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Bioequivalence Evaluation of a Folate-Supplemented Oral Contraceptive Containing Ethinylestradiol/ Drospirenone/Levomefolate Calcium versus Ethinylestradiol/Drospirenone and Levomefolate Calcium Alone

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Abstract

Background: Neural tube defects (NTDs) are congenital malformations that occur during early embryonic development. Suboptimal maternal folate status is a well-known risk factor for the occurrence of NTDs, and periconceptional folic acid supplementation has been shown to reduce the risk of NTDs. Folate-supplemented oral contraceptives (OCs) offer a means of improving folate status in women of childbearing potential by increasing their likelihood of having raised folate levels at the time of conception.

Objective: This study aimed to demonstrate bioequivalence of ethinylestradiol (EE), drospirenone and L-5-methyl-tetrahydrofolate (L-5-methyl-THF; active moiety of levomefolate calcium) when taken as a new folate-supplemented OC containing EE/drospirenone/levomefolate calcium, with the respective OC containing EE/drospirenone and a tablet containing levomefolate calcium only.

Methods: This was a randomized, open-label, three-period crossover study carried out at a single centre in Germany. The study included 45 healthy women (age range 18–38 years). The women were randomly assigned to single doses of (i) EE 0.03 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg (SAFYRAL®), (ii) EE 0.03 mg/drospirenone 3 mg (Yasmin®), and (iii) levomefolate calcium 0.451 mg, administered using a crossover design, with one or more menstrual cycle washout between doses. The primary variables were maximum concentrations (C_{max}) and area under the concentration versus time curve (AUC) values for EE, drospirenone and L-5-methyl-THF.

Results: The bioavailability of EE and drospirenone was similar after administration of EE/drospirenone/levomefolate calcium and EE/drospirenone. The geometric mean ratios (GMRs) and its 90% confidence intervals (CIs) for AUC values and C_{max} were within the pre-specified range (80.00–125.00%) for bioequivalence for EE and drospirenone in both formulations. The bioavailability of L-5-methyl-THF was similar after administration of EE/drospirenone/levomefolate calcium and levomefolate calcium. The respective GMRs and 90% CIs of baseline-uncorrected and -corrected AUC_{last} (AUC from time zero to time of last measurable concentration) and C_{max} were also within the 80.00–125.00% range.

Conclusion: The novel folate-supplemented OC EE/drospirenone/levomefolate calcium is bioequivalent to the established OC Yasmin® (EE/drospirenone components) and to levomefolate calcium (folate component).

Introduction

Neural tube defects (NTDs) are a group of congenital malformations that occur during early embryonic development and are associated with abnormalities in the growth and closure of the neural tube.[1] Suboptimal maternal folate status is a well-known risk factor for the occurrence of NTDs, and periconceptional folic acid supplementation reduces the risk of NTDs.[1-4] In the USA, it is recommended that all women planning or capable of pregnancy should take daily supplementation with folic acid (0.4–0.8 mg). These US recommendations also indicate the need to start folic acid from ≥1 month before conception and to continue daily supplements until the end of the first trimester.[5] In Europe, most countries recommend taking 0.4 mg of folic acid from at least 4 weeks before conception, until up to 8–12 weeks afterwards.[6,7]

The recommendations relating to the timing highlight an important point about periconceptional folic acid supplementation: neural tube development and closure occurs within 28 days after conception, a time when most women are only just aware that they are pregnant.[1] This is relevant because folate levels rise gradually over several weeks,[8,9] and women just beginning folate supplementation may remain at an increased risk of an NTD-affected pregnancy for several weeks.

One novel way to reduce this risk may be through folate-supplemented oral contraceptives (OCs). Delivery via an OC ensures that folate intake is increased in the group that needs it, i.e. women of child-bearing potential. A further benefit is that high levels of compliance are generally seen with OC use (for example, refer to Bachmann et al.[10]). Finally, the use of a folate-supplemented OC means that, for women wishing to become pregnant, it will not be necessary to wait for folate levels to improve upon cessation of their OC. However, in order to maintain adequate folate levels during the periconceptional period, folate supplementation should be continued after stopping a folate-supplemented OC.

There are several different types of folate, one of which is levomefolate calcium. Levomefolate calcium is an alternative form of folate to folic acid and is the stable calcium salt of L-5-methyltetrahydrofolate (L-5-methyl-THF), the major folate transport form in plasma, irrespective of the mode of intake (nutrition or supplementation).[11] Unlike folic acid, levomefolate calcium does not need to be metabolized to be biologically active. It is therefore directly usable by the body, independent of genetic variants of folate-converting enzymes that control the activation of folic acid. Furthermore, levomefolate calcium has less potential than folic acid to mask vitamin B_{12} deficiency symptoms.[12] At equimolar doses, levomefolate calcium and folic acid have also been shown to be similarly effective at increasing red blood cell (RBC) and plasma folate levels in women of child-bearing potential.[8,9,13] Finally, since the pharma-

cology of L-5-methyl-THF is similar to that of folic acid, it would be expected that their effects on NTD risk reduction would also be similar.

