Folic acid : Biomolecular review

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Abstract

After a long period of understanding on its role in the pathogenesis of megaloblastic anemia, increase attentions were given to folic acid in the last decade due to its role in the prevention of the development of various diseases, including neural tube defect, atherosclerosis heart disease, and cancer. Since then, a growing number of studies from community aspects to molecular level were conducted, and considerable progress has been made in our understanding of the metabolic role of folic acid in health and disease, especially its molecular mechanism of action in cell physiology.

Folates are present in various reduced, metabolically active, coenzyme forms, often conjugated in peptide linkage. During the extraction procedures, these labile active forms are either destroyed by oxidation or oxidized and converted to PGA, the most stable form of the vitamin. The effect of folate deficiency on fetal growth and development is the resultant deficiency of dTMP. The thymine deficiency may result in DNA that is subject to breaks and chromosomal instability.

The exact biochemical mechanism(s) by which folic acid affects fetal development is not clearly defined. Inadequate folate intake or a defect in the absorption of folic acid may contribute to the problem. Although the importance for normal brain development of folate intake early in pregnancy is well accepted, the requirements for folate intake late in fetal gestation are not well understood.

To date, the majority of scientific investigations about dietary folate requirements during pregnancy have focused on folate's role in preventing neural tube defects. It was suggested that folate availability also affects brain development long after neural tube closure, and indicates that it may be very important that women ingest adequate intakes of folic acid throughout pregnancy, especially important in those women with genetic polymorphisms in genes of folate metabolism.

Biomolekuler aspek dari asam folat

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Abstrak

Setelah lama dipahami perannya pada patogenesis anemia megaloblatik, perhatian lebih kuat kembali tertuju kepada asam folat pada dekade terakhir ini karena perannya pada pencegahan dari berbagai penyakit, seperti neural tube defect*, penyakit jantung koroner, dan kanker. Kini, banyak penelitian dari tingkat komunitas hingga molekuler dilakukan dengan hasil yang bermakna yang meningkatkan pemahaman kita terhadap peran metabolisme asam folat pada berbagai penyakit, khususnya mekanisme molekuler dari fungsinya ditingkat seluler.*

Asam folat ditemukan di tubuh dalamberbagai bentuk tereduksi, berbagai bentuk koenjim, bahkan banyak dalam bentuk ikatan dengan peptide. Selama proses ekstraksi, bentuk aktif yang tidak stabil ini akan rusak akibat oksidasi, atau berubah dalam bentuk PGA, yang merupakan turunan bentuk paling stabil. Efek defisiensi asam folat pada pertumbuhan dan perkembangan fetus adalah akibat defisiensi dTMP. Defisiensi thymin dapat menyebabkan gangguan sintesis DNA yang dapat berakibat pada instabilitas atau rusaknya kromosom.

Mekanisme biokimiawi dari efek asam folat pada perkembangan fetus belum sepenuhnya dipahami. Asupan asam folat yang tidak adekuat atau gangguan absorpsinya dapat menyebabkan permasalahan. Meskipun peran folat pada perkembangan otak normal telah dapat dipahami, kebutuhan asam folat pada tahap akhir perkembangan fetus belum dimengerti dengan baik.

Kini, kebanyakan penelitian tentang pemenuhan kebutuhan asam folat selama kehamilan ditujukan pada perannya dalam mencegah neural tube defect. Pemenuhan asam folat setelah penutupan tabung neural akan berpengaruh terhadap perkembangan otak. Hal ini menunjukkan pentingnya konsumsi asam folat yang adekuat selama kehamilan, khususnya bagi ibu hamil dengan polimorfisme gena yang berperan pada metabolisme asam folat.

