



The placental transport of folates

Interference by xenobiotics and pathological conditions

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ABSTRACT

Folates are essential nutrients to the developing fetus, these compounds being obtained from the maternal blood through placental transport. Placental transport of folates is a complex process that seems to involve several different transport systems (folate receptor α [FR α], reduced folate carrier [RFC1] and proton-coupled folate transporter [PCFT]) functioning coordinately to ensure the vectorial transfer of folates from maternal-to-fetal circulation.

The strict importance of folates to fetal and pregnancy health is the motor for the current and emerging research on the molecular mechanisms of placental transport of folates. This review summarizes recent findings concerning the placental transport of folates, with a special emphasis on the effect of xenobiotics and pathological conditions in this process. Data obtained so far support the conclusion that the placental transport of folates may be compromised by several dietary substances, therapeutic agents, drugs of abuse and markers of pathological conditions, which may in turn threaten, to some degree, the normal development and growth of the fetus.

INTRODUCTION

The placenta is the main link between mother and fetus, being responsible for the transfer of nutrients from maternal to fetal blood and for the clearance of waste metabolites from fetal blood. This organ is thus determinant for pregnancy success. The functional unit of the placenta is the **syncytiotrophoblast** (STB), a highly differentiated epithelium that is responsible for the endocrine, protective and transport functions of the placenta. The STB is the outermost polarized epithelium of the placental villi, in which the apical membrane is differentiated into numerous microvilli and directly contacts maternal blood, whereas the basal membrane faces fetal circulation.

This mini-review will focus on recent findings concerning the placental transport of folates, and its modulation by **xenobiotics** and pathological conditions.

FOLATES

Folates (vitamin B9) is the generic term given to a family of compounds that facilitate the transfer of one-carbon units in the synthesis of purine and pyrimidine precursors of nucleic acids, in the metabolism of some amino acids (methionine, serine, glycine and histidine), and in the initiation of protein synthesis in mitochondria [1-3]. Members of the family of folates include: a) folic acid (pteroylglutamate; FA), which is the synthetic, fully oxidized, most stable and the parent structure of this family [2, 4], and b) reduced folate derivatives, the active coenzyme forms, of which 5-methyltetrahydrofolate (5-MTHF) is the most abundant form found in plasma and, together with 5-formyltetrahydrofolate (5-FTHF), is the most common folate form present in food [2].

Folates are essential micronutrients critical for normal cellular functions and division, being thus particularly important during pregnancy for normal placental and fetal development

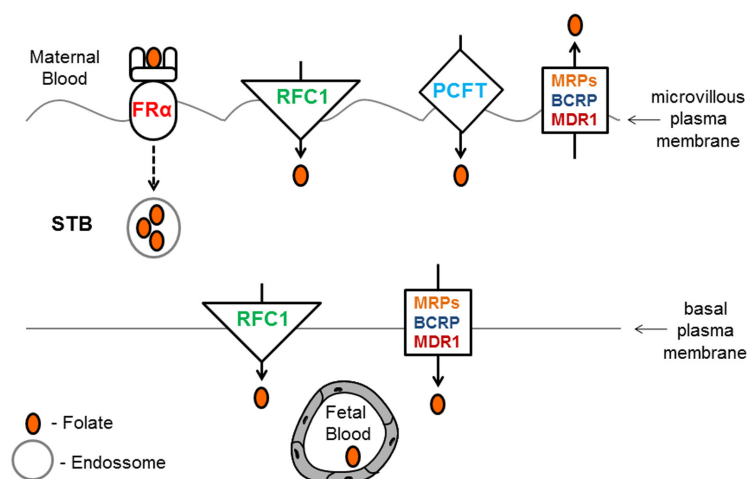


Figure 1: Schematic representation of maternal-to-fetal transport of folates across the human STB. Abbreviations: FR α , folate receptor alpha; PCFT, proton-coupled folate transporter; RFC1, reduced folate carrier 1; MRPs, multidrug resistance proteins; BCRP, breast cancer resistance protein; MDR1, P-glycoprotein.

and growth. This is well demonstrated by the following facts: (a) maternal deficiency of folates, one of the most prevalent vitamin deficiencies in the Western hemisphere, is associated with low birth weight, increased risk of spontaneous abortion, and **neural tube defects** (e.g., spina bifida and anencephaly); and (b) improvement of maternal folate intake, by supplementation during the **periconceptional period**, can reduce the incidence of low birth weight newborns and neural tube defects [1-6].

