

Impact of folate supplementation on the efficacy of sulfadoxine/pyrimethamine in preventing malaria in pregnancy: the potential of 5-methyl-tetrahydrofolate

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Malaria remains the leading cause of mortality and morbidity in children under the age of 5 years and pregnant women. To counterbalance the malaria burden in pregnancy, an intermittent preventive treatment strategy has been developed. This is based on the use of the antifolate sulfadoxine/pyrimethamine, taken at specified intervals during pregnancy, and reports show that this approach reduces the malaria burden in pregnancy. Pregnancy is also associated with the risk of neural tube defects (NTDs), especially in women with low folate status, and folic acid supplementation is recommended in pregnancy to lower the risk of NTDs. Thus, in malaria-endemic areas, pregnant women have to take both antifolate medication to prevent malaria and folic acid to lower the risk of NTDs. However, the concomitant use of folate and antifolate is associated with a decrease in antifolate efficacy, exposing pregnant women to malaria. Thus, there is genuine concern that this strategy may not be appropriate. We have reviewed work carried out on malaria folate metabolism and antifolate efficacy in the context of folate supplementation. This review shows that: (i) the folate supplementation effect on antifolate efficacy is dose-dependent, and folic acid doses required to protect pregnant women from NTDs will not decrease antifolate activity; and (ii) 5-methyl-tetrahydrofolate, the predominant form of folate in the blood circulation, could be administered (even at high dose) concomitantly with antifolate without affecting antifolate efficacy. Thus, strategies exist to protect pregnant women from malaria while maintaining adequate folate levels in the body to reduce the occurrence of NTDs.

Keywords: neural tube defects, NTDs, *Plasmodium falciparum*, antifolate, intermittent preventive treatment during pregnancy, IPTp

Introduction

Malaria and pregnancy

Malaria remains the leading cause of mortality in the world, with its burden being borne primarily by children under the age of 5 years and pregnant women. Each year, it is estimated that over 125 million pregnant women are at risk of malaria,^{1,2} and the disease has serious implications in pregnancy for both mothers and infants. Indeed, placental malaria can lead to placental insufficiency, causing fetal growth restriction and leading to maternal, fetal and infant morbidity and mortality. It is estimated that more than 100 000 infant deaths occur each year as a result of malaria in pregnancy.^{3–5}

To counterbalance the heavy burden of malaria in pregnancy, a strategy known as intermittent preventive treatment during pregnancy (IPTp) has been proposed. This approach is based on the administration of a long-lasting antimalarial drug at its full therapeutic dosage in pregnant women, delivered at specified intervals during pregnancy, regardless of the presence of parasites or clinical symptoms. Drug administration is carried out during

routine contacts with the health authorities at antenatal care visits.^{6,7} IPTp has been conceived as a strategy to substantially reduce or eliminate parasitaemia in infected pregnant women in high malaria transmission areas. It differs from prophylaxis in providing protective antimalarial drug levels in blood for specific periods separated by periods when drug concentration is below the necessary threshold to inhibit parasite growth.^{6,7}

WHO now recommends the implementation of IPTp in all malaria-endemic regions, mainly in sub-Saharan Africa, as part of a focused antenatal care package,⁸ using the antifolate drug sulfadoxine/pyrimethamine (SP) at a dose of 500 mg of sulfadoxine and 25 mg of pyrimethamine per tablet; three tablets in total. Pregnant women receive two or three courses of SP after the onset of fetal movement, the courses being at least 1 month apart.⁸ This strategy has been adopted in 37 countries worldwide, of which 33 are in sub-Saharan Africa,⁸ and in 2011 it was estimated that 25% of pregnant women were using IPTp.⁹ Though this proportion is still low, it nonetheless means that a high number of women are already taking the antifolate SP during pregnancy in Africa. However, with the spread of SP resistance, there is genuine

concern that the efficacy of IPTp could also be compromised.¹⁰ As a result, alternatives are being investigated. However, the paucity of available effective antimalarials makes SP the drug of choice for IPTp for many years to come.

Pregnancy and neural tube defects (NTDs)

Pregnancy is a state of increased requirement of macro- and micro-nutrients, and inadequate dietary intake before and during pregnancy can lead to adverse perinatal outcomes.^{11,12} Among these micronutrients are folate molecules. Folate deficiency in pregnancy is associated with a high rate of NTDs (anencephaly and spina bifida), the second most common birth defects.^{13,14} NTDs result from failure of the neural tube to close properly ~28 days post-conception, and folic acid (FA) has been shown to reduce the risk of NTDs in both high- and low-income countries.^{15,16} A reduction rate up to 70% can be achieved with the appropriate consumption of FA before conception and during early pregnancy.^{17,18} Unfortunately, women are not aware of their pregnancy during the first few weeks, the time when the neural tube closes.^{17–19} As a result, each year 300 000–400 000 infants worldwide are born with an NTD;¹³ however, the exact numbers of cases occurring in Africa are unknown because of lack of proper records. Thus, there is a need to implement the concept of folate supplementation before and during pregnancy to reduce the risk of NTDs, and WHO has recommended a daily supplementation of 0.4 mg of FA for women capable of becoming pregnant.

Conflict between folate supplementation in pregnancy and the use of antifolate in IPTp

As discussed above, WHO has recommended supplementation with FA during the periconceptional and early pregnancy period to minimize the occurrence of NTDs. However, the antifolate SP is also recommended during pregnancy to lower the risk of malaria infection. Thus, pregnant women in Africa are encouraged to take both folate vitamin and antifolate drug concomitantly. A body of evidence indicates that the addition of folate decreases the activity of the antifolate *in vitro*, and the same observation has been made on the efficacy of antifolate *in vivo* (see next sections). Thus, there is genuine concern that the implementation of these two recommendations may be associated with a higher risk of malaria.

