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The Effect of Trimethoprim on Serum Folate Levels in Humans: A Randomized, Double-Blind, Placebo-Controlled Trial

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Trimethoprim antagonize the actions of folate by inhibition of dihydrofolate reductase. This could diminish serum folate levels in humans and causes folate deficiency in some patients. We conducted a randomized, double-blind, placebo-controlled trial, to investigate the effect of trimethoprim on serum folate levels in healthy participants after a 7-day trial period. Thirty young, healthy males were randomly allocated to receive trimethoprim, 200 mg twice daily, and 30 were randomly allocated to placebo. Before trial initiation, participant numbers were given randomly generated treatment allocations within sealed opaque envelopes. Participants and all staff were kept blinded to treatment allocations during the trial. Serum folate was measured at baseline and at end of trial. In the 58 participants analyzed (30 in the trimethoprim group and 28 in the placebo group), 8 had folate deficiency at baseline. Within the trimethoprim group, serum folate was significantly decreased ($P = 0.018$) after the trial. We found a mean decrease in serum folate among trimethoprim exposed of 1.95 nmol/L, compared with a 0.21 nmol/L mean increase in the placebo group ($P = 0.040$). The proportion of folate-deficient participants increased significantly within the trimethoprim group ($P = 0.034$). No serious adverse events were observed. In conclusion, we found that a daily dose of 400 mg trimethoprim for 7 days significantly lowered serum folate levels in healthy study participants.

Keywords: trimethoprim, folate, dihydrofolate reductase

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INTRODUCTION

Folate acts in multiple coenzyme forms in reactions involved in phases of amino acid metabolism and nucleobase synthesis.[1] Cell division and homeostasis depend on adequate folate levels.[1–3] Clinical manifestations of low folate status are wide ranging; megaloblastosis, pancytopenia, and occlusive vascular disease have been described.[2,4] Hyperhomocysteinemia caused by low folate levels has been associated with increased risk of thrombotic and cardiovascular events.[1,2] Furthermore, folate deficiency may be associated with an increased predisposition to neoplasia.[3,4] A recent prospective cohort study reported that supplementary folic acid decreased prostate cancer risk with 12% per 100 μg increase per day.[5]

Trimethoprim is a folate analog that binds to and inhibits the enzyme dihydrofolate reductase, which converts dihydrofolate to more active components.[6] As the enzyme is inhibited, dihydrofolate is catabolized, leading to folate deficiency in bacteria.[7] Trimethoprim also binds to human dihydrofolate reductase, although with a smaller affinity.[2] Studies on the effects of trimethoprim on the human folate status are conflicting. Some have found no association between trimethoprim and folate deficiency,[8–10] and others have found that trimethoprim exposure is associated with diminished folate status.[2,7,11,12] None of these studies were properly randomized.

Trimethoprim exposure during pregnancy has been found to be associated with increased risk of various malformations and miscarriage.[13,14] Two studies suggest that treatment with trimethoprim before pregnancy is associated with congenital malformations.[13,15] It is well documented that periconceptional folic acid intake reduces the risk of congenital malformations.[16–19] Official recommendations regarding folic acid supplementation during pregnancy were introduced in Denmark in 1997.[20]

The indications for use of trimethoprim are urinary, respiratory, and gastrointestinal infections. In Denmark, over 1,106,000 defined daily doses of trimethoprim and 186,000 defined daily doses of sulphamethoxazole in combination with trimethoprim were sold in 2013.[21] Worldwide, trimethoprim is used by millions because it is recommended in daily prophylaxis in AIDS treatment and HIV infections in sub-Saharan Africa.[22] In the light of the wide distribution and the possible adverse effects of trimethoprim on the folate metabolism in humans, this study investigated the effect of trimethoprim on the serum folate levels in healthy study participants.

MATERIALS AND METHODS

Trial design

This trial is a substudy of a larger study (the PENTRIOX study) performed in 3 arms, 1 with trimethoprim, 1 with penicillin, and 1 with placebo. The results from the penicillin arm have no relevance for the hypothesis tested in this study and will not be referred to here. The trial was performed at the Laboratory of Clinical Pharmacology, Copenhagen University Hospital Rigshospitalet, Denmark, from December 2010 to March 2011. Inclusion criteria were; healthy male, white nonsmoker between 18 and 35 years of age with a body mass index above 18 kg/m^2 and below 30 kg/m^2. If the potential participant met inclusion criteria, he received written information. Recruitment was done through advertisement in local media, online, and with posters in public places.