A recent development in the area of NTD risk reduction is a levomefolate calcium containing-formulation based upon a widely used and effective OC that comprises ethinylestradiol (EE) 0.03 mg/drospirenone 3 mg (Yasmin®, Bayer Healthcare Pharmaceuticals, Berlin, Germany), and which is approved in the USA under the name SAFYRAL® (Bayer Healthcare Pharmaceuticals Inc, Wayne, NJ, USA). The regimen comprises 21 days of EE 0.03 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg, followed by a 7-day hormone-free period with tablets containing only levomefolate calcium 0.451 mg. In addition, a second levomefolate calcium-containing formulation, which is based on the OC comprising EE 0.02 mg/drospirenone 3 mg (YAZ®, Bayer Healthcare Pharmaceuticals), has also been approved in the USA under the name Beyaz® (Bayer Healthcare Pharmaceuticals Inc). This regimen comprises 24 days of EE 0.02 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg, followed by a 4-day hormone-free period with tablets containing only levomefolate calcium 0.451 mg. On a molar basis, levomefolate calcium 0.451 mg is equivalent to 0.416 mg L-5-methyl-THF, which is equimolar to 0.4 mg of folic acid.

The current study was conducted to evaluate the possible influence of levomefolate calcium 0.451 mg on the pharmacokinetics of EE and drospirenone, and the possible influence of EE 0.03 mg/drospirenone 3 mg on the plasma pharmacokinetics of L-5-methyl-THF. This was performed by evaluating bioequivalence of the new tablet formulation containing EE 0.03 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg relative to the approved and marketed tablet formulation containing EE 0.03 mg/drospirenone 3 mg and to a tablet containing levomefolate calcium 0.451 mg only.

Methods

Participants and Study Design

Healthy women aged 18–38 years, with a body mass index (BMI) of 19–28 kg/m^2 and a regular menstrual cycle (defined as a 23- to 35-day cycle length) were included if they were willing to use non-hormonal methods of contraception and were free from significant physical, neurological or psychiatric illness or disease. Subjects were excluded if they were smokers, pregnant, lactating or suffering from any condition or disease that might affect the metabolism of the study drugs or had used sex hormone-containing preparations before the study (6 months before for depot preparations and two menstrual cycles for short-acting preparations). Individuals with any clinically relevant findings obtained during the physical or gynaecological examinations were excluded from the study, as were those with hepatic disease or renal insufficiency, thromboembolic disease or increased susceptibility to thromboembolic disease. Exclusion criteria also included the use of herbal preparations, folic acid (supplements or medications) or any agent known to interfere with folate metabolism, or to induce liver enzymes during the two menstrual cycles before the start of the study.

This randomized, open-label study had an intra-individual crossover design with three treatment arms and was conducted at a single centre in Germany between August 2006 and June 2007. The study was approved by the local independent ethics committee (Ethik-Kommision der Ärztekammer, Hamburg, Germany) and was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. All subjects provided written informed consent before entering the study.

Treatments

Each subject received three separate sequential single-dose treatments, with consecutive treatments separated by a washout of at least one menstrual cycle. Each patient received all three treatments:

- EE 0.03 mg/drospirenone 3 mg (treatment A)
- EE 0.03 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg (treatment B)
- Levomefolate calcium 0.451 mg (treatment C).

Subjects were randomly assigned to one of the six possible treatment sequences (ABC, ACB, BAC, BCA, CAB or CBA) in a sequential manner using a computer-generated randomization

list. For each subject, all treatments were started between days three and six of a menstrual cycle.

On the day of each treatment period, all treatments were self-administered under the supervision of an investigator and were taken orally with 240 mL of water. Subjects were instructed to abstain from food and beverages (with the exception of water) from 21:00 hours on the evening prior to the first day of each treatment period. In addition, subjects were not permitted alcohol-, grapefruit- and folic acid-containing foods, drinks and supplements, the latter for at least two cycles prior to first study drug administration. On the morning of treatment, each subject received a standardized diet by consuming a drink containing 85 g of folate-free diet powder (containing 437 kcal [1831 kJ], 4 g protein, 58 g carbohydrate and 21 g fat) in 240 mL of water 2–3 hours before drug administration. This drink was also consumed at approximately 4, 7 and 10 hours after drug intake in accordance with requirements for folate bioequivalence studies.[14] Subjects had nothing by mouth up to 1 hour after drug intake and were then allowed water only and remained upright for 4 hours after drug administration.