One option would be to use a non-folate drug. However, options for antimalarials are limited, and this limitation is compounded by the problem of drug resistance.²⁰ The other option would be to recommend the interruption of folate use when antifolate is administered or vice versa. In poor settings such as those affected by malaria, shifting of drug use is not practical since adherence to the treatments may be compromised. Since the risk of NTDs and malaria in pregnancy bears serious consequences for both the fetus and the mother, appropriate strategies need to be implemented.

Interestingly, as discussed below, detailed observations of published data on folate and antifolate activity in malaria indicate that strategies exist to use a combination of folate derivatives with antifolates in IPTp without affecting drug efficacy, while providing enough folate to prevent the development of NTDs.

General information on folate metabolism in malaria

Folate derivatives consist of a molecule of reduced pterin linked to *para*-aminobenzoate (pABA) and glutamate. The parent biologically active molecule, tetrahydrofolate (THF), is substituted with one-carbon moieties at the N5 and N10 positions of the pteridine ring. These molecules are important cellular cofactors in the synthesis of nucleotides required for DNA replication, in the synthesis and metabolism of the amino acids serine, glycine, methionine, histidine and glutamate, and in the provision of methyl groups for biological methylation reactions, which are essential in epigenetic gene regulation and signalling processes, among other roles.²¹ Folate cofactors are also required for the initiation of protein synthesis in mitochondria through formylation of methionine.²¹ The most important biologically active folate cofactors in mammalian systems are 5-CH₃-THF for the synthesis of methionine, 5,10-CH₂-THF for the formation of the pyrimidine deoxythymidine-monophosphate (dTMP), 10-CHO-THF for the formylation of methionyl-tRNA, 5,10-CH⁺-THF and 10-CHO-THF for the synthesis of purines, and THF and 5,10-CH₂-THF for the metabolism of serine, glycine, histidine and glutamate. 5-CH₃-THF, the folate cofactor central in the synthesis of methionine, is the predominant folate molecule in the blood circulation, representing >95% in populations not fortified with FA.²² It should be noted that FA is not a biologically active form of folate, but is a stable, readily absorbed precursor that is converted into dihydrofolate and thence into THF via the enzyme dihydrofolate reductase (DHFR) (see the next paragraphs in this section).

Mammalian cells do not synthesize folate *de novo*; they acquire it from dietary intake. On the other hand, the malaria parasite can make folate *de novo* from the precursor guanosine triphosphate, and at the same time salvage it from the exogenous environment. Both sources of folate contribute to the parasite's folate requirement. Folate is so critical that inhibition of the *de novo* pathway leads to parasite death, a feature that has been exploited by antifolate drugs such as SP.

Indeed, in the malaria parasite, two enzymes, dihydropteroate synthase (DHPS) and DHFR, have been proved to be critical in the synthesis and conversion of folate (Figure 1). DHPS mediates the condensation of pABA with hydroxymethyl-dihydropyrimidinopyrophosphate to generate dihydropteroate, and DHFR reduces dihydrofolate to THF, the biologically active form of folate that is substituted with one-carbon moieties and used downstream in the synthesis of dTMP from deoxyuridine monophosphate (dUMP).²³

Inhibitors of DHPS are sulpha-based, analogues of pABA; they include a sulphonamide (sulfadoxine) and a sulfone (dapsone). On the other hand, inhibitors of DHFR compete with dihydrofolate in the active site of the enzyme, and the commonly used antifolate antimalarials are pyrimethamine, proguanil and chlorproguanil. Proguanil and chlorproguanil are prodrugs, which are converted *in vivo* into the inhibitors of DHFR (cycloguanil and chlorcycloguanil, respectively).²³ Inhibitors of DHPS and DHFR are synergistic, leading to their use *in vivo* in combination.²³

SP, known also as FansidarTM, has been the most widely used antifolate drug for the treatment of uncomplicated malaria in Africa. This drug was introduced into Africa in the 1990s as a replacement for chloroquine. However, resistance to this drug emerged within a few years,²³ and by the mid-2000s this antifolate drug was replaced by artemisinin combination therapy.²⁴

