COMPARING FOLIC ACID PHARMACOKINETICS AMONG WOMEN OF CHILDBEARING AGE: SINGLE DOSE INGESTION OF 1.1 MG VERSUS 5 MG FOLIC ACID

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ABSTRACT

Background
A 2001 study suggested that supplementation with 5 mg folic acid, among women of childbearing age, is needed to render maximum protection against neural tube defects (NTD). No human study is presently available which examined the pharmacokinetics of 5 mg folic acid.

Objective
To compare the pharmacokinetics of ingesting a single dose of 5 mg versus 1.1 mg folic acid contained in 2 prenatal multivitamins (PregVit® and PregVit-Folic 5®), and to estimate its contribution to steady-state folate levels.

Method
The pharmacokinetics of 1.1 mg folic acid was determined in a previously published study. The method was replicated among 6 healthy, non-pregnant women who were given 5 mg folic acid to ingest. Blood samples were drawn and serum folate concentrations were measured at various time points during 10 hours post-ingestion. Standard pharmacokinetic parameters were determined and compared with Student's t-test, when appropriate.

Results
The mean area under the curve (AUC) of 1.1 mg and 5 mg folic acid were 147.6 ± 52.8 (ng/mL)·hr and 997.5 ± 271.9 (ng/mL)·hr, respectively ($p<0.0002$). An approximate 5-fold difference was detected in the peak concentrations (C_{max}) between the 2 groups ($p<0.0005$), alongside a slight difference in the times to peak (T_{max}) ($p=0.02$). The estimated steady-state serum folate concentrations produced by 1.1 mg and 5 mg folic acid were 6.2 ± 2.2 ng/mL and 41.6 ± 11.3 ng/mL, respectively ($p<0.0002$), prior to its summation with initial (baseline) steady-state levels.

Conclusion
Single dose administration between 1.1 mg and 5 mg folic acid demonstrated linear pharmacokinetics, with approximately a 5-fold difference between the 2 doses in serum folate contribution to steady-state levels, under ideal adherence.

Keywords: *Folic acid; pharmacokinetics; NTD; pregnancy; multivitamin*

The importance of folic acid before and early in pregnancy to reduce the risk of neural tube defects (NTD) such as spina bifida and anencephaly has been well documented.[1-3] Several

studies have also suggested an association between folic acid-containing multivitamins and reducing the risk of other birth defects (i.e. oral cleft palate) and certain pediatric cancers.[4,5] The synthetic folic acid, found in supplements and fortified foods, is metabolized to the biologically active form of folate, which is present in both serum and red blood cells. A 1995 study by Daly and colleagues determined that the risk for NTD increased when maternal plasma folate concentrations were below 7 ng/mL (also applicable to serum folate).[1] For some women of childbearing age, achieving protective folate levels may be challenging, particularly if they consume a low-folate diet or they have poor adherence with supplements. Standard guidelines recommend a minimum of 0.4 mg folic acid daily, although it is common for prenatal supplements to contain 1 mg folic acid. A 2001 study by Wald and colleagues suggests that supplementation with 5 mg folic acid, among women of childbearing age, is needed to render maximum protection against NTD.[6]

One approach to improving adherence and folate levels is with a new prenatal multivitamin formulation which is taken as 2 daily tablets (morning and evening tablets), aimed at separating iron from calcium to avoid their interaction. Consequently, the formulation consists of a reduced iron content (35 mg) to help minimize iron-induced gastrointestinal symptoms (i.e. nausea, heartburn, constipation). Two versions of these prenatal multivitamins are available, containing either 1.1 mg folic acid (PregVit®, Duchesnay Inc., Laval, Quebec)[7] or 5 mg folic acid (PregVit-Folic 5®, Duchesnay Inc., Laval, Quebec)[8]. We have recently reported a study comparing the 2-tablet prenatal multivitamin (PregVit®) to a once-per-day prenatal multivitamin (Orifer F®), which demonstrated no significant difference in adherence, despite the twice-daily dosing regimen.[9] Furthermore, a previously published study determined that there was no circadian variation in folate pharmacokinetics and that folic acid is absorbed similarly whether administered in the morning or evening.[10] This same study also determined the single dose pharmacokinetics of 1.1 mg folic acid.