During the study, smoking and the consumption of food or drinks containing black tea or caffeine were not permitted from 48 hours before study drug administration until after the last blood sample in each treatment period had been taken (up to 24 hours after drug intake). In addition, food and drink containing quinine, poppy seed, grapefruit or charcoal-broiled meat were not permitted from 1 week before study drug administration until after the last blood sample of each period was collected. The consumption of alcohol was not allowed from 48 hours before study drug administration until 2 days afterwards in each treatment period.

During screening and on the day of treatment subjects were reminded to comply with the dietary restrictions detailed above. Subjects completed food diaries to document food and drink intake during the 3 days prior to the first day of each treatment period. These diaries were checked by the investigator and any questions or missing data were discussed with the subjects. Dietary folate intake was estimated based on the mean of the 3-day dietary records.

Pharmacokinetic Tests and Analyses

Concentrations of EE and drospirenone were measured in plasma and L-5-methyl-THF in serum. Blood samples were collected 0.5 hours before (baseline) and then at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 34, 48, 72, 96, 120, 144 and 168 hours after administration of each treatment. For assessment of EE and drospirenone, blood samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA), centrifuged at 3200 *g* for 10 minutes, and plasma stored at –18 to –25°C. For L-5-methyl-THF measurements, blood samples were allowed to clot at room temperature for at least 30 minutes, centrifuged at 1800 *g* for 10 minutes, and the serum mixed with stabilization solution (20% L-ascorbic acid w/v) and stored at –80°C.

Plasma concentrations of EE and drospirenone and serum concentrations of L-5-methyl-THF were determined by a validated liquid chromatography/tandem mass spectrometry analysis with a lower limit of quantification (LLQ) of 4 pg/mL for EE, 0.5 ng/mL for drospirenone and 1.09 nmol/L for L-5-methyl-THF. The performance of the methods during analysis of the clinical samples was assessed by means of quality control samples that were included in each run. Between-run evaluations of the accuracy and precision were expressed in percentages of bias and the coefficients of the variation. For EE, drospirenone and L-5-methyl-THF the bias ranged from –0.7 to –3.0, –0.8 to –3.1 and –0.7 to 2.0, respectively; the coefficients of the variation ranged from 6.2 to 8.4, 4.1 to 7.0 and 3.3 to 4.8, respectively. Measurements of EE and drospirenone were carried out by PRA International – Bioanalytical Laboratory (Assen, the Netherlands) and L-5-methyl-THF measurements were carried out by Anapharm Québec (Québec, Canada).

A commercially available software tool (EP Series™ version 2.6.1 interfacing Kinetica™ Version 4.4.1, Thermo Electron Corporation, Philadelphia, PA, USA) was used to calculate the pharmacokinetic parameters without recourse to model assumptions. The maximum drug concentrations (C_{max}) and the time to reach maximum concentration (t_{max}) were taken directly from the concentra-

tion versus time profiles. A mixed log-linear trapezoidal rule was used when calculating the area under the plasma concentration versus time curve (AUC) from time zero to infinity (AUC_∞) and the AUC from time zero to time of last measurable concentration (AUC_{last}). Any serum or plasma concentrations below the LLQ were set to zero.

The following equation was used to calculate the AUC_∞:

$$AUC_\infty = AUC_{last} - \frac{\text{computed } C_{last}}{\lambda_Z}$$

with computed C_{last} being the concentration calculated for the time point (t_{last}) with the last quantifiable concentration and λ_Z being the terminal disposition rate constant. An AUC_∞ value was only accepted when the extrapolated area corresponded to <20% of the AUC_∞. The λ_Z was calculated using regression analysis of the perceivable linear part of the curve in a semi-logarithmic plot (λ_Z: slope of the regression line). The corresponding terminal half-life ($t_{½}$) was calculated using the following:

$$t_{½} = \frac{\ln 2}{\lambda_Z}$$

Individual values were not accepted if the time range covered by the perceivable linear part of the curve was <2 half-lives. In all cases, at least three data points were used to calculate the half-life.

Safety Analyses

All adverse events (AEs), whether observed, spontaneously volunteered or elicited upon open questioning by an investigator, were documented. Each AE was classified by the investigator in terms of its severity (mild, moderate, severe) and whether or not it was considered to be related to the study drug.

Statistical Analyses

The sample size was based on the known variation in the levels of EE and drospirenone observed during previous pharmacokinetic studies[15,16] and the assumption that the variation in L-5-methyl-THF levels is lower. Therefore, a sample size of six subjects per sequence was considered to be sufficient to establish bioequivalence. Seven subjects were randomized per sequence to allow for one missing value per sequence (dropouts were replaced; the treatment sequence assigned to the dropout was used for the replacement subject).