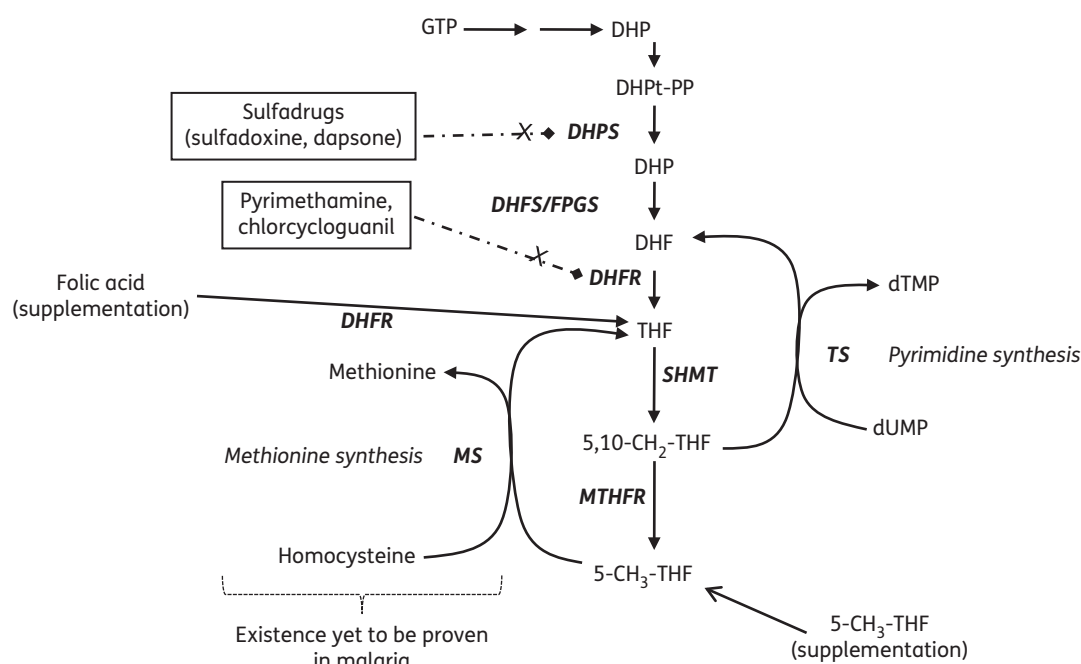


Figure 1. Schematic summary of folate in *Plasmodium falciparum*. Only the most important enzymes are shown. DHPS, dihydropteroate synthase; DHFS, dihydrofolate synthase; FPGS, folylpolyglutamate synthase; DHFR, dihydrofolate reductase; SHMT, serine hydroxymethyl transferase; MS, methionine synthase; TS, thymidylate synthase; MTHFR, methylenetetrahydrofolate reductase; GTP, guanosine triphosphate; DHPT-PP, 7,8-dihydro-6-hydroxymethylpterin pyrophosphate; DHP, 7,8-dihydropteroate; DHF, 7,8-dihydrofolate; THF, tetrahydrofolate; 5-CH₃-THF, 5-methyl-5,6,7,8-tetrahydrofolate; 5,10-CH₂-THF, 5,10-methylene-5,6,7,8-tetrahydrofolate.

SP resistance is associated with parasites that harbour point mutations at codons 108 (Ser to Asn), 51 (Asn to Ile) and 59 (Cys to Arg) of the *dhfr* gene (these are triple mutant parasites). Resistance is increased by the selection of point mutations at codons 437 (Ala to Gly) and/or 540 (Lys to Glu) or 437 and/or 581 (Ala to Lys) of the *dhps* gene. Higher *in vitro* and *in vivo* resistance is observed with the selection of a mutation at codon 164 (Ile to Leu) of *dhfr*.²³ Genotyping of *dhfr* and *dhps* has been used widely to monitor the emergence and selection of resistance to SP in various malaria-endemic areas, including Africa.²⁵

Despite the emergence of SP resistance, as discussed in the previous paragraph, this drug remains the best option for intermittent preventive treatment in pregnancy and in infancy.^{6,26,27} However, in the context of high SP resistance, its efficacy is compromised.¹⁰ Alternative drugs are being evaluated, including the combination of SP with other antimalarials, such as piperaquine and mefloquine;^{28,29} however, as stated earlier, owing to the paucity of available and effective drugs, SP will remain the best choice for IPTp.

Salvage of exogenous folate by malaria parasites and its effect on antifolate

Addition of folate *in vitro* and antifolate activity

In vitro, several studies have clearly demonstrated that the addition of FA or folinic acid and THF decreases the activity of antifolate drugs.^{30,31} However this is not the case with the folate 5-CH₃-THF, even when used at concentrations of 23 µM, which is 1000 times higher than those found in human blood circulation.^{30,31} In the

next section, we shall discuss the implication of this finding in relation to 5-CH₃-THF.

Likewise, other studies have shown that lower folate availability, e.g. due to a decrease in the folate concentration of the medium or a decrease in folate uptake (FA or THF), renders the parasite more susceptible to antifolate drugs both *in vitro* and *in vivo*.^{32–41} Thus, folate derivative concentration in the medium has a strong bearing on the activity of antifolates.³²

Supplementation of folate *in vivo* and SP efficacy

The first clinical evidence of the interaction of FA supplements with antimalarial activity *in vivo* was provided by van Hensbroek *et al.*⁴² in 1995. The authors examined the effects of supplementary treatment with iron, FA or placebo, given together with the antifolate SP and the non-antifolate chloroquine, in 600 Gambian children aged between 6 months and 9 years who were suffering from uncomplicated malaria. Five milligrams of FA or iron was given to children <15 kg, and those with a weight >20 kg received 10 mg of FA or iron. Treatment outcome, measured as parasitological failure rates 7 days after treatment, was significantly higher in the SP/FA group than in the SP/placebo group. Interestingly, the presence of FA did not change the failure rate in children who received chloroquine. This work was the first evidence of the effect of folate *in vivo*.

In 2005, a Kenyan study documented the effect of folate on the efficacy of SP.⁴³ In this report, 303 patients of all ages with uncomplicated malaria were treated with SP and iron and randomized to receive FA or placebo. The dose of FA was 2.5 mg daily if children were <2 years and 5 mg daily if >2 years, and this folate was administered for 30 days. The results showed a significant

reduction in the efficacy of SP in patients taking an FA dose of 5 mg compared with placebo. On the other hand, no difference was observed between placebo and the 2.5 mg FA group.⁴³

A similar study in Zambia investigated the effect of supplementation with a low dose of FA, 1 mg/day for 14 days or placebo, on the activity of SP and Malarone (atovaquone/proguanil) in 183 children (between the ages of 6 and 119 months) suffering from malaria.⁴⁴ In the SP group, parasitological treatment failure was higher in the FA group than in the placebo group. Interestingly, in the Malarone group the use of folate did not affect the drug's efficacy, in line with the known mode of action of Malarone, which does not involve the folate pathways.⁴⁵

In 2005, Dzinjalama et al.⁴⁶ reported a study on the correlation of the total blood folate concentration with SP efficacy in children aged between 6 months and 12 years who were suffering from malaria. No folate supplementation was given. Total folate concentrations in dried blood spots were assessed, and the results showed significantly higher levels of blood folate in children who failed to clear parasitaemia than in those who did clear it ($P=0.026$).

