Pharmacokinetics and Toxicity of Continuous Infusion (6S)-Folinic Acid and Bolus 5-Fluorouracil in Patients with Advanced Cancer


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ABSTRACT

Twenty-seven patients with advanced cancer were entered in a phase I study of bolus i.v. 5-fluorouracil at a dose of 370 mg/m²/day for 5 days combined with a continuous i.v. infusion of (6S)-folinic acid for 5.5 days, starting 24 h in advance of the first 5-fluorouracil dose. The dose of (6S)-folinic acid was escalated in cohorts of patients from 250 mg/m²/day to a maximum of 1000 mg/m²/day. The pharmacokinetics of (6S)-folinic acid were studied in the 3 patients given 250 mg/m²/day and in 6 patients given 1000 mg/m²/day. The mean steady-state plasma concentrations of (6S)-folinic acid and its principal metabolite (6S)-5-methyltetrahydrofolate at the 250 mg/m²/day dose were 2.7 and 1.5 μM, respectively. Both concentrations were comparable to the concentrations produced when (6R,S)-folinic acid was administered as half of a (6R,S)-folinic acid mixture (E. M. Newman et al., Cancer Res., 49: 5755–5760, 1989). At the 1000 mg/m²/day dose of (6S)-folinic acid, the concentration of (6S)-folinic acid was 15.3 μM, more than the 4-fold increase predicted by linear pharmacokinetics, while the concentration of (6S)-5-methyltetrahydrofolate was only 16.5 μM. The change in the ratio of the parent compound to its metabolite was accounted for by a decrease in the nonrenal clearance of (6S)-folinic acid, probably indicating saturation of its metabolism. The toxicities observed in this phase I trial, including stomatitis, diarrhea, neutropenia, and anemia, did not differ in nature or severity from those reported for 5-fluorouracil and (6R,S)-folinic acid when administered on the same schedule. Finally, the degree of toxicity did not appear to depend on the dose of (6S)-folinic acid over the range of doses tested.

INTRODUCTION

Preclinical evidence demonstrates that pharmacological concentrations of folates can potentiate the antineoplastic activity of 5-FUra (1–4). This potentiation is the result of stabilization of the 5-fluoro-2′-deoxyuridylate/thymidylate synthase/5,10-methylenetetrahydrofolate ternary complex (5). Intracellular accumulation of folate cofactors in tissue culture is relatively slow, requiring 12 or more hours to reach maximal concentrations (6, 7). Likewise, in human colon carcinoma xenografts in mice, prolonged infusions of high doses of (6R,S)-folinic acid are necessary to produce the levels of highly polyglutamylated folate cofactors required for maximal stabilization of the ternary complex (8). (6R,S)-Folinic acid and (6R,S)-CH₃-THF appear to produce equivalent potentiation of fluoropyrimidine activity in most cultured cell lines (2, 9). However, one study in xenografts suggests that (6R,S)-CH₃-THF is not as effective as (6R,S)-folinic acid as a precursor of intracellular folate cofactors for some human colon carcinomas (10).

The ability of (6R,S)-folinic acid to enhance the efficacy of 5-FUra has been confirmed in several clinical trials. The objective response rate of patients with colorectal carcinoma after treatment with 5-FUra and high-dose (6R,S)-folinic acid has been reported to be as high as 22% in patients previously exposed to 5-FUra (11) and between 26 and 48% in previously untreated patients (11–16). Comparative trials have demonstrated the superiority of the drug combination over 5-FUra alone (12–16). (6R,S)-Folinic acid, commercially available and used in all of the previous clinical trials, consists of an equimolar mixture of diastereoisomers. While the natural 6S isomer is rapidly metabolized in humans, the unnatural 6R isomer is not metabolized and reaches high concentrations in plasma after i.v. administration of the mixture (17, 18). Theoretical concerns regarding the effects of high concentrations of (6R)-folinic acid (see “Discussion”) led to the development of an i.v. preparation of pure (6S)-folinic acid. We report here the pharmacokinetics and toxicities of high-dose (6S)-folinic acid administered by continuous infusion throughout a 5-day course of bolus 5-FUra. The initial dose of (6S)-folinic acid, 250 mg/m²/day, was equal to the amount of the (6S)-isomer in 500 mg/m²/day of (6R,S)-folinic acid, which we had studied previously in combination with 5-fluorouracil (16, 18–20). The maximum dose was chosen, based on an initial assumption of linear pharmacokinetics, to produce a steady-state plasma concentration of (6S)-folinic acid slightly in excess of 10 μM. None of the preclinical studies suggested that higher concentrations would be of any greater benefit (1–4).