The primary variables were AUC_∞ and C_{max} for EE, drospirenone and L-5-methyl-THF. However, since the AUC_∞ of EE and L-5-methyl-THF was not assessable in the majority of subjects (for the per-protocol set, the number of AUC_∞ assessed as being of 'unacceptable quality' was 45 [55%] for EE and 161 [65%] for L-5-methyl-THF), the AUC_{last} was used for all subjects as the primary AUC variable for EE and L-5-methyl-THF as pre-specified in the study protocol. All other pharmacokinetic variables were considered secondary variables.

The planned analysis set for the primary and secondary variables was the per-protocol set: all treated subjects with no major protocol deviations. Safety data were analysed using the full analysis set: all treated subjects who received at least one dose of study medication and had at least one clinical observation after the start of treatment. Descriptive statistics were used to describe all variables. For the descriptive statistics of pharmacokinetic parameters the pharmacokinetic data set was used and included all subjects who yielded valid measurable values and who completed at least two treatments relevant for the investigated analyte.

The primary variables (AUC_∞ or AUC_{last} and C_{max}) were evaluated using analysis of variance (ANOVA). As these primary variables were assumed to be log-normally distributed,[17] the logarithms were analysed using subject as a random factor and period, treatment and sequence effect as fixed factors.

For each primary variable, bioequivalence was tested by calculating the two-sided 90% confidence intervals (CIs) for the point estimate of the geometric mean ratio between test (EE/drospirenone/levomefolate calcium) and reference (either EE/drospirenone or levomefolate calcium). Two comparisons were used:

(a) The effect of levomefolate calcium on the bioequivalence of EE and drospirenone was

assessed by comparing treatments A (EE 0.03 mg/drospirenone 3 mg; reference) and B (EE 0.03 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg; test).

(b) The effect of EE and drospirenone on the bioequivalence of L-5-methyl-THF was assessed by comparing treatments B (EE 0.03 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg; test) and C (levomefolate calcium 0.451 mg; reference).

The sequence effect was tested on a 10% significance level. The choice of the 10% level was in line with the significance level of bioequivalence tests that use 90% CIs. The geometric mean ratios and respective CIs were derived from the parameter estimations in the ANOVA by antilogarithmic transformation. Bioequivalence was established if the 90% CI for the relative bioavailability was completely within the equivalence interval of 80.00–125.00%.

Results

Of 147 women screened, 48 were randomized to treatment and three discontinued prior to receiving any drug. The remaining 45 subjects received at least one dose of study medication and comprised the full analysis set, of whom 41 completed the study without any major protocol deviations and comprised the per-protocol set (four subjects dropped out due to: withdrawal of consent [n = 2], pregnancy [n = 1] and vasovagal reaction [n = 1]). Drospirenone data sets from two subjects had to be excluded from the pharmacokinetic and bioequivalence analysis as the concentration of drospirenone in the study samples was evaluated against an inappropriate calibration curve. The bioequivalence analyses for drospirenone thus comprised 39 subjects, whereas the descriptive analyses were based on all evaluable subjects.

The baseline characteristics of women who participated in the study are presented in table I. The mean age of subjects was 29.7 years and their mean body weight (63.5 kg) was within the normal range. Mean dietary total folate intake ranged between 226 ± 86 μg/day and 253 ± 161 μg/day in the three study periods. The descriptive analysis of the intra-individual total folate intake over the three periods showed no consistent change over time.

Table I. Baseline characteristics of the full analysis set (n = 45)

Parameter	Mean ± SD	Range
Age, y	29.7 ± 4.6	18–37
Height, cm	167.6 ± 5.3	157–181
Body weight, kg	63.5 ± 7.4	49–80
Body mass index, kg/m^2	22.6 ± 2.3	19–28

SD = standard deviation.

The mean plasma concentration profiles and the mean pharmacokinetic parameters for EE and drospirenone were similar after single oral administration of a tablet containing EE 0.03 mg/drospirenone 3 mg alone and after a tablet containing EE 0.03 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg (figure 1 and table II). Similarly, the mean serum concentration profiles and the mean pharmacokinetic parameters for L-5-methyl-THF (baseline-corrected and -uncorrected) were similar following single oral administration of a tablet containing levomefolate calcium 0.451 mg alone and after a tablet containing EE 0.03 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg (figure 2 and table III). After administration of EE 0.03 mg/drospirenone 3 mg, L-5-methyl-THF levels remained virtually unchanged from baseline levels, indicating that the study design (e.g. nutrition on study drug administration days) had no relevant influence on the endogenous L-5-methyl-THF concentrations. In addition, there was no indication of a circadian fluctuation of L-5-methyl-THF under these study conditions (figure 2).

The mean pharmacokinetic parameters for EE, drospirenone and L-5-methyl-THF (baseline-corrected and -uncorrected) across the three treatment groups are shown in tables II and III. No differences were seen in any of the pharmacokinetic parameters for EE and drospirenone following the administration of both tablets with and tablets without levomefolate calcium 0.451 mg. Similarly, pharmacokinetic parameters for both the baseline-corrected and -uncorrected L-5-methyl-THF were comparable following treatment both with and without EE 0.03 mg/drospirenone 3 mg.

