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A Randomized, Placebo-Controlled Trial in Women of Childbearing Age to Assess the Effect of Folic Acid and Methyl-Tetrahydrofolate on Erythrocyte Folate Levels

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Abstract

Objective: To evaluate the efficacy of a multi-vitamin/mineral preparation (MMP) in achieving erythrocyte folate levels (Fol-E) considered preventive of neural tube defects (NTDs).

Methods: In this single-center, double-blind, randomized, placebo (PBO)-controlled trial, healthy women of childbearing potential (WCBP) took either an oral MMP containing 400 µg folic acid and 451 µg L-5-methyl-tetrahydrofolate (MTHF) or PBO once daily for 16 weeks. Primary endpoints were achievement of Fol-E ≥ 906 nmol/L at Week 4 and maintenance of this level once achieved. Secondary efficacy variables were plasma concentrations of folate, homocysteine, and vitamins B2, B6, and B12 at Week 4, 8, 12, and 16.

Results: Of the 40 women enrolled, 20 were randomized to each study arm. All but one PBO-subject completed the study as planned. Baseline characteristics in both groups were comparable with mean Fol-E around 500 nmol/L. In contrast to only one PBO-subject, all MMP subjects achieved and maintained the target Fol-E (MMP: 100% vs PBO: 5%, p<0.001), 14 (70%) MMP subjects already by Week 4 (means ± SD: 976 ± 186 vs 629 ± 215 nmol/L, p<0.001). In plasma, the only change under PBO was a 10% decrease of vitamin B12 whereas under MMP folate and vitamin B6 significantly increased and homocysteine significantly decreased. There was no serious and only one severe adverse event (AE); most common AEs were gastrointestinal with greater incidence in the MMP group (30% versus 5%).

Conclusion: Supplementation with folic acid and MTHF at equimolar amounts was efficacious in replenishing Fol-E of WCBP within 4 weeks to levels considered protective of NTDs.

Keywords: Folate deficiency; Neural tube defect; Folic acid; Methyl-tetrahydrofolate; Homocysteine

Introduction

Vitamins, minerals, and trace elements are essential nutrients as these cannot be synthesized in the human body. Requirement for such essential micronutrients is increased during pregnancy and lactation as reflected by recommendations for higher daily intakes by pregnant and lactating women worldwide [1-3]. Specifically, inadequacy of folate, a member of the B-vitamin family, is known to increase the risk of NTDs [4,5]. NTDs represent the second most common type of congenital malformations with an average incidence of 1-2 per 1000 live births and are associated with considerable mortality and morbidity [6,7]. To reduce the risk of NTDs a Fol-E level ≥ 906 nmol/L has been proposed [5,6]. This threshold can usually be achieved only by consuming food fortified with folate or by specific supplementation.

The efficacy of periconceptional folic acid supplementation in reducing the risk of NTDs is well established [8], although it might be less efficacious in women with genetic variants of methyl-tetrahydrofolate reductase (MTHFR), an enzyme required to transform folic acid into its active form MTHF [9-11]. Those women may particularly benefit from supplemental MTHF as this bypasses the metabolic activation. Another general advantage of using MTHF instead of folic acid is that it is not masking vitamin B-12 deficiency.

In this study, we aimed to evaluate the efficacy of a MMP containing equimolar amounts of folic acid and MTHF for the achievement and maintenance of Fol-E concentrations that are accepted to reduce the risk of NTDs in healthy young WCBP.

Subjects and Methods

Study design

This was a single-center, double-blind, randomized, placebo-controlled, two-arm, superiority trial in healthy WCBP in Germany. After a screening visit, eligible subjects were randomized to either active or placebo treatment for 16 weeks. Blood samples were drawn for analysis of Fol-E and plasma folate, homocysteine and vitamins B2, B6, and B12 at baseline in intervals of 4 weeks (Week 4, 8, 12, 16). B vitamins and homocysteine are considered indirect indicators of folate status as they interact with one another via several pathways [6]. Co-primary efficacy endpoints were the percentages of (i) women achieving the target level of Fol-E ≥ 906 nmol/L at Week 4 and (ii) of women maintaining a level above this threshold after it had once been achieved. Plasma concentrations of folate, vitamin B2, B6, B12, and homocysteine were secondary efficacy endpoints. The protocol was reviewed and approved by the IEC of the "Landesärztekammer Baden-Württemberg", Stuttgart, Germany.