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Introduction

The term folic acid, which was coined in 1941 by Mitchell et al., was first recognized in 1937 as Wills factor, a factor which was responsible for the development of macrocytic anemia among Hindu women in Bombay. After a long period of understanding on its role in the pathogenesis of megaloblastic anemia, increase attentions were given to folic acid in the last decade due to its role in the prevention of the development of various diseases, including neural tube defect, atherosclerosis heart disease, and cancer. Periconceptional vitamin supplementation with folic acid substantially reduced the risk of women's having neural tube defect-affected pregnancies, and it has been implicated in the reduced risk of several other congenital anomalies. Mechanism underlying this reduced risk has not been fully elucidated. Studies on genetic variations that influence cellular absorption, transport, and metabolism of folic acid may offer better understanding on this unknown mechanism. Since then, a growing number of studies from community aspects to molecular level were conducted, and considerable progress has been made in our understanding of the metabolic role of folic acid in health and disease, especially its molecular mechanism of action in cell physiology. This paper will review the biochemistry and the metabolism of folic acid, and some molecular or genetic defects related to folic acid role in health and disease.

The biochemistry of folic acid

Folic acid is a B vitamin, also known as folate and pteroylglutamic acid. Pteroylglutamic acid (PGA), the common pharmaceutical form of folic acid, can not be found as such in significant amount in either the human body or the various foods form which folates were isolated. Folates are present in various reduced, metabolically active, coenzyme forms, often conjugated in peptide linkage. During the extraction procedures, these labile active forms are either destroyed by oxidation or oxidized and converted to PGA, the most stable form of the vitamin. The major subunits of the molecule are the

pteridine moiety linked by a methylene bridge to p-aminobenzoic acid (PABA), which is joined by peptide linkage to glutamic acid. Crystalline folic acid is yellow with molecular weight of 441.4. The free acid is almost insoluble in cold water, whereas the sodium salt is more soluble. Folic acid is destroyed at a pH below 4 but is relatively stable above pH 5, with no destruction in 1 hour at 100^{o}C.

Folates, in the form of tetrahydrofolate (FH4), are able to donate single carbon units for intracellular reactions. The FH4 molecules that have donated carbon units are subsequently able to accept carbon units for further enzymatic activities. These carbon units are used in the formation of deoxythymidylate monophosphate (dTMP) and purines, necessary components for DNA and RNA synthesis. One explanation for the effects of folate deficiency on fetal growth and development is the resultant deficiency of dTMP. The thymine deficiency may result in DNA that is subject to breaks and chromosomal instability.

FH4 is also involved in the synthesis of amino acids glycine and serine and the conversion of homocysteine into methionine. Homocysteine can be irreversibly converted into the nonessential amino acid cysteine by the enzyme cystathionine synthase (CS). Alternatively, methionine synthase (MS) can revert homocysteine to methionine. FH4 acts as a substrate (carbon donor) for this reaction and cyanocobalamin (B_{12}) as a cofactor. The lack of either B_{12} or folic acid can result in an accumulation of homocysteine, and a decrease in methionine levels. The resultant disruptions in the methylation/demethylation activity may also affect the growth and development of the fetus.

The major point of entry for one-carbon fragments into substituted folates is methylene tetrahydrofolate, which is formed by the reaction of glycine, serine, and choline with tetrahydrofolate. Serine is the most important source of substituted folates for biosynthetic reactions, and the activity of serine hydroxymethyltransferase is regulated by the state of folate substitution and the availability of folate. The reaction is reversible, and in liver it can form serine from glycine as a substrate for gluconeogenesis. Methylene, methenyl, and 10-formyl tetrahydrofolates are interconvertible. When one-carbon folates are not required, the oxidation of formyl tetrahydrofoalte to yield carbon dioxide provides a means of maintaining a pool of free folate.

The methylation of deoxyuridine monophosphate (dUMP) to thymidine monophosphate (TMP), catalyzed by thymidylate synthase, is essential for the synthesis of DNA. The one-carbon fragment of methylene-tetrahydrofolate is reduced to a methyl group with release of dihydrofolate, which is then reduced back to tetrahydrofolate by dihydrofolate reductase. Thymidylate synthase and dihydrofolate reductase are especially active in tissues with a high rate of cell division. Methotrexate, an analog of 10-methyl-tetrahydrofolate, inhibits dihydrofolate reductase and has been used as an anticancer drug. The dihydrofolate reductases of some microorganisms differ from the human enzymes; inhibitors of these enzymes can be used as antibacterial drugs, such as trimethoprim, and anti malarial drugs, such as pyrimethamine.