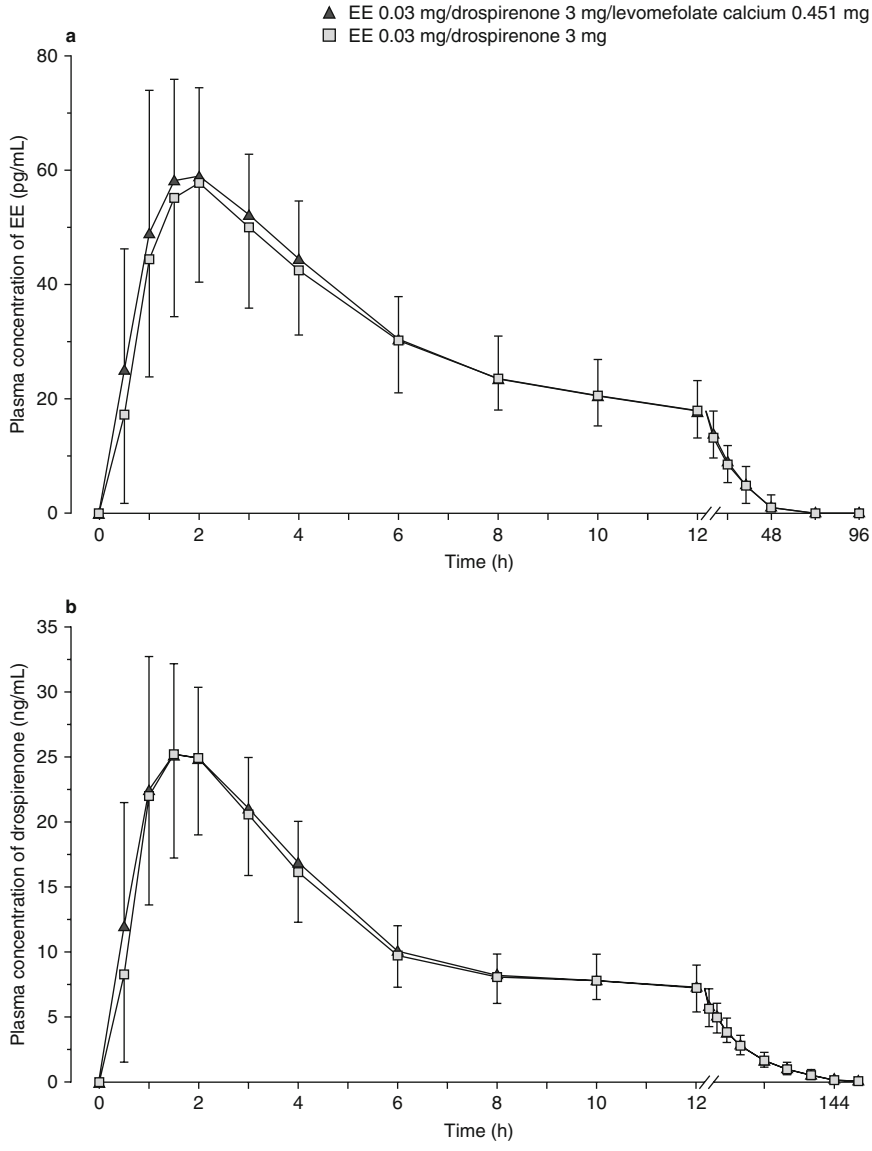

Fig. 1. Mean ± standard deviation (SD) plasma concentrations of (**a**) ethinylestradiol (EE) and (**b**) drospirenone after single oral administration of a tablet containing EE 0.03 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg and of a tablet containing EE 0.03 mg/drospirenone 3 mg. Where SD values cross over, the SD is shown in one direction only.

The absence of an effect of levomefolate calcium on the pharmacokinetics of EE and drospirenone was demonstrated by a comparison of the primary parameters under test and reference treatments. The point estimates and 90% CIs for the geometric mean ratios for C_{max} and AUC_{last} (EE) or AUC_{∞} (drospirenone) were within the predefined bioequivalence interval range of 80.00–125.00% (table IV). The pharmacokinetics of L-5-methyl-THF were also unaffected by concomitant administration with EE/drospirenone and the point estimates and 90% CIs for the geometric

Table II. Pharmacokinetic parameters for ethinylestradiol (EE) and drospirenone after administration of a single-dose formulation of EE 0.03 mg/drospirenone 3 mg with or without levomefolate calcium 0.451 mg (pharmacokinetic data set)[a]

