STUDY ON CIRCADIAN VARIATION IN FOLATE PHARMACOKINETICS

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ABSTRACT

Background
In a new preparation of prenatal multivitamins, PregVit®, two tablets a day (a.m. and p.m.) are given. Folic acid is separated from iron and zinc and is given in the p.m. tablet to overcome problems due to folic acid interactions with iron or zinc, and frequent presence of nausea and vomiting of pregnancy in the morning. The circadian variation of folate in humans has not been investigated. This is the first study attempting to determine whether circadian variation of folate pharmacokinetics exists in humans.

Objectives
To determine whether circadian rhythm of folate pharmacokinetics exists in humans.

Methods
In a crossover design, six healthy, non-pregnant women were randomized to receive 1 tablet of PregVit® p.m., containing 1.1 mg of folic acid, in the morning or evening. Serum folate levels were measured over 10 hours. The area under the concentration-time curve (AUC) was used to compare the extent of absorption between the two time periods.

Results
The mean AUC values for serum folate after administration of PregVit® p.m. were 334.5 ± 119.6 nM*h and 283.1 ± 64.3 nM*h for morning and evening, respectively (P = 0.17). The morning and evening peak serum folate concentrations were also similar (135.3 ± 41.7 nM and 130.3 ± 14.2 nM, respectively) (P = 0.75). Similarly, the time to peak for the morning arm (1 ± 0.5 hour) was similar to evening administration (1 ± 0.4 hour).

Conclusions
There is no evidence of circadian variation in folate pharmacokinetics. Thus, the introduction of folate in PregVit® p.m. will not affect its effectiveness as compared to its routine administration in the morning.

Key Words: *Folate, bioavailability, circadian rhythm, PregVit®, multivitamins, prenatal*

To help prevent the occurrence and recurrence of neural tube defects (NTDs) in pregnancy, folic acid supplementation 3 months before and early in pregnancy is recommended.[1,2] Although still unproven, folic acid supplementation has also been suggested to help prevent other fetal malformations. These include congenital heart defects,[3-6] urinary tract anomalies,[7] limb defects,[4] oral facial clefts[4;7;8] and pyloric stenosis.[3]

The Society of Obstetricians and Gynecologists of Canada (SOGC) approved in 2003 a set of guidelines recommending daily supplementation of folic acid in the periconceptional period and in pregnancy.[9] Women considered at high risk for NTDs, due to previous family history or disease that predisposes them to an increased risk, are recommended to supplement with 4 mg – 5 mg of folic acid daily.[9]

Recent studies have shown a dose response relationship between the amount of folic acid intake and effectiveness of prevention of NTDs.[10;11] In a new formulation of prenatal multivitamin supplements, PregVit®, which is given twice daily (a.m. and p.m.) and 1.1 mg of folic acid is administered in the evening (Table 1).

TABLE 1 Composition of PregVit®

Component	PregVit®
a.m. tablet	
Vit. A	2700 IU (β-Carotene)
Vit. E	30 IU
Vit. C	120 mg
Vit. B_1 (thiamine)	3 mg
Vit. B_2 (riboflavin)	3.4 mg
Niacinamide	20 mg
Vit. B_6	10 mg
Pantothenic acid (calcium pantothenate)	5 mg
Magnesium	50 mg
Iodine	0.15 mg
Iron	35 mg
Copper	2 mg
Zinc	15 mg
p.m. tablet	
Folic Acid	1.1 mg
Vit. B_{12} (cyanocobalamin)	12 μg
Vit. D_3 (cholecalciferol)	250 IU
Calcium	300 mg

The rational for the change of time at which folate is administered was to separate folate from iron and avoid their interaction,[12] as well as folic acid and zinc interaction.[13]

Furthermore, because 50-80% of women suffer from nausea and vomiting of pregnancy (NVP)[14], many of them cannot take folate supplementation in the morning. Moreover, many women with NVP discontinue folic acid intake because their nausea occurs in the morning, when they were used to typically take regular prenatal multivitamins.[15] Hence, the q Various studies have shown that circadian variation can impact pharmacokinetics of xenobiotics in humans.[16-18] Diurnal variations can result in changes in gastric acid secretion and pH, motility, and gastric emptying time, which have been reported to impact absorption of various compounds.[18] Furthermore, the effect of posture on drug disposition can be influenced by the time of day, and is known to influence drug absorption.[18]

