Accumulation of Plasma Reduced Folates After Folic Acid Administration

David G. Priest, John C. Schmitz, and Marlene A. Bunni

The pharmacokinetics of folic acid, and resultant metabolites thereof, have been determined after administration orally and intravenously at 25 mg/m^2 and 125 mg/m^2. Saturation behavior was observed for uptake of folic acid into plasma and with regard to metabolism to methylenetetrahydrofolate and tetrahydrofolate as well as methyltetrahydrofolate. Repetitive oral administration every 6 hours resulted in consistently elevated levels of each metabolite pool with the same general saturation behavior as observed with single dose administration. This repetitive oral administration is concluded to be a suitable means to provide uniform elevation of metabolites that could offer protection from undesirable toxic effects of drugs such as MTA.

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WHILE folic acid (FA) has been used as a vitamin supplement for many years, it has more recently received interest as a means to modulate the activity of antifolates.[1-4] This interest has centered on toxicity reversal, permitting higher dosages of cytotoxic drugs to be administered safely. Much of the basis for use of FA as a pharmacologic modulating agent is associated with studies conducted in animal models.[1,5-7] However, human trials have been recently undertaken to determine the feasibility of this approach in the clinical setting.[3,4]

Folic acid itself is unlikely to be pharmacologically active. It is far more likely that the reduced metabolites of FA are the active species. The predominant reduced folate found in human plasma is 5-methyltetrahydrofolate (5-CH_3FH_4).[8] However, following the administration of FA, other metabolites also arise, albeit to a lesser extent than the major metabolite. The degree to which each of these metabolites participates in the modulatory activity of FA is not clear, but their presence in plasma makes each a candidate. Hence, the dependence of plasma accumulation of each metabolite pool on dose, schedule, and administration route can provide useful information to guide the appropriate administration of FA in conjunction with antifolates.

From the Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC.

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Address reprint request requests to David G. Priest, PhD, Department of Biochemistry and Molecular Biology, Medical University of South Carolina, 171 Ashley Ave, Charleston, SC 29425.

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MATERIALS AND METHODS

Materials

Folic acid was obtained from Lyphomed (Deerfield, IL). Radiolabeled fluorodeoxyuridine monophosphate ([^{3}H]FdUMP) was purchased from Moravek Biochemicals (Brea, CA). Nicotinamide adenine dinucleotide phosphate and all other reagents were purchased from Sigma Chemical Co (St Louis, MO). Thymidylate synthase (3.7 U/mg protein) was purified from an *Escherichia coli* strain that overproduces *Lactobacillus casei* thymidylate synthase.[9] The *E coli* strain was a gift from D. Santi (University of California, San Francisco). 5,10-Methylenetetrahydrofolate reductase (0.62 U/mg protein), 10-formyltetrahydrofolate deacylase (1.1 mU/mg protein), and dihydrofolate reductase (1.25 U/mg protein) were purified from pig liver, beef liver, and methotrexate-resistant *L casei*, respectively.[10-12]

Folic Acid Administration

Ten volunteers were randomly separated into two groups of five each. One group was administered FA at a single dose of 25 mg/m^2; the other group received 125 mg/m^2. Each group was administered FA both orally and intravenously (IV). A 2-week washout period lapsed before the same volunteer was given the same dose by the alternative administration route. In addition, four more volunteers were recruited and administered FA at total daily doses of 100 mg/m^2 in doses of 25 mg/m^2 every 6 hours for 3 days. After a 2-week washout period, the same volunteers received 500 mg/m^2 FA daily in doses of 125 mg/m^2 every 6 hours for 3 days.

Sample Collection and Preparation for Analysis

Blood samples were collected and immediately centrifuged at 400*g* for 5 minutes at 4°C. The resultant plasma was diluted into an equal volume of cold 50 mmol/L Tris-HCl buffer (pH 7.4) that contained 100 mmol/L sodium ascorbate and was stored at -20°C. For routine analysis of folates, plasma samples were placed in a boiling water bath for 5 minutes and centrifuged to remove precipitated protein. The resultant supernatant used for estimation of reduced folates ranged from 0.1 μL (10 μL diluted 100-fold) to 100 μL, depending on FA dose and sampling time.[8]

Estimation of Reduced Folates

The ternary complex assay described previously was used to quantitate reduced folates.[5,13] This assay is based on enzymatic cycling of reduced folates to methylenetetrahydrofolate (CH_2FH_4) followed by entrapment into a stable ternary complex with excess *L casei* thymidylate synthase and [^{3}H]F-