To the best of our knowledge, no human study is presently available which examined the pharmacokinetics of 5 mg folic acid; hence, it remains unclear whether or not there is linear pharmacokinetics of absorption or elimination when folic acid ingestion increases from 1 mg to 5 mg. The objective of the present study was to compare the single dose pharmacokinetics of 5 mg folic acid to the previously determined single dose pharmacokinetics of 1.1 mg folic acid, contained in prenatal multivitamins and administered to women of childbearing age. We further wanted to estimate the contribution of 5 mg versus 1.1 mg folic acid to steady-state folate levels.

METHOD

A previously published study by our group determined the pharmacokinetics of a single dose ingestion of 1.1 mg folic acid; administered within a prenatal multivitamin to 6 healthy, non-pregnant women.[10] The method (i.e. inclusion and exclusion criteria, study design, data collection and analysis) was replicated to determine the pharmacokinetics of a single dose ingestion of 5 mg folic acid. The 2 prenatal multivitamins selected for comparison were PregVit® (Duchesnay Inc., Laval, Quebec), which contained 1.1 mg folic acid, and PregVit-Folic 5® (Duchesnay Inc., Laval, Quebec), which contained 5 mg folic acid; yet, the multivitamins were identical in tablet size, dosing regimen, and other vitamin and mineral content.

Based on the known variability of serum folate and the 5-fold difference between the 2 doses of folic acid, we determined that a sample of 6 subjects for the 5 mg folic acid group, in comparison with the 6 subjects from the 1.1 mg folic acid group of the previous study, would sufficiently detect a 20% difference in AUC, with a power of 80% and alpha of 5%.[10,11] Female volunteers were recruited through the Motherisk Program (The Hospital for Sick Children, Toronto, Canada). Motherisk is a counseling program that provides information to women on the safety or risk to a developing fetus and newborn of maternal exposures to drugs, chemicals, radiation and disease or infections. We included healthy, non-pregnant women between the ages of 18 and 45 years, who did not take multivitamins or folic acid supplements in the 6 months prior to study participation. We excluded

women with chronic medical conditions (i.e. hypertension, diabetes, epilepsy, depression, thyroid problem) and/or taking medication recurrently, including oral contraceptives or folate antagonists such as methotrexate and anticonvulsants. Other exclusion criteria were hypersensitivities to any of the ingredients in the study multivitamins and a family history of NTD. A woman was determined non-pregnant if she responded in the negative when questioned about a current or suspected pregnancy. Upon study enrolment, subjects provided written, informed consent after discussing the study with the research coordinator and agreeing to participate. The study was approved by the Ethics Review Board at The Hospital for Sick Children in Toronto.

At the beginning of the study day, after at least a 6 hour fast, a 5 mL blood sample was collected to measure baseline serum folate concentrations before folic acid was administered. Participants were given 1 evening (blue) tablet of PregVit-Folic 5® to ingest. The morning (pink) tablet of the multivitamin was not administered as it does not contain any folic acid. Blood samples were drawn at 0.5, 1, 2, 3, 4, 6, 8, and 10 hours post-ingestion. All participants were provided with the same meal and snacks, containing a total folate content of approximately 60 micrograms (μg), as prepared by a dietician who was consulted for the study. The meal, which was given 4 hours post-tablet ingestion, consisted of 1 ham sandwich (40 μg folate), yogurt (4.4 μg folate), canned fruit (3-4 μg folate), and apple juice (trace folate). The snacks included grapes (3 μg folate), apple juice (trace folate), 1 slice of cheddar cheese (4 μg folate), shortbread cookies (2 μg folate), jello (no folate), and applesauce (1 μg folate).