The fetus cannot synthesize folates and thus must obtain this vitamin from the maternal circulation, through placental transport. Despite the recognized importance of folates for fetal development, knowledge on the cellular mechanisms involved in the maternal-to-fetal transfer of this vitamin across the placenta is still limited.

PLACENTAL TRANSPORT OF FOLATES

The human placenta expresses the reduced folate carrier (RFC1, SLC19A1) [7-9], the proton-coupled folate transporter (PCFT/HCP1, SLC46A1) [10] and the isoforms alpha (FR α) [11-13] and beta (FR β) [14-16] of the folate receptor (FR). Although both these FR isoforms are detectable in whole placental tissue, only the FR α isoform is selectively expressed in normal trophoblast cells and in choriocarcinoma cells [17-19] (Figure 1).

It is currently accepted that placental transfer of folates involves FR α , RFC1 [7, 20, 21] and

PCFT [22] (Figure 1). FR α is a glycosylphosphatidylinositol-anchored protein located at the apical membrane of the STB. FR α is in direct contact with the maternal blood, binds FA with high affinity (in the nanomolar range) and accomplishes its internalization through receptor-mediated endocytosis [11, 12, 17, 19, 23]. RFC1 is a member of the SLC19 family of facilitative carriers. It is a folate:organic phosphate (OP $^-$) exchanger that is driven by the transmembrane OP $^-$ gradient [7, 15, 24] and has a higher affinity for reduced over non-reduced folates. Finally, PCFT is a high affinity folate:H $^+$ symporter with an optimal activity at acidic pH [10, 24] (Fig. 1).

Recent work from our group suggests that, at physiological pH, both RFC1 and FR α mediate FA apical uptake into the human choriocarcinoma BeWo cell line and into **primary cultured human cytotrophoblasts (TB cells)**. In addition, at acidic pH, uptake seems to be mediated by both RFC1 and/or a low pH-operating transporter, most probably corresponding to PCFT [25, 26]. Our observation that FA is taken up by three distinct transporters (FR α , PCFT and RFC1) [25, 26] was later supported by the demonstration that these three folate transporters are indeed present in the microvillous membranes of the STB [27].

Thus, folate placental transport seems to be a complex and crucial process, which needs redundancy, a property often found in processes essential for life. In the case of placental folate transport, this redundancy may ensure an ad-

equate supply of the vitamin to the placenta and the fetus, even in the case of maternal marginal folate status.

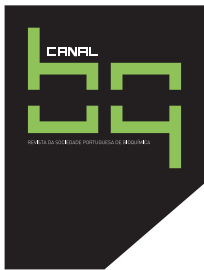
Folate placental homeostasis also seems to be controlled by efflux systems, as folates are substrates of several distinct ABC transporters (multidrug resistance proteins [MRPs] and breast cancer resistance protein [BCRP]), and these transporters are present at the placental level [28, 29]. Accordingly, the involvement of the ABC transporters MRPs and P-glycoprotein (MDR1) in the efflux of FA from both BeWo cells and TB cells was recently hypothesized [28].

EFFECT OF DIETARY BIOACTIVE COMPOUNDS ON THE PLACENTAL UPTAKE OF FA

Knowing that folates in food or supplementation are ingested together with other nutrients and bioactive substances and that the developing fetus obtains folates from the maternal blood through the placenta, it is of major importance to study the interactions between folates and those nutrients/substances that may stimulate or inhibit folate absorption at the placental level.

This was recently investigated by our group, by determining the acute and chronic effects of some **polyphenolic compounds** (catechin, chrysin, epicatechin, epigallocatechin-3-gallate, isoxanthohumol, myricetin, quercetin, resveratrol, rutin and xanthohumol) present in alcoholic (e.g., red wine and beer) and nonalcoholic beverages (e.g., green tea), as well as of some methylxanthines (caffeine and theophylline) present in drinks such as coffee and black tea, upon FA uptake by the BeWo cell line.