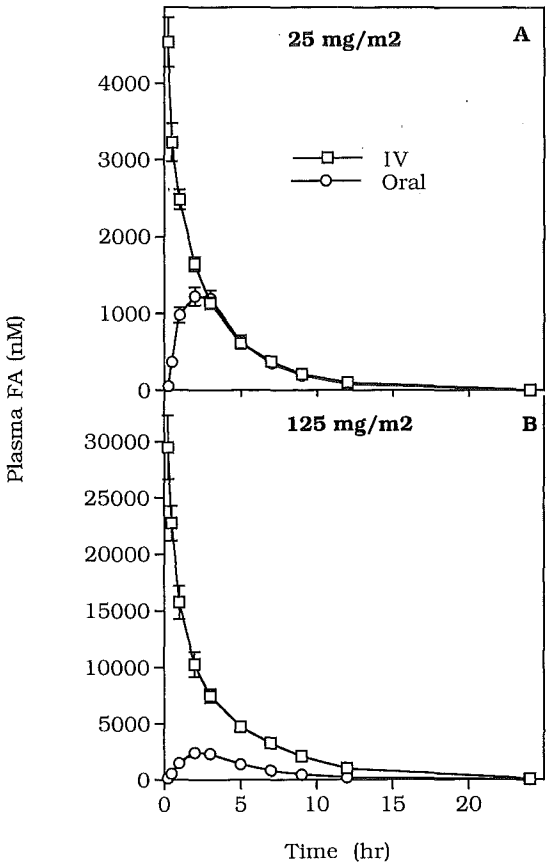

Fig 1. Plasma pharmacokinetics of FA. Each group of five volunteers was given FA IV and orally at doses of 25 mg/m^2 (A) or 125 mg/m^2 (B). Folic acid was estimated by the ternary complex-based assay. Points represent the average from duplicate analyses of samples from five volunteers in each dosage group. Error bars represent SEM.

dUMP.[14] Additional enzymes and cofactors were added as necessary to cycle each reduced folate to the CH_2FH_4 form. Bound [^{3}H]FdUMP, which is equivalent to CH_2FH_4, was determined by scintillation counting following separation on Sephadex G-25 minicolumns. Since CH_2FH_4 can potentially be converted to tetrahydrofolate (FH_4) under these conditions, the sum of these folates is reported.[15]

RESULTS AND DISCUSSION

Folic acid typically has been administered orally at very low doses, consistent with its role as a vitamin.[3] Thus, little information is available regarding its pharmacokinetic properties when administered as a pharmacologic agent. To examine the behavior of FA and its metabolites at pharmacologically relevant doses, human volunteers have been administered FA doses of 25 mg/m^2 and 125 mg/m^2 both orally and IV. Parent compound and corresponding reduced folate metabolites have been evaluated by the ternary complex assay in plasma over a 24-hour period.[8] It can be seen in Fig 1A that at the lower dose, essentially identical levels of FA are achieved after approximately 3 hours, whether FA is administered orally or IV. This is consistent with relatively efficient absorption of FA into the blood stream at this dose. Furthermore, when area under the curve (AUC) values for oral versus IV administration were compared (6,977 ± 747 nmol/L•hr and 11,632 ± 817 nmol/L•hr, respectively), nearly 60% of an oral dose is absorbed into the circulatory system at the 25 mg/m^2 dose. On the other hand, administration of 125 mg/m^2 FA results in much less efficient absorption behavior. Figure 1B shows that at no point is the same plasma level of FA achieved. Likewise, the oral versus IV AUC values