Based on the aforementioned clinical studies, a low dose (<2.5 mg) of FA does not negate SP efficacy, although in one study a dose of 1 mg of FA was associated with reduced drug efficacy.⁴⁴ The next paragraphs will examine the effect of folate in pregnant women suffering from malaria.

In 2006, Ouma et al.⁴⁷ reported the effect of folate on pregnant women in Kenya ($n=488$) with a gestational age between 17 and 34 weeks and presenting with uncomplicated malaria. They were treated with SP and iron supplementation, and were randomized into three arms: 5 mg of FA ($n=161$), 0.4 mg of FA ($n=165$) or placebo ($n=162$). After 14 days, they all received 5 mg of FA daily and were followed up to day 28. At day 14, the rates of treatment failure in the placebo and 0.4 mg FA groups were identical (13.9% and 14.5%, respectively, $P<0.05$), while the failure rate in the 5 mg FA arm was significantly higher than in the placebo group (27.1% versus 13.9%, $P=0.005$).

Using the same study samples, van Eijk et al.⁴⁸ assessed folate blood levels in 467 women and correlated them with drug efficacy. Total plasma folate levels were comparable in all groups at enrolment. However, at day 7 the 5 mg FA group had a significantly higher median increase in plasma folate level (9.2 ng/mL increase) than the 0.4 mg FA group, which had a median increase of 2.7 ng/mL. At day 7, 100 women (2 in the placebo group, 23 in the 0.4 mg group and 75 in the 5 mg group) had folate levels exceeding 15.4 ng/mL, and 48 of these (38 in the 5 mg group and 10 in the 0.4 mg group) failed treatment. Thus, as expected, FA supplement use is associated with increased total folate blood level.

Another study was reported on 1035 primigravidae in Gambia with pregnancies >15 weeks.⁴⁹ They received SP as part of IPTp and were given a low-dose FA treatment of 0.5 or 1.5 mg/day for 14 days. In the FA group, half also received iron supplementation. The rate of treatment failure was similar between the groups treated with 0.5 and 1.5 mg of FA (5.7% versus 4.9%, $P>0.05$), and there was no difference between those who received iron and those who did not. Based on this study, an FA dose as high as 1.5 mg/day does not seem to negate the efficacy of SP in IPTp.

This review of published work, as summarized in Table 1, indicates that supplementation with FA can negate the efficacy of SP as part of normal treatment or IPTp. However, this negation is dependent upon the dose of FA. Clearly, supplementation with 5 mg

of FA daily can significantly decrease the efficacy of SP in both normal treatment and IPTp. On the other hand, 0.4 or 0.5 mg of FA does not affect SP efficacy, though in one study a dose of 1 mg was associated with decreased SP efficacy in the context of normal treatment (but not IPTp). This is important since 0.4 mg is the WHO-recommended daily dose of FA to prevent NTDs in pregnant women. Thus, this low dose could be recommended for supplementation in IPTp. The effect of FA on SP efficacy is dose-dependent, and overall there is no evidence that the implementation of 0.4 mg/day FA supplementation will decrease SP efficacy in the context of IPTp.

5-CH₃-THF and antifolate activity

As discussed above, we also tested the effect of the folate derivative 5-CH₃-THF against the antifolate antimalarial drugs pyrimethamine and chlorcycloguanil. To our surprise, high concentrations of 5-CH₃-THF, up to 1000 times higher than the physiological concentration, did not change the *in vitro* activity of these drugs.³¹ Two hypotheses could be put forward to explain these results. Either 5-CH₃-THF may not be transported into the malaria parasite or this folate is not utilized by the parasite even though it is present in the cell. Detailed observation of folate biochemistry in the parasite indicates that the latter is more plausible. In the folate pathway, 5-CH₃-THF is utilized as a methyl group donor to convert homocysteine into methionine, the only reaction that leads to the synthesis of methionine.²¹ Once the malaria parasite enters a red blood cell, it degrades up to 75% of the haemoglobin, generating an abundance of amino acids, including methionine, to meet its requirement.⁵⁰ In addition, the parasite has developed a transport system to take up amino acids from exogenous medium if its amino acid requirement is not met by haemoglobin degradation. For instance, this is the case for isoleucine, an amino acid that is not found in haemoglobin,⁵¹ and methionine, to supplement its supply from haemoglobin degradation.⁵² Thus, under these conditions, the parasite may not need to synthesize methionine *de novo*.

At the molecular level, two early studies have indicated that a *de novo* methionine synthesis pathway may exist in *Plasmodium falciparum*,⁵³ however, using malaria genome information, genes encoding enzymes that mediate methionine synthesis have not been identified,²¹ and, up to now, to the best of our knowledge, there are no new data supporting the existence of a *de novo* methionine pathway in malaria. Furthermore, since the parasite can obtain methionine from haemoglobin degradation or the exogenous medium,⁵² this pathway is likely to be of little importance, a situation we have already proposed.²¹ The synthesis of methionine also contributes to lowering the concentration of homocysteine, a molecule that can be toxic to the cell at high concentrations. However, the malaria parasite could simply export this molecule from the cell, as it does with the many acids that it does not need following haemoglobin degradation.⁵¹ In addition, a recent study has indicated that the parasite may not salvage 5-CH₃-THF efficiently from the exogenous medium.³⁸ Thus, the inefficiency of the parasite in taking up 5-CH₃-THF and the absence of a methionine pathway may explain why this folate form does not decrease antifolate activity, even at doses 100–1000 times higher than the physiological folate concentration. Thus, as we shall discuss in the next section, this form of folate could be an alternative to FA in IPTp in Africa.