The results obtained showed that FA uptake was significantly reduced by acute (20-min) exposure to epicatechin, isoxanthohumol or theophylline, and that isoxanthohumol seemed



to act as a competitive inhibitor of FA uptake, whereas epicatechin and theophylline caused an increase in both K_m and V_{max} . On the other hand, FA uptake was significantly increased by chronic (48-h) exposure to xanthohumol, quercetin or isoxanthohumol, and this increase did not seem to result from changes in the levels of RFC1 or FR α gene expression [30]. As a whole, our results indicate that distinct polyphenolic compounds possess different effects upon transport of FA and therefore care should be taken when speculating about the effect of a drink based on the effect of a single polyphenolic compound.

EFFECT OF PHARMACOTHERAPY AND DRUGS OF ABUSE ON THE PLACENTAL UPTAKE OF FA

Pregnant women (and consequently the placenta and the fetus) are frequently exposed to several xenobiotics due to pharmacotherapy of maternal or fetal disease or to lifestyle factors such as smoking, drug abuse and alcohol consumption.

Indeed, the need for medication of pregnant women is demonstrated by the fact that 5–10% of pregnant women suffer from hypertension of diverse etiologies [31] and that 2–6% of pregnancies in European women are complicated by gestational diabetes mellitus [32]. Moreover, the US 2009 National Survey on Drug Use and Health (NSDUH) reports that 10% of pregnant women reported current alcohol use, 15% tobacco use and 4.5% illicit drugs abuse (marijuana and cocaine being the most consumed ones) at least one time during the past month [33]. Also, data from a Spanish cohort of 1st trimester pregnant women conducted from 2007–2009 showed that the prevalence of drugs of abuse (mainly cannabinoids and cocaine), cigarette and alcohol consumption was 30, 41 and 36%, respectively. Although dif-

ferent populations were analyzed in these two studies, the Spanish study may give us a better estimate of drug abuse prevalence because it used analytical methods (serum and hair samples) in contrast to structured questionnaires used by the US study [34].

All of these conditions are associated with increased maternal and/or fetal morbidity and mortality. Namely, in pregnant drug abusers, there is a higher risk of preterm delivery, low birth weight, spontaneous abortion and teratogenesis, among other complications [35–38]. Despite the severity of the pregnancy complications induced by drug use during pregnancy, the cellular mechanisms involved are not fully elucidated.

In this context, our group decided to investigate the effect of (a) therapeutic drugs commonly prescribed to pregnant women such as the anti-hypertensives clonidine, α -methyl dopa, atenolol and labetalol and the anti-diabetic insulin; and of (b) the drugs of abuse amphetamine, ecstasy (MDMA), tetrahydrocannabinol (THC), nicotine, cocaine and ethanol (and its metabolite acetaldehyde) upon FA uptake by TB cells and BeWo cells and also upon the gene expression of FA transport systems known to be present at the placental level: RFC1, FR α and PCFT [25, 30, 39].

Using TB cells, we verified that: (a) acutely (20-min), FA uptake was decreased by labetalol, MDMA and amphetamine; and (b) chronically (48-h), FA uptake was decreased by atenolol, nicotine, ethanol, MDMA, amphetamine and THC. Moreover, many of these drugs were cytotoxic and they differentially modulated the mRNA expression of FA placental transport systems [25]. Importantly, all these drugs were tested in concentration ranges comprising their known therapeutic or recreational blood levels. In relation to ethanol's chronic effect, the decrease in FA uptake was associated with a decrease in RFC1 mRNA expression levels in TB cells, and with a reduction in FR α mRNA expression levels in BeWo cells [25, 30]. Alcohol abuse during pregnancy is known to produce severe brain damage and behavioral and learning deficits to the fetus, **fetal alcohol syndrome (FAS)** being the most commonly resulting condition [40–44]. These results thus

suggest that impairment of FA placental uptake may be one of the mechanisms involved in ethanol placento- and fetotoxicity.

In relation to cannabinoids, their effect was investigated more deeply by using BeWo cells, and it was found that not only THC, but several endocannabinoid agonists and antagonists affect FA uptake, both after acute and chronic treatment of the cells [39].

Altogether, our results suggest that inhibition of FA placental uptake may constitute one of the mechanisms involved in the fetotoxicity of many of the compounds tested (amphetamine, MDMA, THC, nicotine and ethanol) reinforcing the harmfulness of their consumption, particularly during pregnancy. Moreover, attention should be drawn to the fact that acute and chronic treatments with the xenobiotics did not always produce parallel results. Therefore, care should be taken when speculating about chronic effects from acute effects, and vice versa.