Parameter	EE/drospirenone/ levomefolate calcium (test)	EE/drospirenone (reference)
EE	(n = 42)	(n = 42)
C_{max} (pg/mL)	61.6 (27.4)	58.5 (31.8)
AUC_{last} (pg•h/mL)	595 (29.5)	573 (31.8)
t_{max} (h)	2.0 (1.0–4.0)	2.0 (0.5–4.0)
$t_{½}$ (h)	12.1 (27.6)[b]	12.0 (24.5)[c]
Drospirenone	(n = 40)	(n = 40)
C_{max} (ng/mL)	27.2 (22.6)	26.3 (30.4)
AUC_{∞} (ng•h/mL)	447 (21.9)	433 (27.9)
t_{max} (h)	1.5 (0.5–3.0)	1.5 (1.0–3.0)
$t_{½}$ (h)	32.7 (25.0)	32.3 (24.1)

a Data are shown as geometric mean values (geometric coefficient of variation [%]) except for t_{max}, where median values (range) are provided.

b n = 17.

c n = 21.

AUC = area under the plasma concentration versus time curve; **AUC_{∞}** = AUC from time zero to infinity; **AUC_{last}** = AUC from time zero to time of last measurable concentration; **C_{max}** = maximum drug concentration; **t_{max}** = time to reach maximum concentration; **$t_{½}$** = terminal half-life.

mean ratios (for baseline-corrected and -uncorrected L-5-methyl-THF) for C_{max} and AUC_{last} were within the predefined 80.00–125.00% range (table IV).

A statistically significant sequence effect (10% significance level) was observed for the AUC_{∞} of drospirenone and the AUC_{last} of EE, respectively, but not for the respective C_{max} values.

Thus, bioequivalence was demonstrated for the rate (C_{max}) and extent (AUC values) of exposure for EE and drospirenone between the tablet formulations containing EE 0.03 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg and those containing only EE 0.03 mg/drospirenone 3 mg.

Similarly, bioequivalence was demonstrated for L-5-methyl-THF between the tablets containing EE 0.03 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg and those containing only levomefolate calcium 0.451 mg for both the baseline-corrected and -uncorrected L-5-methyl-THF geometric mean ratios.

Overall, 88.9% of subjects experienced at least one AE, none of which were considered serious or rated as severe. Most AEs were reported to be of mild or moderate intensity. One significant AE (vasovagal syncope), which was deemed unlikely to be related to study medication, was noted and led to withdrawal from the study. The most frequently occurring AE was decreased serum ferritin, which occurred in 28 women (62.2%). All cases occurred during the follow-up period and were possibly related to the frequent blood sampling (~457 mL of blood) that was conducted during the study. Other commonly reported AEs included nausea (31.1%), headache (28.9%), vomiting (26.7%) and metrorrhagia (22.2%). Bleeding irregularities such as metrorrhagia were to be expected when administering single doses of these sex hormone preparations. There was one unintended pregnancy during the second treatment period (leading to exclusion of the subject), which was considered to be related to insufficient use of non-hormonal methods of contraception, as required by the protocol.

Discussion

This randomized, open-label, three-period crossover study in healthy young women demonstrates that the rate and extent of absorption of EE and drospirenone were not affected by concomitant administration of levomefolate calcium 0.451 mg, nor were there any significant effects on the rate and extent of absorption of L-5-methyl-THF following concomitant administration with EE 0.03 mg and drospirenone 3 mg. Based on these data, which demonstrate bioequivalence, there is no reason to expect that the addition of levomefolate calcium to EE/drospirenone has an impact on the pharmacokinetics of the EE and drospirenone components. The combined safety data for EE/drospirenone, EE/drospirenone/levomefolate calcium and levomefolate calcium alone suggested that they were all generally well tolerated. The proven efficacy and safety obtained in previous studies of EE/drospirenone are, therefore, likely to be representative of the efficacy and safety of EE/drospirenone/levomefolate calcium.