The nonfasting participants were seen 3 times at the trial site. The screening visit included assessment of eligibility and obtainment of written consent from each participant. Routine clinical tests including measurement of weight, height, blood pressure, pulse, and a blood test were performed. In addition, an electrocardiogram was recorded. The second visit at trial site (day 1) included dispensation of test medication. Each participant was handed out 32 tablets of either placebo or trimethoprim 100 mg tablets. From day 1 to 7, the participants would take either trimethoprim, two 100 mg tablets twice daily (a daily dose of 400 mg) or placebo, 2 tablets twice daily. The participants received 2 text messages daily throughout the trial reminding them to take the test medication. These were to be answered by the participant after the medicine was taken. At the third and final visit on day 8, the participants were to bring in excess tablets. At any greater or lesser number than expected, an explanation was noted. A satisfactory compliance for the individual participant was predefined as a tablet intake of at least 65%. Returned medication and any surplus of tablets were destroyed at the trial site. The final visit also included a blood test.

After screening and inclusion by the trial coordinators, participants were numbered in sequence. Before trial initiation, these numbers were given randomly generated treatment allocations within sealed opaque envelopes. The allocation sequence was kept at the trial site in a locked cabinet. Thus, participants, coordinators, investigators, and laboratory personal were kept blinded to treatment allocations.[23] Randomization was done in accordance with the International Conference on Harmonisation guidelines. Each unit of tablets was labeled with a randomization number which correlated with the given participant number to ensure traceability. Production of trial medication and randomization of treatment allocations were done by the Pharmacy of the Capital Region of Denmark.

Before trial initiation, the participants were informed about possible side effects and were told to contact an investigator in case of any side effects. At the final visit, it was ascertained whether the participants experienced any adverse events. To ensure adequate security for the participants in the experiment and to abide with regulation, all adverse events were recorded and were to be reported at the end of the trial to the Danish Health and Medicines Authority and the Danish National Committee on Biomedical Research Ethics.

Study outcome

Blood samples were analyzed at the Laboratory of Clinical Biochemistry, Copenhagen University Hospital Bispebjerg. For the determination of serum folate, a chemiluminescent microparticle folate binding protein assay on the Abbott Architect i System was used. Folate deficiency was defined as a serum folate level below 8.6 nmol/L by the laboratory.

Statistics

Data management and analyses were performed using SAS software, version 9.2 (SAS Institute Inc., Cary, NC). Serum folate was found to have a normal distribution. The mean changes in serum folate in the trimethoprim group and the placebo group were compared using a Student *t* test. Changes in serum folate within the groups were analyzed using a paired *t* test. A χ^2 test was used to analyze the difference in frequency of folate-deficient participants between the trimethoprim group and the placebo group. All tests were two-sided and *P*-values <0.05 were considered statistically significant.

Ethics

The study was conducted pursuant to the Danish Medicines Act,[24] the Declaration of Helsinki,[25] and Good Clinical Practice (GCP).[26] The study was monitored by the GCP unit at Copenhagen University Hospital and registered at the European Clinical Trials Database (reference number: 2010-022762-27)[27] and at clinicaltrials.gov (reference number: NCT02188472). The study was approved by the Danish Health and Medicines Authority (approval number: 2612–4390), the Danish National Committee on Biomedical Research Ethics (H-1-2010-099), and the Danish Data Protection Agency (BBH-2010-10).

RESULTS

Study population

Participant flow for the trial is presented in Figure 1. One hundred twelve potential participants were screened and 22 of these were excluded. Reasons for exclusion were hypercholesterolemia,[11] high blood pressure (2), elevated alanine aminotransferase (2), hypertriglyceridemia (1), hypokalemia (1), diagnosis of cardiac arrhythmia (1), heart murmur (1), use of illicit drugs (1), and withdrawal (2). Sixty participants were included in this study; 30 were randomized to the trimethoprim group and 30 were randomized to placebo (Figure 1). Baseline demographic and clinical characteristics of the trial participants are presented in Table 1. One participant withdrew because of personal issues. Overall compliance of tablet consumption was 99%. Four participants forgot to take 2 of 28 tablets leading to individual compliances of 93%. One participant in the placebo group only consumed 18 of 28 tablets, leading to noncompliance. Data from this participant were not included in the analyses.