An earlier study demonstrated that circadian rhythm has an effect on serum folate levels in mice embryos[19] with 2 daily peaks - at 8:00 a.m. and at 8:00 p.m. with no parallel effect in maternal serum. However, no published human studies have ever investigated the potential role of circadian variation on folate pharmacokinetics, which are important in the context of the new supplementation, given in the evening. The objective of this study was to determine whether circadian rhythm of folate pharmacokinetics exists in humans.

METHODS

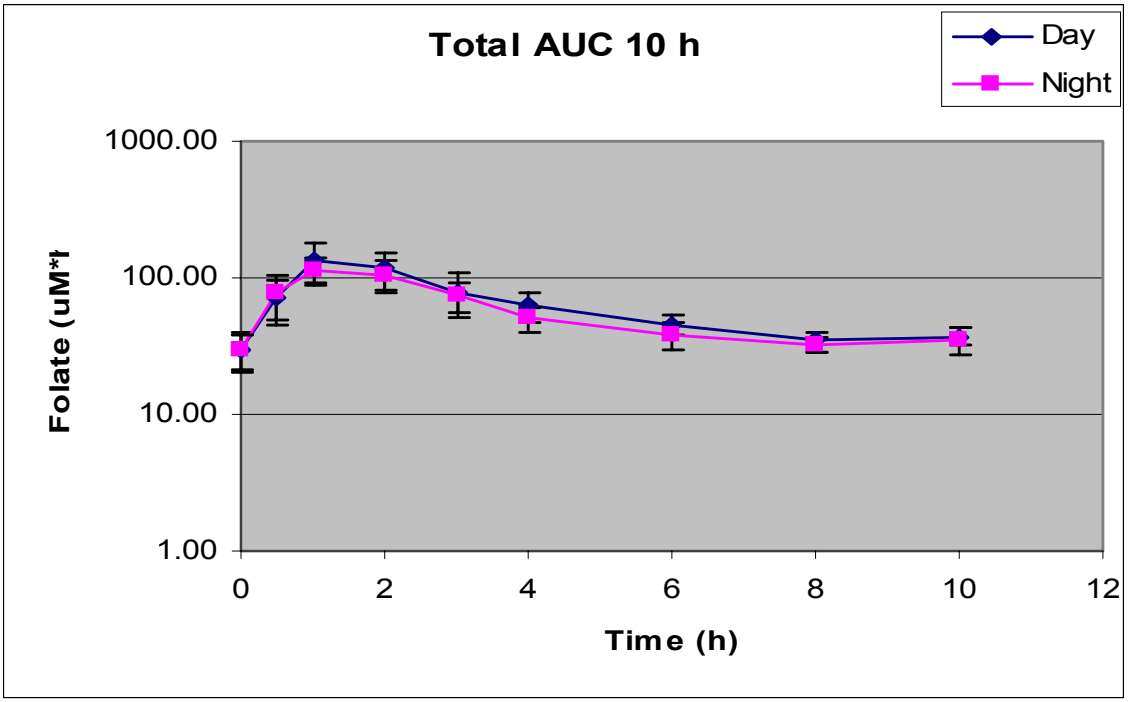
Six healthy, non-pregnant women between the ages of 18 and 45 years enrolled in this study. Each participant agreed to enter the study and signed a written consent form. The Ethics Review Board at The Hospital for Sick Children in Toronto approved the protocol.