Blood samples were collected in 4 mL EDTA Vacutainer® tubes (Becton, Dickinson and Co., Franklin Lakes, New Jersey), and each blood sample was left to clot at room temperature for 30 minutes. The samples were then centrifuged at 1500 rpm for 15 minutes at 4°C. The serum was harvested and immediately stored at -20°C. To minimize analytical variability, all samples were analyzed as a batch within 2 months. Serum folate concentrations were measured using an Access Analyzer (Beckman Coulter Inc., Fullerton, CA). The coefficient of variation of the method was 6.6% - 15.9%. The lower limit of detection for serum folate was 0.49 ng/mL. Using the serum folate concentrations measured for the previous study (1.1 mg folic acid ingestion) and for the present study (5 mg folic acid ingestion), we plotted the data to generate concentration-time curves. Applying the trapezoid rule, we calculated the area under the curve (AUC), and we also determined the peak concentrations (C_{max}) and the times to peak (T_{max}). Apparent clearance was calculated as the dose (1.1 mg folic acid or 5 mg folic acid) divided by the AUC. Based on the assumption that supplemental folic acid ingested after fasting has a bioavailability of 100%, a bioavailability factor of 1 can be applied in clearance calculations.[12] The mean values of AUC, C_{max}, T_{max}, and apparent clearance were compared between the 2 groups, using Student's t-test, for unpaired data. P-value of less than 0.05 was considered statistically significant.

The estimated steady-state serum folate concentrations produced by the supplemental folic acid (prior to its summation with baseline serum folate levels) were calculated as the dose rate divided by the apparent clearance, whereby dose rate was either 1.1 mg per 24 hours or 5 mg per 24 hours. The overall estimated steady-state serum folate concentration was calculated as follows: (estimated steady-state serum folate concentration produced by supplemental folic acid) + (baseline serum folate concentration). To convert from nanograms per milliliters (ng/mL) to nanomoles per litre (nmol/L), divide by 0.441. Unit conversions were applied when appropriate, and the mean values of the estimated steady-state serum folate concentrations were compared between the 2 groups, using unpaired Student's t-test. P-value of less than 0.05 was considered statistically significant.

RESULTS

The present study compared the single dose pharmacokinetics of 5 mg versus 1.1 mg folic acid ingestion among healthy, non-pregnant women of childbearing age. The mean ages of the 5 mg and 1.1 mg folic acid groups were 32.5 ± 6.5 years and 24.3 ± 1.0 years, respectively (Table 1).

TABLE 1 Single dose pharmacokinetic comparison of 5 mg versus 1.1 mg folic acid ingestion, among non-pregnant women of childbearing age

	5 mg folic acid (n=6)	**1.1 mg folic acid (n=6)**	**p-value (Student's t-test)**
Age*, in years (range)	32.5 ± 6.5 (27 – 44)	24.3 ± 1.0 (24 – 26)	0.002
Weight*, in pounds (range)	160.8 ± 38.0 (120 – 220)	124.2 ± 16.6 (100 – 145)	0.09
Baseline (fasting) serum folate concentration, at time=0 (ng/mL)	11.2 ± 3.9	13.2 ± 4.0	0.41
Area under the curve, AUC [(ng/mL)·hr]	997.5 ± 271.9	147.6 ± 52.8	<0.0002
$C_{max,}$ peak serum folate concentration (ng/mL)	273. 3 ± 56.3	59.7 ± 18.4	<0.0005
$T_{max,}$ time to achieve peak concentration (hr)	1.8 ± 0.4	1.2 ± 0.4	0.02
Apparent clearance (mL/min)	91.7 ± 37.2	143.6 ± 66.6	0.13
Estimated steady-state serum folate concentration produced by supplemental folic acid (ng/mL)	41.6 ± 11.3	6.2 ± 2.2	<0.0002
Overall estimated steady-state serum folate concentration¶(ng/mL)	52.8 ± 12.6	19.3 ± 4.2	<0.0001