Study population

Eligibility criteria included healthy WCBP aged 18 to 35 years, body mass index (BMI) of 17-30 kg/m^2, and a normal baseline Fol-E (318 to 799 nmol/L) [5]. Subjects had to be neither pregnant nor lactating, to use a medically acceptable form of contraception, and to sign an informed consent prior to enrolment.

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Study medication

The study medication was formulated in film-coated tablets (Elevit® film coated tablets, Bayer Health Care Consumer Care, Basel Switzerland). Tablets for active treatment contained equimolar amounts of folic acid (400 µg; Caesar and Loretz GmbH, Hilden, Germany) and MTHF-Ca (451 µg; Metafolin®, Merck Eprova, Schaffhausen, Switzerland) as well as vitamins A (2566 IU), B1 (1.4 mg), B2 (1.4 mg), B6 (1.9 mg), B12 (2.6 µg), C (85 mg), D3 (200 IU), E (15 IU), calcium-pantothenate (6 mg), biotin (30 µg), nicotinamide (18 mg), calcium (125 mg), magnesium (100 mg), iron (45 mg), copper (1 mg), manganese (2 mg), zinc (11 mg), iodine (220 µg) and selenium (50 µg). Matching placebo tablets were identical in appearance but contained neither vitamins nor any of the minerals above. All subjects were instructed to take one tablet of their assigned trial medication once a day, with a glass of water during breakfast, for 16 weeks. Compliance of supplement intake was assessed by pill counting upon return of blisters at each study visit.

Analytical methods

Folate concentrations were determined in lithium heparin blood and plasma by TNO Quality of Life, Netherlands using a GLP validated microbiological assay. Fol-E was calculated according to the formula: Fol-E=([whole blood folate × 100]-[plasma folate × (100-hematocrit)])/ hematocrit [12]. Plasma homocysteine and vitamin B12 levels were measured using commercial immunoassays and plasma concentrations of vitamins B2 and B6 were determined using validated HPLC methods [13].

Statistical methods

A power calculation based on effect sizes observed in an earlier study [13] revealed 30 evaluable subjects (15 per group) to be required. Assuming a drop-out rate of 25%, 40 subjects had to be randomized (20 per group). For statistical analysis, primary efficacy parameters (number of responders, i.e., women with a Fol-E ≥ 906 nmol/L (i) at week 4 and (ii) at any visit that did not fall below this threshold thereafter) were tested using Fisher's exact test. Secondary efficacy parameters (plasma levels of folate, vitamins B2, B6, B12 and homocysteine) were tested for temporal changes using a mixed model ANOVA appropriate for the treatment comparison of parallel groups with repeated measures of subjects over the weeks. Mixed-models were evaluated based on plots of scaled residuals vs. predicted values and Q-Q plots of scaled residuals. All tests were 2-sided at a significance level of 0.05. Areas under the curves (AUC) were calculated using non-compartmental methods in SAS®, version 9.2.

Results

Eighty female volunteers were screened for this study, 40 of whom were enrolled between 15 Feb 2010 and 5 Apr 2011. Subjects were randomized 1:1 to receive either active treatment (n=20) or PBO (n=20). One subject of the PBO group missed visit 3 and dropped out of the study (Figure 1).

On average, volunteers were 26.8 years old, had a mean BMI of 22.7 kg/m^2 and a Fol-E of about 500 nmol/l at baseline without any significant differences between both groups (Table 1).

Efficacy

In the active treatment group, 14 out of 20 subjects exceeded the target erythrocyte folate threshold of 906 nmol/l by week 4 as compared to 1 in 19 subjects of the placebo group (70% vs 5.3%, Fisher's exact test p<0.0001). Moreover, all 20 subjects of the active treatment group showed a sustained response, i.e., Fol-E did not fall below this threshold after it once was reached, in contrast to again only one subject in the placebo group (100% vs 5.3%, Fisher's exact test p<0.0001). While mean folate concentrations in erythrocytes increased until the end of the treatment period, those in plasma reached a plateau at about Week 8. By contrast during treatment with placebo, mean concentrations for both remained at baseline levels throughout (Figure 2; AUCs, MMP: 138.4 ± 18.6 vs PBO: 70.8 ± 23.7 days·µmol/L).