MATERIALS AND METHODS

Patient Eligibility. All patients entered in this study had histologically proven cancer which was metastatic or locally unmanagable by conventional therapy, an Eastern Cooperative Oncology Group performance status of 2 or better, a WBC count ≥4000/μl, a platelet count ≥130,000/μl, total bilirubin <3.0 mg/dl, serum creatinine <3.0 mg/dl, and age <80 years. (Consistent with laws prohibiting age discrimination, persons >80 years of age were not excluded if they had intact cognitive function and a physical condition consistent with completely independent self-care.) Prior radiotherapy, if any, was completed at least 4 weeks before study entry. Patients were excluded if they had received nitrosourea or mitomycin C, more than 2 chemotherapy regimens, or radiation therapy to >50% of hematopoietically active bone marrow. Patients were also excluded if they had a second primary malignancy within 5 years prior to entry in this study or active central nervous system metastases. Patients were >18 years of age and signed informed consent in accord with federal, state, and local guidelines.

Pretreatment and Safety Evaluations. Before initiation of chemotherapy, all patients underwent a complete history and physical examination. Laboratory studies included a complete blood cell count, platelet count, 18-function blood chemistry profile, urinalysis, and electrocardiogram. A chest X-ray, a bone scan, or a liver computerized tomogram were obtained only if clinically indicated or required for measurement.
or scans needed for measurement of a lesion were repeated bimonthly. The complete blood cell count and platelet count were repeated weekly, blood chemistries were repeated monthly, and X-rays or scans needed for measurement of a lesion were repeated bimonthly. Laboratory and clinical assessments were carried out more frequently if clinically warranted. Laboratory values exhibiting clinically significant changes were repeated until they returned to normal, returned to pretreatment values, or stabilized. Toxicities were graded using the Common Toxicity Scale of the National Cancer Institute.

If a single patient had grade 3 toxicity, 3 additional patients were entered at each dose level, an additional 6 patients were treated at this dose in order to better describe the degree of toxicity encountered. Pharmacokinetics were evaluated at the lowest and highest doses of (65)-folinic acid. Efficacy was determined in patients with bidimensionally measurable lesions.

Treatment Plan. All patients received the same initial dose of 5-FUra, 370 mg/m²/day for 5 days, by bolus i.v. injection. (65)-Folinic acid was given by continuous i.v. infusion beginning 24 h before the first dose of 5-FUra and continuing for 12 h beyond the last dose of 5-FUra (5.5 days); the dose levels were 250, 400, 700, and 1000 mg/m²/day. Second and subsequent courses of (65)-folinic acid could be administered at 4-week intervals if the patient had recovered from any toxicity and if there was no evidence of disease progression (see "Efficacy Evaluation"). The dose of 5-FUra for subsequent courses was reduced if the patient had moderate or severe toxicity in the prior course.

Pharmacokinetics. Plasma and urine samples were obtained from the 3 patients given 250 mg/m²/day of (65)-folinic acid and from 6 of the patients given 1000 mg/m²/day. Plasma samples were obtained at the following times: prior to the infusion; 15 min, 30 min, 1 h, 2 h, 3 h, 5 h, 7 h, 15 h, 23 h, and each 24 h after the start of the infusion; 15 min, 30 min, 1 h, 2 h, 3 h, 5 h, 7 h, 15 h, and 23 h after the end of the infusion. At these times, the Cₚ of 5-FUra was negligible (18) and did not interfere with the microbiological assay of the folates. Urine was collected during two 24-h periods beginning at least 23 h after the start of the infusion, except that a single urine collection was obtained from the first patient. Aliquots of fresh plasma at the beginning and end of each urine collection were centrifuged through Ultrafine-MC filter units with a nominal cutoff of M, 10,000 (Millipore Corporation) to separate free from protein-bound drug before the addition of ascorbic acid. Cₚ was estimated by the means of samples from each patient obtained between 23 and 119 h. Clearances were calculated in individual patients at steady state using the following equations:

\[
CL_p = \frac{\text{Dose rate}}{C_u}
\]

\[
CL = \frac{\text{Amount excreted} \times 24 \text{ h}}{\text{Plasma concentration} \times 24 \text{ h}}
\]

\[
CL_{uw} = CL_p - \text{mean } CL
\]

Clearances were normalized based on the body surface area of each patient. For comparison, pharmacokinetic parameters from a previously reported study of (6R,5)-folinic acid (18) were recalculated in a like manner, using only the data at steady state.

