of them received periconceptionally Folan pill, containing 400 µg of folic acid, alone daily in accordance with international recommendations (31).

The samples of human milk were obtained from mothers of term infants on the 1st and 2nd day (colostrum) and 30th day of lactation. The first day of lactation was defined as the first day of production of transitional milk, and was either the second or the third day after delivery. The milk samples were obtained fresh, after the infant had suckled for 5 minutes with a manual breast pump (Ginevri, Milan, Italy). The samples of human milk were stored at -20°C until arginase activity and MDA concentration analyses were done.

The arginase activity was measured with spectrophotometric method on the basis of the determination of the amount of liberated ornithine from arginine as substrate (32). The amount of malondialdehyde,
presenting the level of lipid peroxidation, was determined by the spectrophotometric method, using thio-barbbituric acid purchased from "Sigma"(33,34). The MDA standard was prepared with 1,1,3, and 1-tetramethoxypropane (Sigma) (35).

The study was approved by the the Ethical Committee for Medical Research, Clinical Center, Faculty of Medicine, University of Nis (protocol number 01-3565-1). Informed consent was obtained for every enrolled nursing mother.

Statistics: The obtained results were statistically analyzed using Student's t-test applying the SPSS computer statistical program. All the results are presented as mean ± SD.

Results

The obtained results suggest that in colostrum and mature human milk samples of breastfeeding mothers, supplemented with folic acid during pregnancy, arginase activity increases compared to the enzyme activity in milk samples taken from breastfeeding mothers who were not on folate supplementation (Fig. 1).

![Figure 1. Effect of folic acid on arginase activity in colostrum and mature human milk (a - without folic acid; b - with folic acid); ###p <0.001 in comparison with colostrum and milk samples without added folic acid](image1)

Arginase activity in colostrum and mature human milk samples of mothers, supplemented with folic acid, was significantly increased (62.6 ± 3.02 U/L, p <0.01; 51.8 ± 2.90, p <0.001; 47.9 ± 2.71, p <0.05, respectively) compared to the enzyme activity in milk samples taken from mothers who were not on folate supplementation (55.9 ± 5.56 U/L; 43.7 ± 3.23; 41.9 ± 2.97, respectively).

After folate supplementation (Fig. 2), the significant decrease of MDA amount was observed in colostrum on 1st and 2nd day of lactation (8.30 ± 0.99 µmol/L, p <0.001; 7.12 ± 1.23, p <0.001, respectively) and mature human milk on 30th day of lactation (5.35 ± 1.05 µmol/L, p <0.001), compared to the sample of mothers who were not on folate supplementation (10.4 ± 1.07; 9.15 ± 0.83; 6.76 ± 0.62 µmol/L, respectively).

![Figure 2. Folic acid effects on MDA levels in colostrum and mature human milk (a - without folic acid; b - with folic acid); ###p <0.001, **p <0.01, #p <0.05 Compared to the values without folic acid](image2)

Discussion

Human milk is the main source of nutrients for newborn infants during the first months of life. The enzyme systems normally present in mothers’ milk may influence the health and nutrition of the newborn infant. There is a possibility that the sources of milk enzymes are mother’s blood and the mammary glands (36). Studies with animals show that colostrum intake influences plasma enzyme activities of the newborns, since colostral enzymes are absorbed. The physiological importance of the absorbed enzymes in the newborns is not clear at present. The activity of many enzymes can vary significantly due to diet, the stage of lactation, or other factors (37,38).

Currently, it is believed that folate deficiency affects DNA stability principally through two potential pathways: DNA hypomethylation and uracil misincorporation into DNA (3,4). 5,10-methylenetetrahydrofolate donates a methyl group to uracil, converting it to thymine, which is used for DNA synthesis and repair. Alterations in DNA methylation, with resulting changes in gene expression, can have important consequences for embryogenesis (3-6,22,39). If folate is limited, imbalances in DNA precursor pool occur, and uracil may be misincorporated into DNA. Folate deficiency causes massive incorporation of uracil into human DNA and chromosome breaks (40,41).
subsequent misincorporation and repair may lead to double strand breaks and chromosomal damage. Moreover, folate affects gene expression by regulating cellular S-adenosylmethionine (SAM). DNA-methylation is catalyzed by DNA methyltransferases that transfer methyl groups from SAM to cytosine (23,24). In the past decade, the literature data has established that low or inadequate folate status may contribute to congenital malformations (spina bifida and anencephaly), an important factor of fetal and infant mortality (17). Experimental studies, epidemiological data and clinical trials showed the necessity of sufficient quantities of folic acid for normal embryogenesis and fetal development for the prevention of neural tube defects and other neurological malformations and spontaneous abortion (39,42). Also, folic acid may have important roles in physiological pathways needed for successful pregnancy, including angiogenesis and vasculogenesis (21,43).