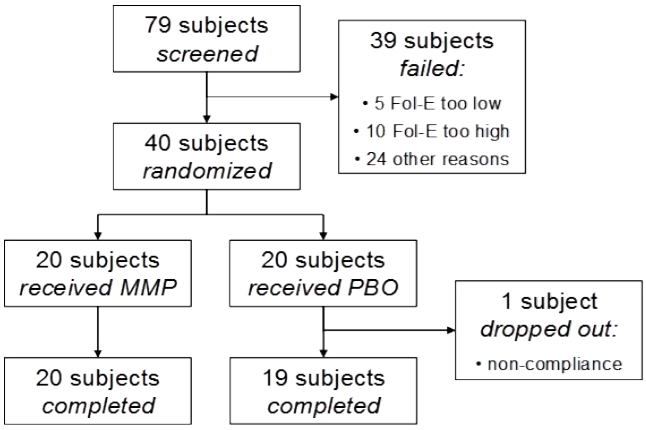

Figure 1: PConsolidated Standards of Reporting Trials (CONSORT) flow diagram

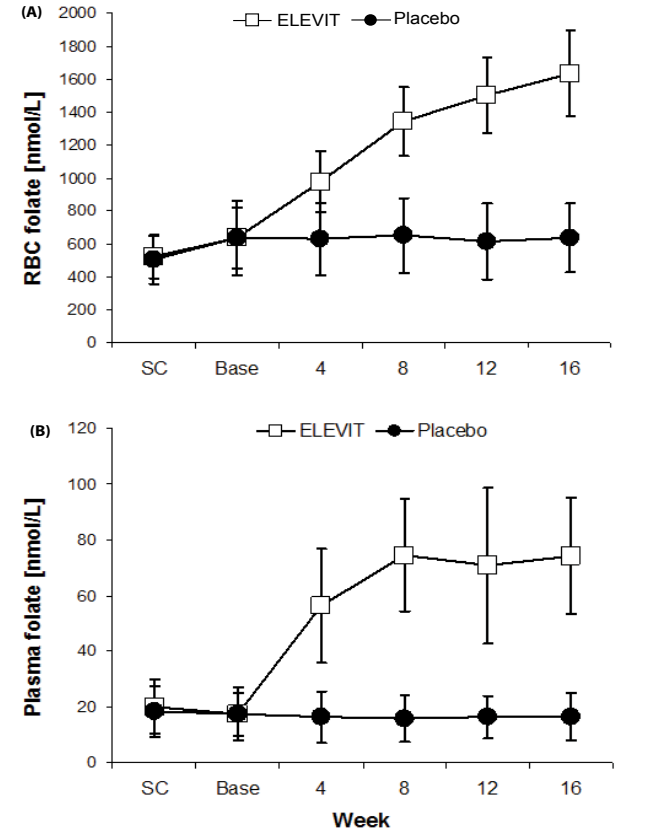

Figure 2:A and B. Folate concentrations (means ± standard deviation) in erythrocytes (on the top) and plasma (on the bottom) during the 16 weeks of treatment.
SC: screening
RBC : red blood cell

Plasma levels of vitamin B6 increased similarly to those of folate in the active treatment group only, by about 50% after week 4 (Table 2; AUCs, MMP: 3.6 ± 1.2, PBO: 2.1 ± 0.8 days·mg/L). By contrast, levels varied by <10% around baseline in the PBO group as did plasma vitamin B2 levels in general, regardless of treatment (Table 2; AUCs, MMP: 13.1 ± 2.0), PBO: 13.4 ± 2.1 days·mg/L). Vitamin B12 levels either did not change under active treatment whereas they decreased by about 10% with PBO (Table 2; AUCs, MMP: 31.0 ± 8.0 PBO: 24.1 ± 7.3 days·nmol/L). Vice versa plasma homocysteine levels decreased by about 20% already 4 weeks after initiation of treatment with MMP, and remained constant with PBO (Table 2; AUCs, MMP: 0.93 ± 0.14, PBO: 1.19 ± 0.44 days·mmol/L).

Safety and tolerability

In total, there were 129 treatment-emergent AEs, of which 12 (9.3%) were considered treatment-related. Eighty-seven and 42 AEs occurred in 15 (75.0%) MMP- and 16 (80.0%) PBO-subjects, respectively (Table 3). No AE was serious or led to study discontinuation, and only one was severe (vomiting), but deemed unrelated by the investigator. Most commonly affected system organ classes were infections, nervous system and gastrointestinal disorders with headache, nasopharyngitis, and nausea as most common AEs (Table 3). Gastrointestinal AEs were significantly more common with active treatment (30.0% versus 5.0% with PBO) and also more frequently considered drug-related. All but one AE (unrelated vitiligo at both hands) had resolved at the end of the study, in 24 out of the 31 subjects after using drug treatment, mainly for headache and infections.