Table 1. Summary of studies carried out in Africa in which the efficacy of antimalarials, primarily SP, was tested in the context of FA supplementation in children, adults and pregnant women suffering from malaria

Year of study	Country	Study population	Antimalarial(s) tested	FA supplementation	Main findings	Reference
1992	Gambia	children between 6 months and 9 years (<i>n</i> = 600)	SP, chloroquine, placebo	5 or 10 mg daily	treatment failure was significantly higher in the SP/folate group than the SP/placebo group	⁴²
1998–99	Kenya	patients of all ages, but not pregnant women (<i>n</i> = 303)	SP	2.5 or 5 mg daily	significant reduction in SP efficacy in the 5 mg FA group	⁴³
2000	Malawi	children between 6 and 60 months (<i>n</i> = 191)	SP	no supplementation was carried out	high blood folate level was associated with SP failure	⁴⁶
2000–02	Zambia	children between 6 and 119 months (<i>n</i> = 183)	SP, atovaquone/proguanil	1 mg daily for 14 days	prevalence of parasitological failure was higher in the SP/folate group than in the SP/placebo group; FA had no effect on atovaquone/proguanil efficacy	⁴⁴
2002–03	Gambia	primigravidae at gestational age >15 weeks (<i>n</i> = 1035)	SP, placebo	0.5 or 1.5 mg daily	FA supplementation did not reduce SP efficacy in either group	⁴⁹
2003–05	Kenya	pregnant women at gestational age 17–34 weeks (<i>n</i> = 488)	SP	0.4 or 5 mg daily for 14 days or placebo	significantly higher treatment failure in the SP/5 mg folate group than in the SP/placebo and SP/0.4 mg folate groups; no difference in SP efficacy between the SP/0.4 mg folate group and the SP/placebo group	⁴⁷
2002–05	Kenya	pregnant women with unrestricted age of pregnancy (<i>n</i> = 467)	SP	0.4 or 5 mg daily for 14 days	SP failure was associated with the use of 5 mg folate supplementation	⁴⁸

Folate supplementation with FA or 5-CH₃-THF in pregnant women with malaria

Pharmacokinetic considerations

A number of studies have compared the acute and long-term bioavailability of 5-CH₃-THF with FA, using various methodologies (for reviews see Obeid *et al.*⁵⁴ and Pietrzik *et al.*⁵⁵). It is now well established that with low doses (i.e. equivalent to 0.4 mg of FA), FA and 5-CH₃-THF have similar bioavailability, although the time required for emergence of FA into serum is somewhat longer than that for 5-CH₃-THF, consistent with the notion discussed earlier that FA must be converted into 5-CH₃-THF prior to emergence in serum.⁵⁶ In one high-dose study, equimolar amounts of FA and 5-CH₃-THF (equivalent to 15 mg of FA daily) were administered for 12 weeks to dialysis patients.⁵⁷ Both compounds were equally effective in increasing the total plasma folate concentrations, but a distributional analysis found that with 5-CH₃-THF ingestion all folate was present as 5-CH₃-THF, whereas with FA ingestion nearly 50% of the total folate was present as unmetabolized FA.⁵⁷ Thus, 5-CH₃-THF has better pharmacological properties than FA.

Current folate treatment in Africa

In most African countries, 5 mg of FA daily is commonly used as supplementation to prevent anaemia in pregnancy. It is used at 5 mg because this is the available form of folate tablet commonly found in Africa. The WHO recommends the low dose of 0.4 mg/day for all women in the periconceptional and early pregnancy period, in order to reduce the risk of NTDs [see also the earlier section, Pregnancy and neural tube defects (NTDs)]. The currently available dose of 5 mg of FA is probably much higher than what is needed and a lower dose of 0.4 mg would be adequate for all women of child-bearing age.

As our review indicates, the use of a low dose of FA (<0.5 mg) does not affect the antimalarial activity of SP in the context of normal and IPTp treatment. Thus, the WHO-recommended dose of 0.4 mg/day to lower the risk of NTDs could be implemented for IPTp in Africa. However, if this is to be implemented the currently available 5 mg FA tablet should be phased out in Africa; this is a challenge on its own since FA is widely available and sold over the counter as a food supplement. Attempting to control its manufacture so as to make only 0.4 mg tablets available in the market

would be an impossible task since this vitamin is consumed by the general population as well (not only pregnant women). One possible scenario would be that both tablets would be available in the market, but, in this instance, adherence to the low dose of 0.4 mg in pregnancy may not be fully observed since women would take whichever form is available at the time they need FA. Thus, the implementation of a 0.4 mg dose will remain a challenge in the African or developing world context.

Advantages of using the alternative of 5-CH₃-THF

In vitro work indicates that 5-CH₃-THF may be a better alternative to FA, both in IPTp and prevention of NTDs. Firstly, this folate form does not increase the parasite folate pool, however high the dose may be. Its use will not affect the activity of the antifolate, allowing full protection of pregnant women against malaria. Secondly, 5-CH₃-THF will provide enough folate in the blood of the host (pregnant women) to lower the risk of NTDs.