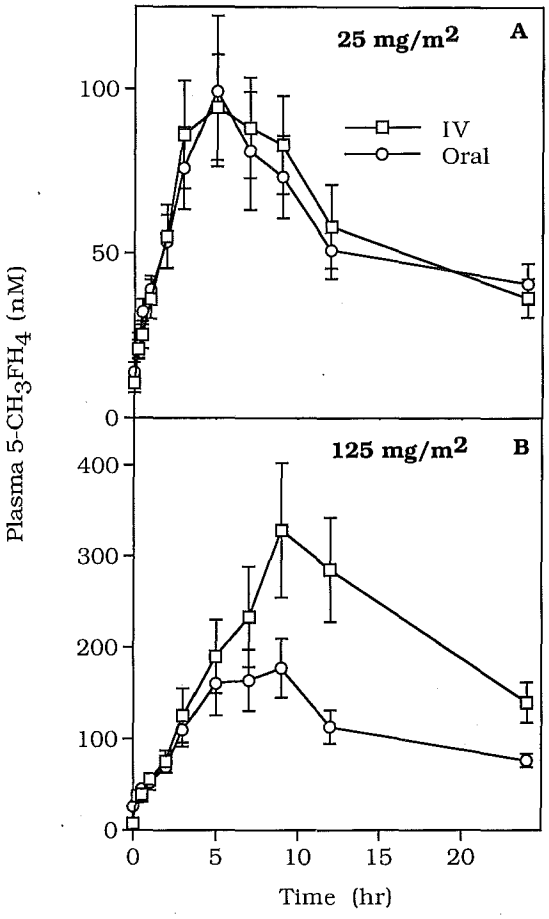

Fig 2. Plasma accumulation of 5-CH_3FH_4 after administration of FA IV and orally at doses of 25 mg/m^2 (A) or 125 mg/m^2 (B). 5-CH_3FH_4 was estimated by the ternary complex-based assay. Points represent the average from duplicate analyses of samples from five volunteers in each dosage group. Error bars represent SEM.

(14,127 ± 1,790 *v* 83,066 ± 6,663) yield an estimate of absorption of only 17%. Hence, there is clear saturation of FA absorption into the blood stream as the dose is increased from 25 mg/m^2 to 125 mg/m^2.

5-CH_3FH_4, the predominant circulating reduced folate, became elevated substantially following FA administration (Fig 2). At the lower dose (Fig 2A), the accumulation of this metabolite is essentially identical whether FA is given IV or orally. This is true with regard to both peak accumulation (C_{max}) and AUC. At the higher dose, oral administration results in both C_{max} and AUC values that are only approximately half those for IV administration. When comparison is made for C_{max} or AUC between FA oral doses of 25 mg/m^2 versus 125 mg/m^2, it can be seen that the fivefold increase in dose results only in approximately a twofold increase in this metabolite pool. These results are consistent with saturation of FA absorption as a major component of the limited increase in 5-CH_3FH_4 elevation as the dose is increased from 25 to 125 mg/m^2. However, there is also some saturation of metabolism at the higher dose because when administered IV, wherein the entire dose is available for metabolism, 5-CH_3FH_4 C_{max} is only increased approximately threefold and AUC approximately fourfold, as a result of the fivefold increase in dose (25 mg/m^2 *v* 125 mg/m^2). Hence, there is a dose-dependent limitation of 5-CH_3FH_4 accumulation after FA administration that is largely due to uptake saturation with a smaller contribution from saturation of metabolic capacity.

In addition to 5-CH_3FH_4, the methodology used permits evaluation of the more labile, but potentially more important, reduced folate pool, CH_2FH_4 + FH_4 (Fig 3). This pool behaves pharmacokinetically much like the 5-CH_3FH_4 pool (Fig 2) and achieves maximal accumulation that is approximately half that of the more stable metabolite pool. As such, this pool can have a significant quantitative importance to FA modulation. The CH_2FH_4 + FH_4 pool, like the 5-CH_3FH_4 pool, shows a strong dose-dependence for the time required to achieve maximal accumulation. This is true for both IV and oral dosing and suggests that metabolic capacity is the origin of this delay in peak achievement.

While the prolonged elevation associated with the relatively long time to peak for all metabolite pools after a single dose results in an expanded