Changes in serum folate

Of the 58 participants analyzed, 8 (14%) were serum folate deficient at baseline (Table 2). Among trimethoprim exposed, 25 (83%) had a decrease in serum folate compared with 12 (43%) in the placebo group ($P = 0.001$) (Table 2). We found a statistically significant decrease in serum folate within the trimethoprim group of −1.95 nmol/L ($P = 0.018$) compared with a nonsignificant mean increase of 0.21 nmol/L in the placebo group ($P = 0.76$). These changes were significantly different (2.16 nmol/L, 95% confidence interval = 0.10 nmol/L–4.24 nmol/L) ($P = 0.040$) (Table 2). In the trimethoprim group, 10 participants (33.3%) were folate deficient after the trial, compared with 4 (14.3%) in the placebo group ($P = 0.090$) (Table 2). Post hoc, we used McNemar test to analyze the increased frequency of folate deficiency within the trimethoprim group after the trial. This increase was statistically significant ($P = 0.034$).

Adverse events

Six (20%) adverse events were reported in the trimethoprim group, compared with 9 (32%) in the placebo group. Gastrointestinal symptoms (loose stools, constipation, and decreased appetite) were the most commonly reported adverse events in both the trimethoprim (10%) and the placebo (18%) groups. Post hoc, we compared proportions of reported gastrointestinal adverse events using Fisher exact test. No significant difference was found ($P = 0.464$). In both groups, all adverse events were mild in intensity and no code breach was necessary.

DISCUSSION

We found that trimethoprim administered twice daily for 7 days significantly lowered serum folate levels. In addition, we found a significant increase in the number of folate-deficient test participants within the trimethoprim group. This study is, to our knowledge, the first to investigate the effect of a moderate-dose, short-term exposure to trimethoprim on serum folate levels in healthy trial participants, using a randomized controlled design. Our findings differ from a few other studies.[8–10] Hjortshoj et al[9] conducted a study on 10

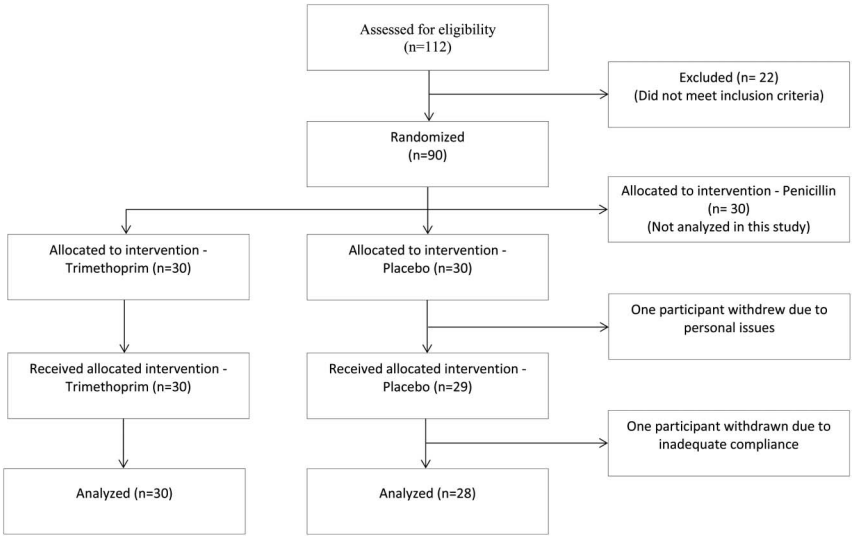

FIGURE 1. Participant flow for the trial.

patients with various bacterial infections treated long-term with trimethoprim and sulphadiazine and found no evidence for diminished folate status. Owing to the small sample size, Hjortshoj et al may not have had sufficient statistical power to detect significant changes. However, because of different study populations, our results may not be directly comparable. Kahn et al found no significant decrease in median plasma or red blood cell folate activity after high-dose trimethoprim treatment in nursing home dwellers.

Table 1. Clinical and demographic characteristics of the study populations.

	Placebo (n = 28)	Trimethoprim (n = 30)
Age, yr	24 (2.90)	25.2 (2.89)
Weight, kg	78.5 (8.9)	81.3 (9.2)
Height, m	1.84 (0.05)	1.87 (0.07)
Body mass index, kg/m^2	23.3 (2.1)	23.4 (2.3)
Systolic blood pressure, mm Hg	129 (7)	130 (8)
Diastolic blood pulse, mm Hg	77 (8)	77 (7)
Pulse, bpm	66 (9)	68 (13)
Total cholesterol, mmol/L	4.4 (0.7)	4.4 (0.7)
Serum creatinine, mmol/L	79 (12)	79 (9)
Ferritin, μmol/L	126 (81)	121 (72)
Hemoglobin, mmol/L	9.5 (0.5)	9.4 (0.6)
Iron, μmol/L	19 (7)	22 (5)
Transferrin saturation	0.28 (0.12)	0.34 (0.09)
C-reactive protein, mg/mL	1 (0)	1 (1)

Values are presented as mean and SDs.