The evidence summarized above shows that, in humans, low-dose FA is readily converted into THF via the enzyme DHFR and thence into 5-CH₃-THF, the most dominant folate form in the circulation. Thus, the administration of low-dose FA leads rapidly to an increase in plasma 5-CH₃-THF, the form that the parasite does not utilize. On the other hand, in the context of high-dose FA, the host's DHFR activity is overwhelmed and unmetabolized FA appears in the plasma.⁵⁸ This form would be salvaged by the parasites (in the case of malaria infection) and will increase the parasite folate pool, leading to a decrease in antifolate activity. Supplementation with a low dose of FA is equivalent to providing the parasite with 5-CH₃-THF, a form that it does not utilize, explaining why the use of a low dose of FA does not affect the activity of antifolate. Thus, a high dose of FA (5 mg) could be replaced by any dose of 5-CH₃-THF. This will provide a sufficient folate level to protect pregnant women against NTDs while maintaining the activity of antifolate.

Another argument to support the use of 5-CH₃-THF is its advantage over FA in preventing the masking of megaloblastic anaemia due to vitamin B12 deficiency. Deficiency of either folate or vitamin B12 can cause severe haematological symptoms, described as megaloblastic anaemia. Vitamin B12 deficiency can also result in an irreversible neurological lesion. Inappropriate treatment of an individual suffering from vitamin B12 deficiency with FA can mask the haematological symptoms of the disease.⁵⁹ As a result, people suffering from pernicious anaemia and under FA supplementation may only be detected late in the disease progression, leading to a higher risk of neurological deterioration, a situation that compromises effective intervention.⁵⁹ This risk of masking the anaemia of vitamin B12 deficiency has been used as an argument against the use of FA fortification in food.⁶⁰ On the other hand, 5-CH₃-THF does not interfere with the development of megaloblastic symptoms in people with vitamin B12 deficiency and thus it is a good alternative to FA in the population. So the inability of 5-CH₃-THF to mask the anaemia of vitamin B12 deficiency and to negate antifolate efficacy make this folate form a better alternative to FA in pregnancy in Africa.

Concluding remarks

The prevention of malaria and NTDs during pregnancy needs to be addressed in Africa. The antifolate SP is used for malaria prevention

and FA supplementation in NTD prevention. Thus, *a priori*, the implementation of these two strategies is counter-productive. However, we have provided evidence that FA can be used at the recommended dose of 0.4 mg/day without affecting the efficacy of SP in IPTp while providing a sufficient blood folate level to prevent NTDs. On the other hand, 5-CH₃-THF, the most dominant form of folate, could be used even at high dose to prevent NTDs while not affecting SP efficacy. This folate form is an important alternative to FA as it does not mask megaloblastic anaemia. However, 5-CH₃-THF has two main limitations: it is relatively more expensive than FA, and is less stable. However, a stable supplementary form of 5-CH₃-THF as a calcium salt has become available.⁶¹ Thus, cost remains the major limitation for its use in routine supplementation. However, since the benefit of this folate form in pregnancy outweighs this limitation, it is imperative to develop strategies to implement both WHO recommendations of antifolate SP in IPTp for the protection against malaria and supplementation with 5-CH₃-THF for the prevention of NTDs in pregnant women in malaria-endemic areas.

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Transparency declarations

None to declare.

References

- 1 Dellicour S, Tatem AJ, Guerra CA *et al.* Quantifying the number of pregnancies at risk of malaria in 2007: a demographic study. *PLoS Med* 2010; **7**: e1000221.
- 2 Rogerson SJ, Hviid L, Duffy PE *et al.* Malaria in pregnancy: pathogenesis and immunity. *Lancet Infect Dis* 2007; **7**: 105–17.
- 3 Desai M, ter Kuile FO, Nosten F *et al.* Epidemiology and burden of malaria in pregnancy. *Lancet Infect Dis* 2007; **7**: 93–104.
- 4 Desai M, Dellicour S. Effects of malaria and its treatment in early pregnancy. *Lancet Infect Dis* 2012; **12**: 359–60.
- 5 Uneke CJ, Ogbonna A. Malaria and HIV co-infection in pregnancy in sub-Saharan Africa: impact of treatment using antimalarial and antiretroviral agents. *Trans R Soc Trop Med Hyg* 2009; **103**: 761–7.
- 6 Bardaji A, Bassat Q, Alonso PL *et al.* Intermittent preventive treatment of malaria in pregnant women and infants: making best use of the available evidence. *Expert Opin Pharmacother* 2012; **13**: 1719–36.
- 7 Menendez C, D'Alessandro U, ter Kuile FO. Reducing the burden of malaria in pregnancy by preventive strategies. *Lancet Infect Dis* 2007; **7**: 126–35.
- 8 WHO Antenatal Care Trial Research Group. *WHO Antenatal Care Randomized Trial: Manual for the Implementation of the New Model*. 2002. http://www.who.int/reproductivehealth/publications/maternal_perinatal_health/RHR_01_30/en/ (3 June 2013, date last accessed).
- 9 van Eijk AM, Hill J, Alegana VA *et al.* Coverage of malaria protection in pregnant women in sub-Saharan Africa: a synthesis and analysis of national survey data. *Lancet Infect Dis* 2011; **11**: 190–207.