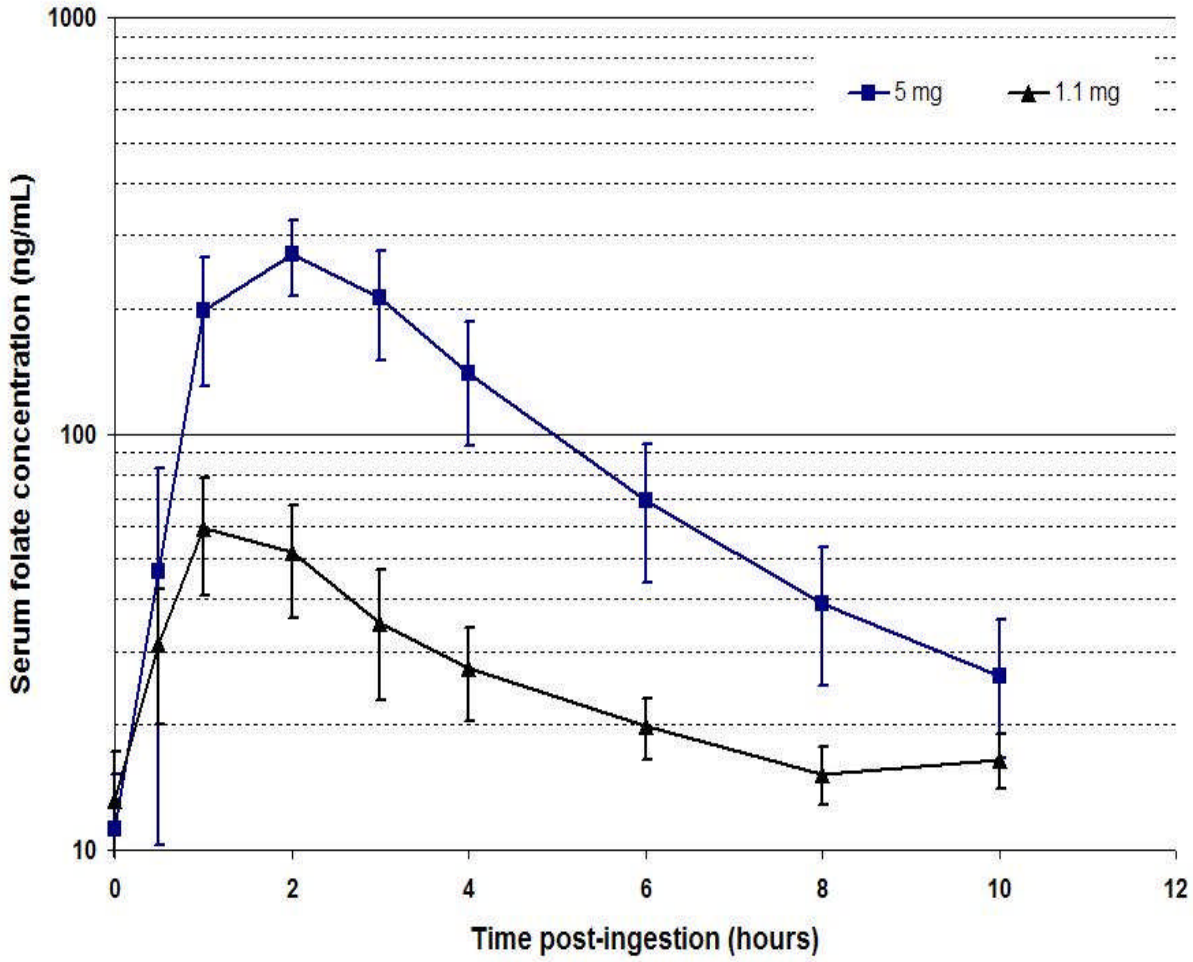
Note: Data presented as mean ± standard deviation.
* Used Mann-Whitney U test for significance.
¶ Overall estimated steady-state serum folate concentration = baseline concentration + (estimated steady-state serum folate concentration produced by supplemental folic acid).

Although a significant difference in age was detected (p=0.002), all women participating in the study were of childbearing age, as per the inclusion criteria (18-45 years). We ruled out age as having a potential confounding effect as there was no correlation between age and the various pharmacokinetic parameters such as AUC or apparent clearance (results not shown). The mean weights of the 5 mg and 1.1 mg folic acid groups were 160.8 ± 38.0 pounds and 124.2 ± 16.6

pounds, respectively (Table 1). There was no significant difference in weight. Furthermore, the 5 mg folic acid group had 2 smokers and the 1.1 mg folic acid group reportedly had no smokers.