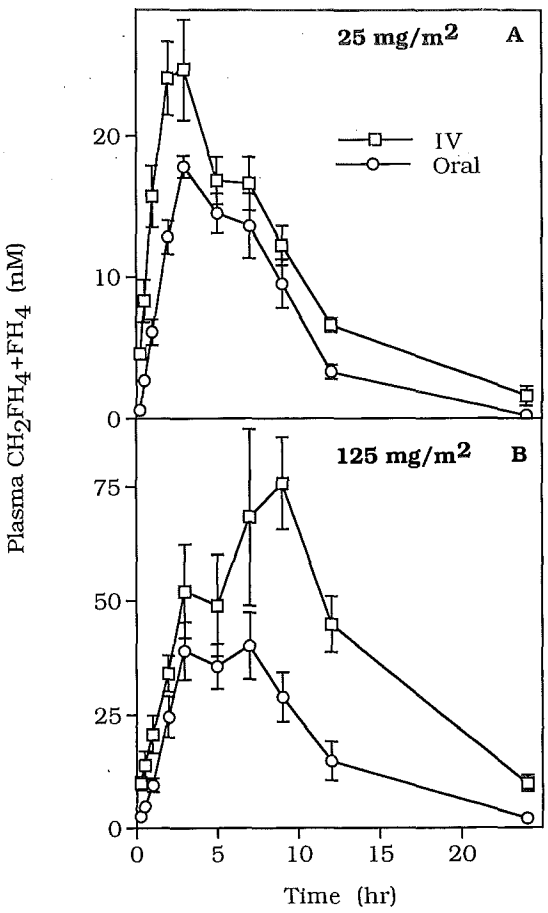

Fig 3. Plasma accumulation of CH_2FH_4 + FH_4 after administration of FA IV and orally at doses of 25 mg/m^2 (A) or 125 mg/m^2 (B). CH_2FH_4 + FH_4 was estimated by the ternary complex-based assay. Points represent the average from duplicate analyses of samples from five volunteers in each dosage group. Error bars represent SEM.

opportunity for administration of other drugs whose modulation is sought, single daily doses do not result in consistent elevation over the entire period. Hence, multiple doses were evaluated to further extend this modulatory window. Folic acid was administered to volunteers orally every 6 hours for 3 days at total daily doses of 100 mg/m^2 and 500 mg/m^2. Metabolite pools, as well as parent compound, were monitored (Fig 4). This administration schedule resulted in achievement of relatively constant metabolite elevation at approximately twofold higher levels than were observed at C_{max} for equivalent single doses. Parent compound behavior was less uniform than metabolite pools and could suggest some circadian dependence of uptake or elimination. Generally, the same saturation of uptake is observed as when single doses

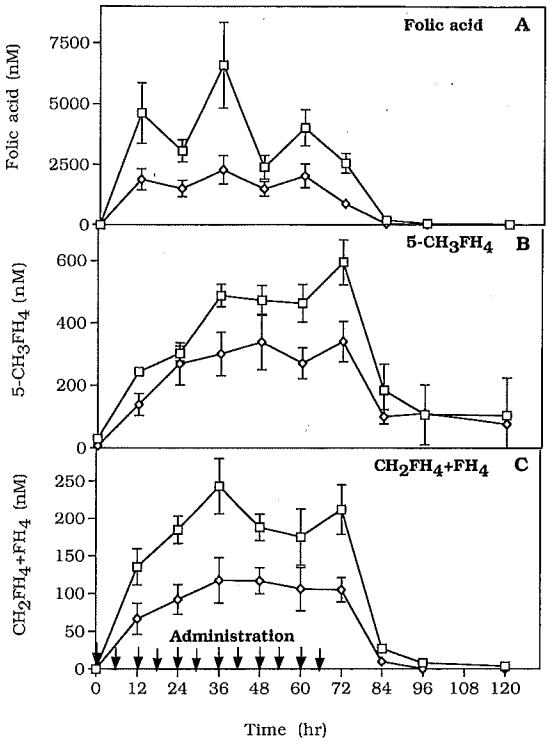

Fig 4. Plasma accumulation of FA (A), 5-CH_3FH_4 (B), and CH_2FH_4 + FH_4 (C) following administration of oral FA to four volunteers. Folic acid was administered at a total daily dose of 100 mg/m^2 (open diamonds) given four times daily (25 mg/m^2/dose) for 3 days. After a 2-week washout period, the same volunteers received a total dose of 500 mg/m^2 FA (open boxes) given four times daily (125 mg/m^2/dose) for 3 days. Plasma folates were estimated by the ternary complex assay. Points represent the average from duplicate analyses of samples from four volunteers in each dosage group. Error bars represent SEM.

are increased from 25 mg/m^2 to 125 mg/m^2. Hence, this schedule permits a substantially higher sustained metabolite level, which may, in turn, provide a more consistent means for modulating the antitumor activity of drugs that target reduced folate-metabolizing enzymes.

In summary, FA administered orally exhibits dose-dependent saturation of absorption and, to a lesser extent, metabolism. The relatively prolonged elevation of plasma-reduced folate metabolites can be extended further with multiple dosing, which also leads to achievement of twofold higher levels than can be achieved maximally with a single dose. Hence, FA administered in convenient oral doses can be used to provide sustained elevation of reduced folate metabolites which likely are the agents that modulate the toxicity of antifolates such as MTA.

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