Discussion

Since the early 1990s, there is high level evidence based on prospective controlled trials, that periconceptional folic acid supplementation decreases the incidence of NTDs [14-16]. A Fol-E threshold of 906 nmol/L has been established for WCBP to minimize the risk of NTDs [5]. Consequently, periconceptional folate supplementation has been included in international guidelines and is recommended to WCBP worldwide [6]. In some countries such as the United States, Canada, and Brazil mandatory food fortification with folic acid was implemented after which the incidence of NTDs indeed declined [17-20]. Still, periconceptional folate supplementation is the most reliable measure to meet increased requirements at conception and in the first months of pregnancy and to reduce the risk of adverse pregnancy outcomes.

Despite this evidence and the widely accepted key role of folate in early pregnancy, during screening for the study we still observed a high number of WCBP with Fol-E levels below the NTD protective 906 nmol/L in supposedly healthy women in Germany. In 1999, about 75% of WCBP in Germany were reported to have at least Fol-E levels >500 nmol/L [21] and in another small German study comparable to ours 10 years later, 41% of women had to be excluded due to Fol-E values >800 nmol/l [13]. In fact, this percentage was significantly lower in our study (13%) and about 5% were even below the general normal range for adults. We are not aware of any other study that recently investigated prevalence of folate deficiency in Germany, however, in Italy regionally 49% of WCBP and 33% of pregnant women were reported to have Fol-E levels even below normal [22]. Thus, dissemination and implementation of the WHO guidelines on achieving optimal Fol-E levels in WCBP to prevent NTDs still appear insufficient in Europe.

Parameter	MMP (N=20)	PBO (N=20)
Age (years)	26.4 ± 5.0 (19-35)	27.2 ± 5.0 (18-35)
Body mass index (kg/m^2)	22.1 ± 1.9 (19-26)	23.4 ± 2.9 (19-29)
Erythrocyte folate (nmol/L)	521 ± 132 (322-793)	502 ± 143 (318-780)
Women consuming low amounts of:		
• Nicotine	6 (30%)	5 (25%)
• Xanthine	11 (55%)	17 (85%)
• Alcohol	13 (65%)	12 (60%)

Table 1: Summary of baseline characteristics (means ± standard deviation, range or n [%]).

		Week				
Vitamin	**Group**	**0**	**4**	**8**	**12**	**16**
B_2 [µg/L]	MMP, N=20	121 ± 21	122 ± 19	115 ± 27	112 ± 19	115 ± 16
	PBO, N=19	124 ± 23	128 ± 27	113 ± 20	114 ± 21	125 ± 16
B_6 [µg/L]	MMP, N=20	20.7 ± 13.5	34.0 ± 14.1	33.7 ± 13.0	34.8 ± 12.9	34.4 ± 12.5
	PBO, N=19	18.5 ± 8.6	18.4 ± 10.1	20.4 ± 10.5	17.4 ± 8.5	19.8 ± 9.5
B_{12} [pmol/L]	MMP, N=20	271 ± 80	285 ± 72	280 ± 80	273 ± 78	270 ± 75
	PBO, N=19	236 ± 90	217 ± 81	203 ± 55	216 ± 60	211 ± 67
Homocysteine [µmol/L]	MMP, N=20	10.0 ± 1.7	8.1 ± 1.5	8.0 ± 1.1	8.2 ± 1.5	8.1 ± 1.4
	PBO, N=19	10.7 ± 3.0	10.5 ± 4.7	10.2 ± 3.4	10.9 ± 4.9	10.9 ± 3.3

Table 2: Plasma concentrations of secondary efficacy parameters during the 16 weeks of treatment.