There was no significant difference in baseline (fasting) serum folate concentrations between the 2 groups, and no significant difference was detected in the apparent clearance rates (Table 1). There was approximately a 5-fold difference between the mean AUCs of the 5 mg folic acid group [997.5 ± 271.9 (ng/mL)·hr] and the 1.1 mg folic acid group [147.6 ± 52.8 (ng/mL)·hr] ($p<0.0002$, Figure 1, Table 1).

FIG. 1 Serum folate concentration-time curves, comparing single dose ingestion of 5 mg versus 1.1 mg folic acid



An approximate 5-fold difference was also detected between the peak concentrations (C_{max}) of the 1.1 mg folic acid group (59.7 ± 18.4 ng/mL) and the 5 mg folic acid group (273. 3 ± 56.3 ng/mL) ($p< 0.0005$). The times to achieve the peak concentrations (T_{max}) were 1.2 ± 0.4 hr and 1.8 ± 0.4 hr for the 1.1 mg and 5 mg folic acid groups, respectively ($p=0.02$). No subjects reported adverse events such as nausea, heartburn, constipation, abdominal pain, or any other gastrointestinal irritability.

The estimated steady-state serum folate concentrations produced by supplemental 1.1 mg and 5 mg folic acid were calculated as 6.2 ± 2.2 ng/mL and 41.6 ± 11.3 ng/mL, respectively ($p<0.0002$, Table 1). Steady-state concentrations are additive, thus the supplemental folic acid increased the overall estimated steady-state serum folate levels to 19.3 ± 4.2 ng/mL for the 1.1 mg folic acid group and 52.8 ± 12.6 ng/mL for the 5 mg folic acid group, from baseline ($p=0.0001$, Table 1). At the beginning of the study, all subjects had baseline serum folate concentrations above the protective level of 7 ng/mL for prevention of NTD. However, if these individuals had baseline serum folate levels of 3 ng/mL or less, then 2/6 (33%) of those receiving 1.1 mg folic acid daily would not achieve the protective level. Moreover, in a recently published study, we have shown that the mean adherence rate among pregnant women taking prenatal multivitamins was 55%, although pill intake ranged from 0% (no adherence) to 100% (complete adherence).[9] We determined from this adherence data that at the 75th, 50th, 25th, and 10th percentiles, pregnant women had a pill intake of 90%, 63%, 24%, and 5%, respectively. Pill intake was defined as pills ingested out of pills prescribed. Repeating the above steady-state calculations and adjusting for adherence rates (as observed in our previous study) revealed that the estimated steady-state serum folate concentrations produced by supplemental folic acid would decrease for both groups as adherence decreased (Table 2). However, the estimated steady-state serum folate levels produced by 5 mg folic acid would remain above 7 ng/mL, even with adherence as low as 24% pill intake.

TABLE 2 Estimated steady-state serum folate concentrations produced by supplemental 1.1 mg and 5 mg folic acid, at various adherence rates

	Estimated steady-state serum folate concentrations (ng/mL)	
Adherence Rates (% pill intake)	**5 mg folic acid**	**1.1 mg folic acid**
100%	41.6 ± 11.3	6.2 ± 2.2
90% (75th percentile)	37.4 ± 10.2	5.5 ± 2.0
63% (50th percentile)	26.2 ± 7.1	3.9 ± 1.4
24% (25th percentile)	10.0 ± 2.7	1.5 ± 0.5
5% (10th percentile)	2.1 ± 0.6	0.3 ± 0.1

Note: Data presented as mean ± standard deviation. The percentiles of pill intake were acquired from a previously published study conducted by our group.

DISCUSSION

To the best of our knowledge, this is the first formal comparison of folic acid pharmacokinetics when taken at a commonly dispensed dose (i.e. 1.1 mg) versus 5 mg folic acid, which is recommended for women at high risk for an NTD pregnancy (i.e. previous pregnancy with NTD, taking anticonvulsant medication). Although alternative study designs were applicable (i.e. randomized, 2-arm, cross-over), our 2-arm comparative, parallel design was appropriate considering that the method employed by us in the previous 1.1 mg folic acid study was replicated for the present 5 mg folic acid study. However, with our limited sample size, it precluded measurements and analysis of the effects of various diets and socioeconomic status.