Both vitamin B_{12} and folic acid are required for synthesis of thymidylate and thus of DNA. A vitamin B_{12}-containing enzyme removes a methyl group from methyl folate and delivers it to homocysteine, thereby converting homocystein to methionine (methyl homocysteine) and regenerating THF, from which the 5, 10-methylene THF involved in thymidylate synthesis in human serum and liver and probably also in other body storage depots for folate. Because methyl folate may only return to the body's folate pool via a vitamin B_{12}-dependent step, patients with vitamin B_{12} deficiency have much of their folate "trapped" as methyl folate, which is metabolically inactive. This "folate trap" hypothesis helps explain the hematologic damage of vitamin B_{12} deficiency that is not clinically distinguishable from that of folate deficiency. In both instances, the hematologic defect results from lack of adequate 5,10-methylene THF, whose methyl group is transferred in the conversion of deoxyuridylate to thymidylate used for DNA synthesis during the S (Synthesis) phase. In either deficiency, inadequate DNA synthesis causes hematopoietic cells to die in the bone marrow, possibly without ever completing the S phase of cell replication, which in turn known as a form of "ineffective erythropoiesis".

The DNA damage caused by folate (or vitamin B_{12}) deficiency increases the risk of cancer. Megaloblastosis (the presence of giant germ cells) can be due to slowed DNA synthesis of any cause. The finely stippled sieve-like open chromatin in megaloblasts suggests a defect in nuclear maturation. The precise molecular basis of megaloblastic maturation is not really understood. Poor thymidylate synthesis (due to folate and/or

vitamin B_{12} deficiency) may fail to promote elongation of DNA chains in the presence of a relatively normal capacity to initiate DNA synthesis. This process occurs, presumably, because the lowered thymidylate concentrations suffice for "initiation" but not for "elongation" of the DNA chain by polymerase. Alternatively, the defect may permit "forbidden" incorpation of thymidylate precursors (such as deoxyuridylate) into DNA, with subsequent cleavage of the DNA containing the forbidden/wrong nucleotide.

Folate metabolism.

Foods deliver folate mostly in the "bound" form – that is, combined with a string of amino acids (glutamate), known as polyglutamate. The folates in foods may have up to seven additional glutamate residues linked by γ-peptide bonds. In addition, all of the one carbon substituted folates may also be present in foods. The intestine prefers to absorb the "free" folate form with only one glutamate attached (the monoglutamate form). Enzymes on the intestinal cell surfaces hydrolyze the polyglutamate to monoglutamate and several glutamates. Then the monoglutamate is attached to a methyl group (CH_3). Special transport systems deliver the monoglutamate with its methyl group to the liver and other body cells. In order for the folate coenzyme to function, the methyl group needs to be removed. The enzyme that removes the methyl group requires the help of vitamin B_{12}. Without that help, folate becomes trapped inside cells in its methyl form, unavailable to support DNA synthesis and cell growth. To dispose of excess folate, the liver secretes most of it into bile and ships it to the gallbladder. Thus folate returns to the intestine in an enterohepatic circulation route like that of bile itself.

Food folate is absorbed primarily from the proximal third of the small intestine, although it can be absorbed from the entire length of the small bowel. Folate in food is primarily in polyglutamate form. Before absorption, the "excess" glutamates must be split off the side chain of the vitamin molecule by enzyme conjugases (pteroylpolyglutamate hydrolase). The products of conjugase action are detectable in the intestinal lumen before absorption and are due to a surface-active brush border conjugase that is functionally and chromatographically distinguishable from intracellular conjugase. A small but relatively unchanging percentage of ingested folate is absorbed by passive diffusion after deconjugation, as is the case with vitamin B_{12}. Passive diffusion may also account for

absorption of unreduced synthetic PGA eaten as a bolus in excess of 266 µg. The altered activity of the brush border folate hydrolase in certain diseases and following exposure to drugs such as salicylazosulfapyridine, alcohol, and diphenylhydantoin appears to play a significant role in causing malabsorption and eficiency of folate. The discovery that jejunal folate hydrolase shares gene homology with a prostate-specific protein and a neural enzyme implies folate hydrolase may relate to both brain development and carcinogenesis.