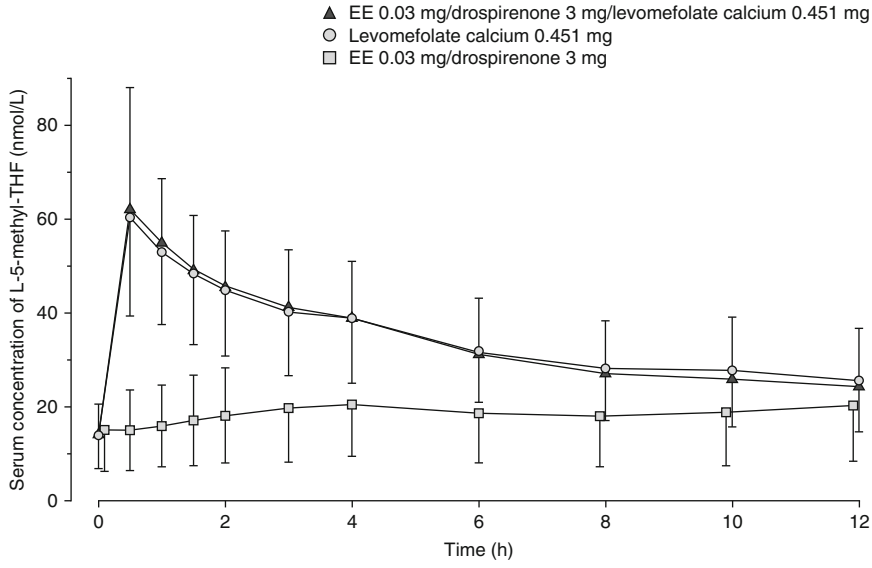

Fig. 2. Mean ± standard deviation (SD) serum concentrations of L-5-methyl-tetrahydrofolate after single oral administration of a tablet containing ethinylestradiol (EE) 0.03 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg, a tablet containing EE 0.03 mg/drospirenone 3 mg and a tablet containing levomefolate calcium 0.451 mg. Where SD values cross over, the SD is shown in one direction only.

This single-dose study was designed to assess bioequivalence under standardized conditions in accordance with relevant regulatory guidelines. An intra-individual crossover design in which the administration of the treatments was within a small time-window of the menstrual cycle was used to reduce variation in pharmacokinetic parameters. In order to ensure that all agents administered in previous treatments had been eliminated prior to the next treatment period and to avoid putative interference with physiological changes occurring during the natural menstrual cycle, a long washout phase of at least one menstrual cycle was used.

A significant sequence effect was observed for the AUC_{∞} of drospirenone and AUC_{last} of EE; however, since this was a single-dose study, EE and drospirenone are non-endogenous entities, and all EE and drospirenone baseline concentrations were below the LLQ (indicating an adequate washout period between treatments), a study inherent carryover effect can be excluded. As the study was performed with a balanced distribution of sequences, the significant sequence

Table III. Pharmacokinetic parameters for L-5-methyl-tetrahydrofolate (L-5-methyl-THF) after administration of a single-dose formulation of levomefolate calcium 0.451 mg with or without ethinylestradiol (EE) 0.03 mg/drospirenone 3 mg (pharmacokinetic data set)[a]

Parameter	EE/drospirenone/ levomefolate calcium (test)	Levomefolate calcium (reference)
L-5-methyl-THF, baseline uncorrected	n = 41	n = 43
C_{max} (nmol/L)	65.2 (30.7)	61.8 (29.2)
AUC_{last} (nmol • h/L)	393 (32.8)	390 (33.2)
L-5-methyl-THF, baseline corrected	n = 41	n = 43
C_{max} (nmol/L)	51.7 (30.6)	48.7 (30.4)
AUC_{last} (nmol • h/L)	236 (26.3)	239 (26.5)

a Data are shown as geometric mean values (geometric coefficient of variation [%]).

AUC_{last} = area under the serum concentration versus time curve from time zero to time of last measurable concentration; **C_{max}** = maximum drug concentration.

Table IV. Point estimates and 90% confidence intervals (CIs) for the geometric mean ratios for the primary pharmacokinetic parameters for ethinylestradiol (EE), drospirenone and L-5-methyltetrahydrofolate (L-5-methyl-THF; baseline-uncorrected and -corrected) after oral administration of single-dose formulations of EE 0.03 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg, EE 0.03 mg/drospirenone 3 mg and levomefolate calcium 0.451 mg (per-protocol set)

Analyte (treatment comparison)	n	Geometric mean ratio test/reference (%)	90% CI (%)
EE (EE/drospirenone/levomefolate calcium vs EE/drospirenone)			
AUC_{last} (pg • h/mL)	41	101.13	97.65, 104.75
C_{max} (pg/mL)	41	102.26	96.65, 108.20
Drospirenone (EE/drospirenone/levomefolate calcium vs EE/drospirenone)			
AUC_{∞} (ng • h/mL)	39	99.45	96.70, 102.28
C_{max} (ng/mL)	39	98.66	93.37, 104.24
L-5-methyl-THF, baseline-uncorrected (EE/drospirenone/levomefolate calcium vs levomefolate calcium)			
AUC_{last} (nmol • h/L)	41	99.63	95.73, 103.69
C_{max} (nmol/L)	41	104.90	98.83, 111.34
L-5-methyl-THF, baseline-corrected (EE/drospirenone/levomefolate calcium vs levomefolate calcium)			
AUC_{last} (nmol • h/L)	41	97.98	94.19, 101.93
C_{max} (nmol/L)	41	106.19	99.18, 113.68

AUC = area under the concentration versus time curve; **AUC_{∞}** = AUC from time zero to infinity; **AUC_{last}** = AUC from time zero to time of last measurable concentration; **C_{max}** = maximum drug concentration.

effect observed is not expected to have a misleading influence on the final outcome of the study nor was it considered to indicate a bias in the estimation of bioequivalence.