- 10 Gutman J, Kachur SP, Slutsker L *et al.* Combination of probenecid-sulphadoxine-pyrimethamine for intermittent preventive treatment in pregnancy. *Malar J* 2012; **11**: 39.
- 11 Abu-Saad K, Fraser D. Maternal nutrition and birth outcomes. *Epidemiol Rev* 2010; **32**: 5–25.
- 12 de Onis M, Villar J, Gulmezoglu M. Nutritional interventions to prevent intrauterine growth retardation: evidence from randomized controlled trials. *Eur J Clin Nutr* 1998; **52** Suppl 1: S83–93.
- 13 Christianson A, Howson CP, Modell B. *March of Dimes Global Report on Birth Defects: The Hidden Toll of Dying and Disabled Children*. 2006. http://www.marchofdimes.com/glue/files/Birth_Defects_Report-PF.pdf (1 June 2013, date last accessed).
- 14 Daly LE, Kirke PN, Molloy A *et al.* Folate levels and neural tube defects. Implications for prevention. *JAMA* 1995; **274**: 1698–702.
- 15 Blencowe H, Cousens S, Modell B *et al.* Folic acid to reduce neonatal mortality from neural tube disorders. *Int J Epidemiol* 2010; **39** Suppl 1: i110–21.
- 16 Wolff T, Witkop CT, Miller T *et al.* Folic acid supplementation for the prevention of neural tube defects: an update of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med* 2009; **150**: 632–9.
- 17 Botto LD, Moore CA, Khoury MJ *et al.* Neural-tube defects. *N Engl J Med* 1999; **341**: 1509–19.
- 18 Mosley BS, Hobbs CA, Flowers BS *et al.* Folic acid and the decline in neural tube defects in Arkansas. *J Ark Med Soc* 2007; **103**: 247–50.
- 19 Chandler AL, Hobbs CA, Mosley BS *et al.* Neural tube defects and maternal intake of micronutrients related to one-carbon metabolism or antioxidant activity. *Birth Defects Res A Clin Mol Teratol* 2012; **94**: 864–74.
- 20 Sa JM, Chong JL, Wellems TE. Malaria drug resistance: new observations and developments. *Essays Biochem* 2011; **51**: 137–60.
- 21 Nzila A, Ward SA, Marsh K *et al.* Comparative folate metabolism in humans and malaria parasites (part I): pointers for malaria treatment from cancer chemotherapy. *Trends Parasitol* 2005; **21**: 292–8.
- 22 Kelly P, McPartlin J, Goggins M *et al.* Unmetabolized folic acid in serum: acute studies in subjects consuming fortified food and supplements. *Am J Clin Nutr* 1997; **65**: 1790–5.
- 23 Nzila A. The past, present and future of antifolates in the treatment of *Plasmodium falciparum* infection. *J Antimicrob Chemother* 2006; **57**: 1043–54.
- 24 Cui L, Su XZ. Discovery, mechanisms of action and combination therapy of artemisinin. *Expert Rev Anti Infect Ther* 2009; **7**: 999–1013.
- 25 Sridaran S, McClintock SK, Syphard LM *et al.* Anti-folate drug resistance in Africa: meta-analysis of reported dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*) mutant genotype frequencies in African *Plasmodium falciparum* parasite populations. *Malar J* 2010; **9**: 247.
- 26 Oyibo WA, Agomo CO. Scaling up of intermittent preventive treatment of malaria in pregnancy using sulphadoxine-pyrimethamine: prospects and challenges. *Matern Child Health J* 2011; **15**: 542–52.
- 27 Chico RM, Chandramohan D. Intermittent preventive treatment of malaria in pregnancy: at the crossroads of public health policy. *Trop Med Int Health* 2011; **16**: 774–85.
- 28 Briand V, Bottero J, Noel H *et al.* Intermittent treatment for the prevention of malaria during pregnancy in Benin: a randomized, open-label equivalence trial comparing sulfadoxine-pyrimethamine with mefloquine. *J Infect Dis* 2009; **200**: 991–1001.
- 29 Cisse B, Cairns M, Faye E *et al.* Randomized trial of piperazine with sulfadoxine-pyrimethamine or dihydroartemisinin for malaria intermittent preventive treatment in children. *PLoS One* 2009; **4**: e7164.
- 30 Kinyanjui SM, Mberu EK, Winstanley PA *et al.* The antimalarial triazine WR99210 and the prodrug PS-15: folate reversal of in vitro activity against *Plasmodium falciparum* and a non-antifolate mode of action of the prodrug. *Am J Trop Med Hyg* 1999; **60**: 943–7.
- 31 Nduati E, Diriyee A, Ommeh S *et al.* Effect of folate derivatives on the activity of antifolate drugs used against malaria and cancer. *Parasitol Res* 2008; **102**: 1227–34.
- 32 Metz J. Folic acid metabolism and malaria. *Food Nutr Bull* 2007; **28**: S540–9.
- 33 Wang P, Sims PF, Hyde JE. A modified in vitro sulfadoxine susceptibility assay for *Plasmodium falciparum* suitable for investigating Fansidar resistance. *Parasitology* 1997; **115**: 223–30.
- 34 Watkins WM, Mberu EK, Winstanley PA *et al.* The efficacy of antifolate antimalarial combinations in Africa: a predictive model based on pharmacodynamic and pharmacokinetic analyses. *Parasitol Today* 1997; **13**: 459–64.
- 35 Sirotnak FM, Moccio DM, Young CW. Increased accumulation of methotrexate by murine tumor cells in vitro in the presence of probenecid which is mediated by a preferential inhibition of efflux. *Cancer Res* 1981; **41**: 966–70.
- 36 Sirotnak FM, Wendel HG, Bornmann WG *et al.* Co-administration of probenecid, an inhibitor of a cMOAT/MRP-like plasma membrane ATPase, greatly enhanced the efficacy of a new 10-deazaaminopterin against human solid tumors in vivo. *Clin Cancer Res* 2000; **6**: 3705–12.
- 37 Nzila A, Mberu E, Bray P *et al.* Chemosensitization of *Plasmodium falciparum* by probenecid in vitro. *Antimicrob Agents Chemother* 2003; **47**: 2108–12.
- 38 Salcedo-Sora JE, Ochong E, Beveridge S *et al.* The molecular basis of folate salvage in *Plasmodium falciparum*: characterization of two folate transporters. *J Biol Chem* 2011; **286**: 44659–68.
- 39 Wang P, Wang Q, Sims PF *et al.* Characterisation of exogenous folate transport in *Plasmodium falciparum*. *Mol Biochem Parasitol* 2007; **154**: 40–51.
- 40 Sowunmi A, Adediji AA *et al.* Comparative effects of pyrimethamine-sulfadoxine, with and without probenecid, on *Plasmodium falciparum* gametocytes in children with acute, uncomplicated malaria. *Ann Trop Med Parasitol* 2004; **98**: 873–8.
- 41 Sowunmi A, Fehintola FA, Adediji AA *et al.* Open randomized study of pyrimethamine-sulphadoxine vs. pyrimethamine-sulphadoxine plus probenecid for the treatment of uncomplicated *Plasmodium falciparum* malaria in children. *Trop Med Int Health* 2004; **9**: 606–14.
- 42 van Hensbroek MB, Morris-Jones S, Meisner S *et al.* Iron, but not folic acid, combined with effective antimalarial therapy promotes haematological recovery in African children after acute falciparum malaria. *Trans R Soc Trop Med Hyg* 1995; **89**: 672–6.
- 43 Carter JY, Loolpapit MP, Lema OE *et al.* Reduction of the efficacy of antifolate antimalarial therapy by folic acid supplementation. *Am J Trop Med Hyg* 2005; **73**: 166–70.
- 44 Mulenga M, Malunga P, Bennett S *et al.* Folic acid treatment of Zambian children with moderate to severe malaria anemia. *Am J Trop Med Hyg* 2006; **74**: 986–90.
- 45 Barton V, Fisher N, Biagini GA *et al.* Inhibiting *Plasmodium* cytochrome bc1: a complex issue. *Curr Opin Chem Biol* 2010; **14**: 440–6.
- 46 Dzinjalimala FK, Macheso A, Kublin JG *et al.* Blood folate concentrations and in vivo sulfadoxine-pyrimethamine failure in Malawian children with uncomplicated *Plasmodium falciparum* malaria. *Am J Trop Med Hyg* 2005; **72**: 267–72.
- 47 Ouma P, Parise ME, Hamel MJ *et al.* A randomized controlled trial of folate supplementation when treating malaria in pregnancy with sulfadoxine-pyrimethamine. *PLoS Clin Trials* 2006; **1**: e28.
- 48 van Eijk AM, Ouma PO, Williamson J *et al.* Plasma folate level and high-dose folate supplementation predict sulfadoxine-pyrimethamine