Folic acid absorbed from the intestine as physiologic concentration is largely converted in the gut lumen and enterocytes to reduced forms and then methylated or formylated; at higher concentrations, it is transported through the enterocytes without such modification. However, reduced and formylated or methylated forms of folate are transferred faster from the intestine to the circulation than is folic acid. How folate is transported from the enterocytes to the lamina propria of the villi or the circulation is poorly understood; a carrier mediated system was suggested and is now identified. Their physiologic function has not yet been demonstrated, but they may play a role in (a) delivering folate to liver, similar to the role of haptocorrin in vitamin B_{12} transport; (b) controlling folate distribution, breakdown, and excretion in deficient states; and (c) transporting oxidized folates from cerebrospinal fluid to blood. They are also present in milk, where they may enhance folate absorption from gut, and they may be related to membrane protein-mediated folate uptake. High-affinity folate-binding protein found in human serum appears to have a higher affinity for folic acid (PteGlu) than for reduced folate. Folate-binding proteins in several tissues closely resemble the serum binders.

This complicated system for handling folate is vulnerable to GI tract injuries. Since folate is actively secreted back into the GI tract with bile, it has to be reabsorbed repeatedly. If the GI tract cell are damaged, then folate is rapidly lost form the body. Such is the case in alcohol abuse; folate deficiency rapidly develops and, ironically, damages the GI tract further. The folate coenzymes are active in cell multiplication, and the cells lining the GI tract are among the most rapidly renewed cells in the body. Unable to make new cells, the GI tract deteriorates and not only loses folate, but also fails to absorb other nutrients.

Folate is delivered to bone marrow cells, reticulocytes, liver, cerebrospinal fluid, and renal tubular cells against a concentration gradient in a manner that suggests energy-dependent carrier-mediated transport. Normal total-body folate stores range from 5 to 10 mg (11.3 – 22.6 μmol), of which approximately half is in the liver. It has been suggested that the enterohepatic circulation, which transports about 0.1 mg (0.226 μmol) of biologically active folate daily, is important in the maintenance of serum folate levels.

Folate is excreted in urine and bile in metabolically active and inactive forms. Urinary excretion of the biologically active material occurs after glomerular filtration of the free fraction and reabsorption of some filtered folate by active transport across the tubular cell wall. The principal breakdown product of folate in urine, acetamidobenzoyglutamate, suggests that the principal route of folate catabolism occurs through oxidative cleavage of the folate molecule at the 9-10 bond, with acetylation of the ρ-aminobenzoyl moiety in the liver before excretion.

Studies have been conducted to determine if gastrointestinal absorption of folate is a factor in the development of NTDs. Supplements of 400 μu/d of folate begun before conception result in a significant reduction in the incidence of neural tube defects as found in spina bifida. The inability to break down complex folates or to transport the folate could result in folate deficiency. Deficiency of folic acid itself-or deficiency of vitamin B_{12}, which leads to functional folic acid deficiency-affects cells that are dividing rapidly because they have a large requirement for thymidine for DNA synthesis.

Genetic and molecular defect related to folate metabolism, taking NTD as the case.

The exact biochemical mechanism(s) by which folic acid affets fetal development is not clearly defined. Inadequate folate intake or a defect in the absorption of folic acid may contribute to the problem. Disruption in the intracellular activities of folic acid is another area under investigation. Genetic defects that alter the enzymes involved in folic acid absorption or the enzymes involved in the intercellular activities of folic acid have been implicated in NTDs. Sufficient evidence had accumulated linking a reduction in NTDs with folic acid intake. The activities of folic acid in the cell have been examined for defects and alterations that are associated with NTD occurrences.