Mammalian cells are devoid of the enzymatic capacity for folate biosynthesis and thus are absolutely dependent on folate uptake from exogenous dietary sources. The reduced folate carrier (RFC), a bidirectional anion transporter, is the major uptake route of reduced folates essential for a spectrum of biochemical reactions. Therefore, the insufficiency of RFC may exacerbate the pathological status of the embryos caused by the folate deficiency (44).

A metabolic effect of folate deficiency is an elevation of blood homocysteine concentrations. To date, the best evidence from human studies indicates that impairments in the homocysteine remethylation, as the cause of low concentrations of dietary and circulating folate, can impair normal embryonic development and increase risks for NTD, infant low birth weight, and fetal growth retardation (2,10,21,42,45,46). Elevated concentrations of homocysteine can be reduced effectively by folic acid supplementation (47).

Hyperhomocysteinemia individuals are characterised by raised plasma levels of asymmetric dimethylarginine (ADMA), a novel risk factor for teratogenesis. Folic acid treatment reduces elevated plasma levels of asymmetric dimethylarginine in hyperhomocysteinaemic subjects.

Also, increasing evidence suggests that the beneficial effect of folate may be related to improved function of methionine synthase, a vitamin B12-dependent enzyme that converts homocysteine to methionine (49). Homocysteine itself is located at a branch-point of metabolic pathways: either it is irreversibly degraded via the transsulfuration pathway to cysteine or it is remethylated back (23,50,51). One of the primary functions of folic acid is to regenerate the essential, sulfur-containing, amino acid, methionine. Methionine adenosyltransferase (EC 2.5.1.6) converts methionine to SAM (the active methylating agent for DNA methylation) (23,24). As a stabilizer of cystathionine b synthase, SAM promotes the transulfuration pathway that generates GSH, while decreasing its precursor homocysteine (53,54).

Of the many enzymes present in milk our attention has been directed to arginase. In the available literature data there is no scientific data about the folic acid effects on arginase activity except that folic acid suppressed arginase activity (55). Arginase, through ornithine, is involved in the synthesis of polyamines, which control cell proliferation and collagen production. Proline, produced by arginase activity, is necessary for the production of collagen, which is needed for growth and development of newborns, after absorption from milk in digestive baby tract (56,57). Our present results suggest that folic acid increases arginase activity in human milk, which could downregulate the levels of nitric oxide (NO) and divert L-arginine metabolism toward cell proliferation and/or tissue regeneration by providing L-ornithine, which is the substrate of polyamine biosynthesis (58-60), needed for normal pregnant uterus and embryo development.

Folic acid has important roles in angiogenesis and vasculogenesis occurring during physiological pregnancy (21,43,60). Supplementation with folic acid during the very early stages of pregnancy may be beneficial by promoting extravillous trophoblast (EVT) invasion, which could be of benefit in preventing pregnancy disorders. Inadequate EVT invasion is associated with defective placental development, and intrauterine growth restriction. Placental angiogenesis is critical for development of a normal placental circulation, and consequently normal development of the baby (21,43).

Folic acid is able to induce angiogenesis, in part via a NO dependent mechanism (60); also, ornithin, the product of arginase activity, the precursor of polyamines synthesis is implicated in process of angiogenesis (61).

Folate has antioxidant properties, scavenging several reactive oxygen species in vitro and inhibiting lipid peroxidation (62). Folic acid may decrease oxidative stress in human diseases, significantly lowering the levels of free radicals in liver, brain, and kidney; and increasing the content of SH-groups (63,64).

Our study was performed to determine the effects of folate supplementation on the concentration of MDA in human milk. After folate supplementation, the significant MDA decrease was observed in colostrum and mature human milk. In mothers receiving Folan, the decrease of MDA in colostrum and mature milk was probably due to methionine increase in mothers' organisms, which is directly responsible for maintaining of reduced glutathione (GSH), the important antioxidant.

Taking in consideration the increased arginase to SAM.
activity, another mechanism of MDA lowering could be the production of higher amount of glutamate, the constitutive amino acid in glutathione structure. Our results are in agreement with the literature data suggesting that pharmacological doses of folate supplementation lower plasma homocysteine and serum malondialdehyde levels, probably by increasing glutathione (64). Thus, it is apparent that folate availability is important during the first few weeks of pregnancy, especially during embryogenesis, by providing the sufficient amount of methyl groups for metabolic processes in human body, especially DNA-methylation, which can regulate tissue-specific expression of certain genes.

The effects of folic acid supplementation on central nervous system growth processes are not restricted only to the embryonic period, but can also be effective for enhancing growth and repair in the later phases of pregnancy and during lactation (11).

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