After a six hour fast, the women were randomized to either receive one tablet of PregVit® p.m. in the morning (8:00 a.m.) or in the evening (6:00 p.m.), in a cross-over design. A 5 mL blood sample was obtained at the start of the study day to measure serum folate levels before the dose. The participants were then given PregVit® and had additional 5 mL blood samples drawn at 1, 2, 3, 4, 6, 8, and 10 hours after ingestion of the tablet. The participants were given a standard meal four hours after swallowing the multivitamin. This consisted of one scrambled egg, 2 rolls with butter, and a non-carbonated, non-caffeinated beverage, which altogether contained 40 µg of folate. Participants repeated this procedure in the subsequent study period, which was scheduled so that both days were on the same day of the woman's menstrual period.[20]

The blood samples were collected in Vacutainer® tubes (Becton, Dickinson and Co., Franklin Lakes, New Jersey). The tubes were allowed to clot at room temperature for 30 minutes. They were subsequently centrifuged at 1500 rpm for 15 minutes at 4 °C. The serum was stored at -20 °C immediately. To minimize the analytical variability, all samples were analyzed as a batch within 2 months. Serum folate samples were measured using an Access Analyzer (Beckman Coulter Inc., Fullerton, CA). The coefficient of variation of the method was 6.6% - 15.9%. The trapezoid rule was used to determine the AUC of folate.[21]

From this curve we also determined peak concentrations (Cmax) as well as time to peak (Tmax). AUC values were compared between the two study periods using the paired Students' *t* test. The same statistical method was employed to compare peak concentrations as well as the time to peak of serum folate between the two study periods. Given the known variability in serum folate levels, a sample of 6 subjects would allow detection of 20% difference in AUC with power of 80% and alpha of 5%(22).

FIGURE 1 Mean concentration time values (± SD) for serum folate during the day compared to at night.



RESULTS

The mean AUC values for serum folate after administration of PregVit® p.m. were 334.5 ± 119.6 nM*h and 283.1 ± 64.3 nM*h for morning and evening, respectively (P =0.17) (Figure 1). Comparing the Cmax, the morning and evening peak serum folate concentrations were 135.3 ± 41.7 nM and 130.3 ± 14.2 nM, respectively (P = 0.75). The Tmax for the morning (1 ± 0.5 hour) was similar to that in the evening (1 ± 0.4 hour).

DISCUSSION

This is the first study to investigate potential circadian variation in folate pharmacokinetics in humans. The issue was raised when a new generation of prenatal multivitamin supplement, PregVit®, was designed such that folic acid is administered in the evening to allow separation from iron and zinc, which are given in the morning. Folic acid is separated from iron in PregVit® because concurrent ingestion of folic acid may form stable complexes with iron, decreasing their intestinal absorption.[12] There is evidence that zinc may also attenuate the absorption of folic acid.[13] Iron ingestion at high doses is associated with adverse effects such as nausea, constipation, fatigue, diarrhea, and headache[23;24], which can impede on the daily consumption of prenatal multivitamins.[23]

Previous studies on regular multivitamin supplements with high doses of iron have found that these intestinal adverse effects may cause 10% to 40% of pregnant women to discontinue daily consumption of these supplements.[24;25] PregVit® has a reduced content of iron and due to its new formulation, enhanced bioavailability of iron that helps to reach iron blood levels of regular multivitamins (unpublished data). By separating folic acid from iron in PregVit®, women sensitive to iron may still be able to take the folic acid tablet without experiencing iron associated adverse effects. A previous study in mice embryos demonstrated circadian variation affecting serum folate levels.[19] This was shown to be clinically relevant for the protective effects of

the folate metabolite, folinic acid (5-CHO-THF), on valproic-acid induced teratogenicity.

However, in the same study maternal serum folate levels did not change significantly over the course of the day. This is the first study attempting to determine whether circadian variation of folate pharmacokinetics exists in humans. In our investigation, the mean AUC, Cmax, and Tmax values were all similar in the morning and evening, which provides support, that circadian variation do not affect folic acid absorption, distribution and elimination. However, similar to the animals, in the future it would be of interest to determine if circadian rhythms affect folate concentrations in the human embryos.

While circadian rhythm does not influence folate pharmacokinetics, other factors have been shown to affect its absorption. Dawson et al.[22] conducted a study comparing folate bioavailability from two prenatal multivitamin supplements. They showed that different formulations among prenatal multivitamin supplements also affects absorption.[22] The standardized meal given 4 hours after dose, containing 40 µg of folate, was chosen to minimize the amount of folate given and to exclude any molecules known to inhibit the absorption of folate.

In conclusion this study demonstrates that there is no circadian variation in folate pharmacokinetics. Thus, the folic acid contained in PregVit® p.m. will be absorbed similarly whether given in the morning or in the evening.

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