The results of the present study are consistent with those of a similar recent bioequivalence study with another EE/drospirenone/levomefolate calcium-containing OC; in this study, single doses of an OC containing EE 0.02 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg were compared with an established OC containing EE 0.02 mg/drospirenone 3 mg and levomefolate calcium 0.451 mg alone.[18] AUC and C_{max} values were similar in the two studies, with the exception of the EE values, which were approximately two-thirds of those seen in the current study, reflecting the lower EE dose in EE 0.02 mg/drospirenone 3 mg.

Although many countries, including the USA and various European countries, recommend that women start folic acid supplementation at least 4 weeks before conception and continue for at least 8 weeks afterwards, evidence suggests that it may be of value to start folic acid supplementation even earlier. A recent study of US women (where food fortification is mandatory) found that plasma folate levels reached a plateau after approximately 8 weeks in women treated with a novel OC containing EE 0.02 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg.[19] Supplementation studies in populations without food fortification have shown that plasma folate levels reach a plateau after 12 weeks.[8,20,21] For RBC folate, on the other hand, data suggest that a plateau is approached but not completely reached after 24 weeks.[8,9,20-22] Indeed, using a kinetic model, it was postulated that RBC folate steady state would only be reached after 40 weeks.[9] Thus, the time required for folate levels to substantially improve after initiation of dosing may be longer than the guidance offered by some countries. Indeed, many women may not be aware of this aspect of folate metabolism and may not realise that folate status will not improve immediately upon beginning supplementation.

For women who are planning pregnancy at some point in the future, a folate-supplemented OC formulation ensures that they would receive the recommended daily dose of folate considerably in advance of their pregnancy, which should reduce the risk of NTD-associated births. Moreover, women use OCs because they are sexually active, and therefore a folate-supplemented OC will help to ensure improved folate status in individuals who would benefit most in the event of an unplanned pregnancy. The high level of compliance associated with OC usage is also another important factor that favours the use of OCs for folate delivery. For example, a large clinical study of more than 1000 subjects followed over more than 11 100 cycles of treatment with an OC containing EE 0.02 mg/drospirenone 3 mg observed that the number of subjects who took the prescribed number of pills ranged between 92.6% and 95.7%.[10]

The present study was a single-dose bioequivalence study and, therefore, was not designed to

establish how long folate levels remain elevated following discontinuation of EE/drospirenone/levomefolate calcium. However, data from a pharmacodynamic study in European women[23] show that RBC and plasma folate levels remain above baseline in a substantial proportion of women for several weeks following cessation of EE/drospirenone/levomefolate calcium; in this study, women received 24 weeks of EE 0.03 mg/drospirenone 3 mg/levomefolate calcium 0.451 mg, followed by EE 0.03 mg/drospirenone 3 mg for 20 weeks. At 20 weeks, RBC and plasma folate levels remained above baseline in 89.3% and 41.3% of women, respectively.[23] These findings, together with the fact that as many as 46% of women in an observational study who deliberately stopped OC use to become pregnant became pregnant within three cycles of stopping their OC,[24] support the role of a folate-supplemented OC for improving folate levels in advance of conception. However, in order to maintain elevated folate levels during the periconceptional period, women should be advised to continue folate supplementation upon folate-supplemented OC cessation.

The use of levomefolate calcium in fixed combination with an OC offers several benefits. Subjects treated with equimolar amounts of levomefolate calcium and folic acid show comparable increases in plasma folate and RBC folate concentrations, as well as similar plasma folate concentration over time profiles and reductions in homocysteine levels.[12] In addition, levomefolate calcium has been shown to produce a long-term folate status at least as high as that observed with equimolar doses of folic acid.[8,13,25] Thus, levomefolate calcium offers an alternative to folic acid for the improvement of folate status and should be expected to be at least as effective as folic acid in reducing the risk of NTD-affected pregnancies.

Conclusion

Concomitant administration of EE 0.03 mg/drospirenone 3 mg with levomefolate calcium 0.451 mg in a single-dose administration has no effect on the bioavailability of the oestrogen, progestogen or folate components, demonstrating the absence of an interaction between EE/drospirenone-containing OCs and folates. Therefore, the new tablet formulation of EE/drospirenone/levomefolate calcium is bioequivalent to the established OC containing EE/drospirenone.

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Conflicts of Interests

Herbert Wiesinger, Dietmar Trummer, Hartmut Blode, Beate Rohde and Konstanze Diefenbach are employees of Bayer HealthCare Pharmaceuticals. Urte Eydeler is an employee of Scope International GmbH, the contract research organization that conducted the study, and has no other conflicts of interest to declare. Frank Richard was an employee of Bayer HealthCare Pharmaceuticals at the time that the study was conducted; Frank Richard has no other conflicts of interest to declare.

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