Another limitation was the use of prenatal multivitamins containing folic acid, as opposed to administering folic acid on its own. However, our approach was justified by the fact that folic acid is most commonly combined with other micronutrients in a prenatal preparation. The formulation of a prenatal multivitamin containing 5 mg folic acid is new and uncommon, as there is only one such product available in Canada. However, we would not expect the pharmacokinetics to differ whether the folic acid was ingested by itself or as part of a multivitamin, as the comparison in the present study was between 2 different doses of folic acid in the same type of preparation with the same content of other micronutrients.

Our data confirmed that a proportional relationship exists between folic acid dosing and pharmacokinetics such that an increase from 1.1 mg to 5 mg folic acid produced an approximate 5-fold increase in AUC, C_{max}, and estimated steady-state folate concentrations. A proportional relationship suggests that within this dose range there is minimal saturation in the absorption or elimination of folate. There was a slight, but statistically significant, difference in the time-to-peak (T_{max}), suggesting that the extent of folic acid absorption is not affected, but the rate of absorption may be marginally limited by the higher dose. Nevertheless, single dose ingestion of 1.1 mg and 5 mg folic acid essentially demonstrated linear pharmacokinetics.

Folic acid toxicity is generally not a concern considering that folic acid is a water-soluble B-vitamin, thus it can undergo renal elimination.[13] Studies involving other population groups such as neonates or the elderly have suggested that ingesting folic acid at a dose which exceeds the recommended range may lead to undesirable adverse events.[14,15] Interestingly, some literature have reported folate deficiency as a form of toxicity.[16-19] In general, folic acid is considered non-toxic across a range of recommended doses (0.4 – 5 mg/day) among healthy individuals.

Based on the single dose kinetics, it appears that under absolute adherence (i.e. 100% pill intake) with either 1.1 mg or 5 mg folic acid, women should achieve the protective serum folate level of 7 ng/mL. However, we have recently shown in a cohort of 167 pregnant women randomized to take one of two types of prenatal multivitamins, that the mean adherence rate was 55% (range: 0 to 100%), despite supervision and reminders.[9] Our present study suggests that under conditions of partial adherence, a proportion of women may not achieve protective folate levels with supplements containing 1.1 mg folic acid. Our analysis further suggests that 5 mg folic acid may yield protective folate levels at most adherence rates (i.e. pill intake of 100%, 60%, 20%). This estimation corroborates recent findings that in 2005-2006, 40% of healthy non-pregnant women and 36% of healthy pregnant women in Ontario had red blood cell (RBC) folate concentrations below 906 nmol/L (400 ng/mL) – the minimum level needed for full protection against NTDs - which may be attributed to partial adherence with 1 mg or less of supplemental folic acid.[1,20] Our study involved only serum folate measurements because a single dose of folic acid would produce rapid changes only in serum (or plasma) folate, while measurable changes in RBC folate would require multiple dosing (i.e. daily supplementation) and a time lapse of approximately 120 days, the turnover rate of red blood cells.[21] A study by Ray and colleagues reported the mean serum folate concentration for a sample of Ontario women of childbearing age to be approximately 6 ng/mL which borders at the protective level of 7 ng/mL.[22]

It is unlikely that all women of childbearing age monitor their folate levels; yet, it may not be practical for all of these women to undergo blood

tests to measure folate concentrations prior to pregnancy (particularly with unplanned pregnancies). Recently, the Society of Obstetricians and Gynaecologists of Canada (SOGC) moved to recommend 5 mg folic acid supplementation under a broader list of indications, including when adherence cannot be ensured, which may assist more women of childbearing age to achieve protective folate levels in time for pregnancy.[23] Current American and European guidelines for daily folic acid supplementation remains at 0.4 mg for planning and pregnant women, further advising women with a family history of NTD or women undergoing anticonvulsant treatment to take 5 mg folic acid daily.[24,25]

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