System Organ class ■ Preferred term	MMP	PBO
Subjects, total Subjects, with an adverse event	20 (100%) 15 (75%)	20 (100%) 16 (80%)
Cardiac disorders	**0**	**1 (5%)**
Ear and labyrinth disorders	**1 (5%)**	**1 (5%)**
Gastrointestinal disorders	**11 (55%)**	**3 (15%)**
■ Abdominal pain	2 (10%)	1 (5%)
■ Abdominal pain upper	3 (15%)	0
■ Diarrhea	4 (20%)	0
■ Feces discolored	2 (10%)	0
■ Flatulence	4 (20%)	0
■ Nausea	6 (30%)	1 (5%)
General disorders and administration. site conditions	**2 (10%)**	**0**
■ Pyrexia	2 (10%)	0
Infections and infestations	**8 (40%)**	**7 (35%)**
Injury, poisoning and procedural complications	**1 (5%)**	**0**
Investigations	**2 (10%)**	**0**
Metabolism and nutrition disorders	**0**	**1 (5%)**
Musculoskeletal and connective tissue disorders	**1 (5%)**	**1 (5%)**
Nervous system disorders	**8 (40%)**	**7 (35%)**
■ Dizziness	2 (10%)	1 (5%)
■ Headache	7 (35%)	6 (30%)
Reproductive system and breast disorders	**0**	**3 (15%)**
■ Dysmenorrhea	0	3 (15%)
Respiratory, thoracic and mediastinal disorders	**2 (10%)**	**1 (5%)**
■ Nasopharyngitis	4 (20%)	5 (25%)
Skin and subcutaneous tissue disorders	**1 (5%)**	**3 (15%)**
■ Pruritus	0	2 (10%)

Table 3: Number of subjects (%) with treatment-emergent adverse events by system organ class and treatment group, with preferred terms provided, if >1 subject affected.

In view of this epidemiological data there might be an increasing need to replenish body folate stores quickly, given the high number of unplanned pregnancies and the early neural tube closure during the first 4 weeks of pregnancy [23]. In this regard, the tested equimolar combination of folic acid and MTHF proved to be efficacious with 70% of women reaching the Fol-E target level within 4 weeks and a continuous Fol-E increase over 16 weeks. This data accords well with that for a folic acid-only preparation using the same daily dose [13] whereas studies using lower doses up to the currently recommended 400 µg/day showed considerably longer periods to achieve the target level [12,24]. At these lower doses, folate either reached no plateau in plasma at all or it took considerably longer than the 8 weeks observed with the 800 µg-dose (Figure 2b), regardless of the preparation [13]. Of note, Fol-E is representative of tissue concentrations and therefore considered to more reliably reflect the maternal folate status, but plasma levels more closely vary with recent intakes and the amounts the embryo is actually exposed to [5]. In addition, plasma folate is known to inversely correlate with homocysteine of which high levels on their own have been associated with the risk of adverse pregnancy outcomes, including NTDs [25-27]. Vitamin B supplementation is known to decrease homocysteine levels, in our study to 20% lower levels by week 4. This effect size is in line with other studies and does neither appear to depend on the supplemented folate form nor the dose [13,28].

Other B vitamin plasma levels at baseline were also lower than reported in the other German study evaluating a folic acid-only MMP in 2009 [13], but >95% of our baseline values remained at least within normal ranges. Both MMPs contained the same amounts of vitamins B2, B6, and B12, i.e., 1.4 mg, 1.9 mg, and 2.6 µg, respectively, which are more or less representing current dietary reference intakes for pregnant women in the US [29]. However, whereas in the earlier study supplementation over 16 weeks resulted in corresponding plasma level increases of 12%, 72%, and 25%, respectively, we observed a comparable increase for B6 levels only; B2 and B12 remained mostly unchanged. Whether this deviation might be linked to the use of the combination of micronutrients in the product remains open. Otherwise available data indicate both MMPs to be equivalent in their potential to increase Fol-E, although it may still warrant further investigation whether the combination provides superior benefit to women with genetic variants of MTHFR. Interestingly, in countries with mandatory folic acid fortification folate deficiency today appears to be less prevalent, however in a Brazilian study, pregnant women with the MTHFR 677TT genotype appeared to have significantly lower plasma folate levels than women with the wildtype enzyme [30].

In conclusion, the MMP containing an equimolar combination of folic acid and MTHF at daily doses of 800 µg was well tolerated and proved to be efficacious in replenishing folate stores of WCBP to levels proposed to minimizing the risk of NTD within 4 weeks. The lower Fol-E values than observed in earlier studies in Germany still points at the need for improving dissemination of official recommendations to reach the target group of WCBPs.

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Conflict of interest

Authors currently are (ES, GB, SM, LB) or have been (ÖS until December 2013) full-time employees of Bayer Consumer Care, the marketing authorization holder of the tested MMP.

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