However, the study reported other hematological abnormalities which reversed after supplements with folinic acid, a dihydrofolate reductase product analog, and concluded that the toxicity noted was caused by impaired utilization of folate induced by the actions of trimethoprim.[10] Naderer et al[12] found that 10 days high-dose trimethoprim therapy significantly lowered serum folate levels in healthy volunteers, and Smoulders et al[2] found a significant decrease in mean plasma folate after 2 weeks of moderate-dose trimethoprim exposure in healthy male volunteers, which is in accordance with our observations. They also observed a rise in plasma homocysteine levels after a few days of trimethoprim exposure, which led to the conclusion that an increased risk of thrombosis in vulnerable patients could be possible after short-term trimethoprim exposure.[2]

Patients receiving trimethoprim in clinical practice may differ from our study participants in a number of ways. They are likely to be older, have a higher prevalence of chronic diseases, and poorer nutritional status. It is to be expected that folate deficiency in these patients would be more profound after trimethoprim exposure compared with healthy participants. Long-term trimethoprim treatment is prevalent as prophylaxis against urinary tract infections in elderly patients,[28] which is likely to further increase the risk of folate deficiency. It has previously been observed that depleted plasma folate levels returned to baseline values by day 50 after 2 weeks of moderate-dose trimethoprim exposure.[2] These observations, together with the findings that trimethoprim exposure before conception is associated with an increased risk of congenital malformations,[13] indicate a prolonged effect of trimethoprim on folate status.

Table 2. Serum folate at baseline and after the trial in placebo-exposed and trimethoprim-exposed study participants.

Placebo (n = 28)	Trimethoprim	(n = 30)	*P*
Serum folate before exposure, nmol/L	14.65 (7.3)	14.42 (5.8)	
Change in serum folate, nmol/L	0.21 (3.6)	−1.95 (4.3)	0.040
Serum folate decrease	12/28 (42.9%)	25/30 (83.3%)	0.001
Folate deficiency before exposure	4/28 (14.3%)	4/30 (13.3%)	
Folate deficiency after exposure	4/28 (14.3%)	10/30 (33.3%)	0.090

Results are presented as mean (SDs) or in absolute numbers (percentages).

Trimethoprim is recommended in AIDS prophylaxis in sub-Saharan Africa. Therefore, millions of people worldwide are exposed to trimethoprim everyday. It is important to note that prophylaxis with trimethoprim-sulphamethoxazole for HIV or AIDS reduces morbidity and mortality.[29–31]

Limitations

This study was designed to detect differences in response to exposures. Owing to a relatively small sample size, our study was not designed to detect small differences in response by characteristics of participants, such as polymorphisms in genes associated with folate metabolism. We set out to investigate the effects of a moderate dose of trimethoprim on serum folate. We did not measure red blood cell folate, and the dose–response relationship was not assessed. Our trial period was relatively short, with blood tests obtained before and after the trial period. As a consequence, we were unable to detect changes in serum folate during the trial period and to measure when folate levels returned to baseline values.

Participants were nonfasting when the blood tests were performed. This could have influenced our results because it is well known that serum folate levels fluctuate with, for example, dietary intake. However, because of the randomized design of this study, it must be assumed that a potential effect of the diet was equal in the 2 treatment groups. Participants were furthermore told to be consistent in their diet during the trial period.

Common gastrointestinal symptoms were the most frequent reported adverse events. It is however unlikely that these adverse events were sufficient to cause inanition to a degree that would result in folate deficiency in itself. Only 3 participants in the trimethoprim group reported gastrointestinal symptoms during the trial.

Strengths

The main strengths of this study are the randomized, placebo-controlled design, and a high degree of compliance, ensuring that observed effects on serum folate levels were caused by exposure to trimethoprim.

CONCLUSIONS

In this double-blind, placebo-controlled clinical trial, we found that a moderate-dose trimethoprim exposure for 7 days significantly lowered serum folate levels in healthy study participants. We find our results to be biologically plausible. Low serum folate levels indicate the first stage of negative folate balance and precede tissue folate depletion. In light of the increased risk of adverse events caused by low levels of folate and the possible prolonged suppression of folate levels by trimethoprim, our study underlines the importance of assessing the folate status when trimethoprim is necessary in patients at risk of deficiency. Further well-designed clinical studies are needed to clarify the possible prolonged effect of trimethoprim on folate metabolism and the effects of certain polymorphisms on folate metabolism during treatment with trimethoprim.

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