EFFECT OF PATHOLOGICAL CONDITIONS ON THE PLACENTAL UPTAKE OF FA

Finally, the effect of some markers of pathological conditions common in pregnancy were also investigated by our group, namely high serotonin and high glucose levels as markers for preeclampsia and diabetes, respectively. Interestingly enough, chronic (48-h) exposure of TB cells to high levels of glucose (30 mM) caused a 30% decrease in FA uptake, which was accompanied by a similar decrease in the mRNA expression levels of FR α . This leads us to conclude that hyperglycaemia chronically inhibits FA uptake by TB cells, most probably because it inhibits FR α gene expression. These results strengthen the widely accepted notion of hyperglycaemia as a teratogen [45].

PLACENTAL CELLULAR MODELS - GENERAL REMARKS

Many of the studies on human placental transport refer to results obtained with placental cellular models such as the BeWo and the JAR human chorionic carcinoma cell lines and TB cells. These are known models of the human

STB and as such are useful tools to study placental function and transport [46-50]. For example, BeWo cells exhibit morphological and functional properties of normal trophoblasts and they rapidly form a confluent and polarized monolayer, being particularly attractive for functional studies on the transplacental barrier [46]. With respect to TB cells, they are also considered to be a suitable model to study human placental transport and function [47, 49, 50], because these cells spontaneously differentiate into a functional and polarized STB-like structure that resembles the *in vivo* STB [49-53]. Also, our observations that FA uptake characteristics and modulation are very similar in BeWo and TB cells further validate these cell types as good models to study placental transport and function.

CONCLUDING REMARKS

Placental transport of folates is a complex process that seems to involve several different transport systems (FR α , RFC1 and PCFT) functioning coordinately to ensure the vectorial maternal-to-fetal transfer of folates. This functional redundancy is likely to contribute to an efficient transfer of this vitamin through the placenta, ensuring an adequate supply of folates to the developing fetus even in cases of maternal marginal vitamin status.

Also emergent is the idea that placental transport of folates may be compromised by several dietary substances, therapeutic agents, drugs of abuse and markers of pathological conditions, and that this may, in some degree, threaten the normal development and growth of the fetus.

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GLOSSARY

BeWo cell line: epithelial cell line derived from human choriocarcinoma.

Fetal alcohol syndrome (FAS): a severe condition that results from alcohol exposure during fetal development, being one of the most common causes of mental retardation. It is defined by poor growth, central nervous system abnormalities, including impaired brain growth or abnormal structure, and specific dysmorphic facial features. Poor growth beginning *in utero* continues throughout infancy and childhood. During infancy, central nervous system manifestations of FAS include irritability, jitteriness, and an exaggerated response to noise. During childhood, characteristic manifestations of FAS include hyperactivity, developmental delay, hypotonia, learning disabilities, auditory and visual impairment, seizure disorders, and intellectual disability (mental retardation) [54].

Folates: or vitamin B₉, is the generic name given to a family of compounds that facilitate the transfer of one-carbon units in the synthesis of nucleic acids precursors, metabolism of some amino acids, and in the initiation of protein synthesis in mitochondria [1-3].

Neural tube defects (NTDs): one of the most prevalent congenital anomalies that result from the incomplete closure of the neural tube during the early phases of human development.

Periconceptional period: a period of time that extends from few months before conception to the first weeks of pregnancy.

Polyphenolic compounds: a class of organic compounds, widely distributed in most foods of vegetal origin, structurally characterized by the presence of a variable numbers of phenol rings.

Preeclampsia: a syndrome characterized by the onset or first recognition of hypertension and proteinuria after 20 weeks of gestation in a previously normotensive woman [55].

Primary cultured human cytotrophoblasts (TB cells): primary cultures of cytotrophoblastic cells isolated from human placentas.

Syncytiotrophoblast (STB): the functional layer of the human placenta which is a polarized epithelium in which the apical membrane is differentiated in numerous microvilli and directly contacts the maternal blood, whereas the basal membrane faces fetal circulation.

Xenobiotic: is a chemical compound which is found in an organism but which is not synthesized endogenously.

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