Alterations in enzymes involved in the carbon transfer of folate result in methionine synthesis and homocysteine accumulation, a condition that can lead to the development of NTD. Genetic defects in MTHFR (methylenetetrahydrofolate reductase), Methionine synthase (MS), and Cystathionine synthase (CS) have the potential to result in increased levels of homocysteine. Elevations of homocysteine levels have been associated with NTDs. In women, maternal dietary methionine has demonstrated an inverse relationship with risk for NTDs. The genes for MTHFR and MS have been studied in association with NTDs. A common alteration in the MTHFR gene is a thymine for cytosine substitution at the 677 nucleotide. However, this alteration in the MTHFR gene accounts for a small portion (~13%) of NTDs. Other genetic alterations in the MTHFR gene may also be responsible for disrupted folate metabolism in NTD mothers. Another gene that has been implicated in the NTD/folic acid relationship is MS. One mutation of MS has an adenine substituted for a guanine at nucleotide 2756. This mutation of MS may also be involved in the occurrence of NTD. However, other alterations in the enzymes involved in the carbon transfer of folate, and the resultant decrease in methionine synthesis and homocysteine accumulation, remain potential sources of the genetic components of NTD occurrences. NonHispanic women of reproductive age with the TT genotype for the MTHFR 677C→T polymorphism who consume low folate diets are at greater risk for impaired folate status than women with the CC genotype. Of particular concern is the potential negative impact of chronic consumption of low folate diets coupled with the TT genotype for the MTHFR 677C→T polymorphism on reproductive health.

There are multiple causes of neural-tube defects, including drugs (especially antifolate and antiepileptic agents), chromosomal abnormalities, and environmental and genetic factors. Studies showing a reduction in the incidence of neural-tube defects of approximately 70 percent with periconceptional folic acid supplementation provide evidence that supplementary folate circumvents either an impaired intracellular folate-dependent enzyme pathway or an inhibitor of the cellular uptake of folate. However, the genetic variants of folate-pathway enzymes or of folate receptors identified in women who have pregnancies complicated by a neural-tube defect do not account for the 70 percent reduction in neural-tube defects associated with folate supplementation.

The occurrence of the autoantbody might explain the observed benefit of periconceptional folic acid supplementation. The autoantibody-mediated blocking of cellular folate uptake by folate receptors could be bypassed by folic acid, because it is reduced and methylated in vivo and is transported into cells by the reduced folate carrier. The mechanism by which folate receptors might become self antigens is not known. Since the risk of neural-tube defects may increase after abortions or miscarriages, autoimmunity may be induced by epitopes of the folate receptors exposed as a result of injury and proteolysis of the reproductive tissues, which together with host genetic factors, may trigger the generation of autoantibodies.

During the perinatal period, progenitors of neurons and glia divide, unnecessary cells die by apoptosis, whereas others migrate to reach their final destinations within various brain regions, creating the structures of the brain and setting the stage for brain function or dysfunction later in life. During this period, the brain is sensitive to the supply of essential nutrients. Maternal dietary supplementation with folic acid in the periconceptional period significantly reduces the risk of neural tube defects. Folate plays a central role in DNA synthesis through de novo purine and thymidine biosynthesis necessary for mitotic cell division and folate is important in the transfer of methyl groups. Although the importance for normal brain development of folate intake early in pregnancy is well accepted, the requirements for folate intake late in fetal gestation are not well understood.

Folate is interrelated metabolically to choline metabolism; both methyltetrahydrofolate and betaine (derived from choline) can methylate homocysteine to produce methionine. Maternal dietary choline intake during late pregnancy modulated mitosis and apoptosis in progenitor cells of the fetal rat hippocampus and septum and altered the differentiation of neurons in the fetal hippocampus. Mothers fed choline-deficient diets during late pregnancy had offspring with diminished progenitor cell proliferation and increased apoptosis in the fetal hippocampus, insensitivity to long-term potentiation when they were adult animals, and decremented visuospatial and auditory memory. Because choline and folate metabolism are interrelated, maternal dietary folate intake during late gestation might similarly influence neurogenesis in developing mouse brain.

Summary

To date, the majority of scientific investigations about dietary folate requirements during pregnancy have focused on folate's role in preventing neural tube defects. This has led to recommendations that pregnant women take supplemental folic acid before and during the first weeks of pregnancy. It was suggested that folate availability also affects brain development long after neural tube closure, and indicates that it may be very important that women ingest adequate intakes of folic acid throughout pregnancy. This may be especially important in those women with genetic polymorphisms in genes of folate metabolism.

Further reading

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