treatment failure in pregnant women in Western Kenya who have uncomplicated malaria. *J Infect Dis* 2008; **198**: 1550–3.

49 Mbaye A, Richardson K, Balajo B *et al.* Lack of inhibition of the anti-malarial action of sulfadoxine-pyrimethamine by folic acid supplementation when used for intermittent preventive treatment in Gambian primigravidae. *Am J Trop Med Hyg* 2006; **74**: 960–4.

50 Loria P, Miller S, Foley M *et al.* Inhibition of the peroxidative degradation of haem as the basis of action of chloroquine and other quinoline antimalarials. *Biochem J* 1999; **339**: 363–70.

51 Martin RE, Kirk K. Transport of the essential nutrient isoleucine in human erythrocytes infected with the malaria parasite *Plasmodium falciparum*. *Blood* 2007; **109**: 2217–24.

52 Cobbold SA, Martin RE, Kirk K. Methionine transport in the malaria parasite *Plasmodium falciparum*. *Int J Parasitol* 2011; **41**: 125–35.

53 Asawamahaskda W, Yuthavong Y. The methionine synthesis cycle and salvage of methyltetrahydrofolate from host red cells in the malaria parasite (*Plasmodium falciparum*). *Parasitology* 1993; **107**: 1–10.

54 Obeid R, Holzgreve W, Pietrzik K. Is 5-methyltetrahydrofolate an alternative to folic acid for the prevention of neural tube defects? *J Perinat Med* 2013; **41**: 469–83.

55 Pietrzik K, Bailey L, Shane B. Folic acid and L-5-methyltetrahydro-folate: comparison of clinical pharmacokinetics and pharmacodynamics. *Clin Pharmacokinet* 2010; **49**: 535–48.

56 Wright AJ, Dainty JR, Finglas PM. Folic acid metabolism in human subjects revisited: potential implications for proposed mandatory folic acid fortification in the UK. *Br J Nutr* 2007; **98**: 667–75.

57 Bostom AG, Shemin D, Bagley P *et al.* Controlled comparison of L-5-methyltetrahydrofolate versus folic acid for the treatment of hyperhomocysteinemia in hemodialysis patients. *Circulation* 2000; **101**: 2829–32.

58 Bailey SW, Ayling JE. The extremely slow and variable activity of dihydrofolate reductase in human liver and its implications for high folic acid intake. *Proc Natl Acad Sci USA* 2009; **106**: 15424–9.

59 Babior BM. Chapter 37, *The Megaloblastic Anemias*. <http://medtextfree.wordpress.com/2011/12/27/chapter-37-the-megaloblastic-anemias/> (1 June 2013, date last accessed).

60 Wald NJ, Law MR, Morris JK *et al.* Quantifying the effect of folic acid. *Lancet* 2001; **358**: 2069–73.

61 Houghton LA, Sherwood KL *et al.* [6S]-5-Methyltetrahydrofolate is at least as effective as folic acid in preventing a decline in blood folate concentrations during lactation. *Am J Clin Nutr* 2006; **83**: 842–50.