Analytical Methods. The procedures for sample processing and the determination of (65)-folinic acid and (6S)-CH₃-THF are described in detail elsewhere (18, 21). Ascorbic acid was added to plasma, ultrafilters, and urine, which then were stored at ~70°C until assayed. (6S)-Folinic acid was quantitated by the growth of *Pediococcus cerevisiae* (detection limit, 0.0003 μM), and (6S)-CH₃-THF was quantitated by the growth of *Lactobacillus casei* after separation from other folates by high-performance liquid chromatography (detection limit, 0.03 μM).

Statistical Methods. Unless otherwise indicated, data are presented as the means ± SE. The t statistic was used to determine the two-tailed P that mean values were not different.

RESULTS

Patient Characteristics. Twenty-four patients were entered in this study at the following dosages of (65)-folinic acid: 3 at 250 mg/m²/day, 6 each at 400 and 700 mg/m²/day, and 12 at 1000 mg/m²/day. The primary sites of disease were 13 gastrointestinal (48%), 3 cervical (11%), 3 unknown (11%), and 8 other (30%). The demographic features of the patients are shown in Table 1. The patients were of good performance status. All but one patient had prior surgery, and the majority had prior chemotherapy, typically with one multiagent regimen (6 contained cisplatin, 5 contained doxorubicin, and 4 contained 5-FUra).

Toxicity. Overall, the toxicity of this regimen was mild. Grade 3 stomatitis or diarrrhea was encountered in one patient at each dose level beyond the first. Only one patient developed grade 3 leukopenia. In addition, at 700 mg/m²/day of (65)-folinic acid, one patient had a grade 4 hemorrhage attributed to disease progression, and preexisting eczema of one patient increased to grade 3 following chemotherapy. There was not a statistically significant association between any toxicity and the dose of (65)-folinic acid by analysis of variance (Table 2). Even at the highest dose level, the toxicities were no different in type and severity from those observed at lower dose levels.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients</th>
</tr>
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<tr>
<td>Patients entered</td>
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</tr>
<tr>
<td>M:F</td>
<td>14:13</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>Median 57, Range 43-78</td>
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<tr>
<td>Performance status*</td>
<td>0 (26%), 1 (59%), 2 (15%)</td>
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<tr>
<td>Prior therapy</td>
<td>Surgery (96%), Radiotherapy (41%), Hormonal therapy (7%), Biological response modifier therapy (4%), Chemotherapy (63%)</td>
</tr>
</tbody>
</table>
| [Eastern Cooperative Oncology Group Criteria.]

* Eastern Cooperative Oncology Group Criteria.
Courses of therapy were administered. Eight patients (30%) treated with the high dose of (6S)-folinic acid and (6S)-CH3-THF accounted for >95% of the total bioactive folates. For patients treated with the high dose of (6S)-folinic acid, derived from the Ctr and the amount of drug excreted at steady state, are also listed in Table 3. In accord with the greater than expected increase in the Ctr of (6S)-folinic acid at the higher dose, its CLp is significantly lower at the high dose than at the low dose. The change in the CLp is accounted for by a significant decrease in the CLwar while the change in the CLr between the two doses is not statistically significant and in the wrong direction to account for the decrease in the CLp.

The percentages of (6S)-folinic acid and (6S)-CH3-THF free from binding to plasma proteins were determined as the ratios of the concentrations of the drugs in plasma ultrafiltrates to the concentrations in plasma. The percentage of free over total (6S)-folinic acid did not differ significantly at the lowest and highest doses (64 ± 8% at 250 mg/m2/day and 72 ± 4% at 1000 mg/m2/day). This finding was also true for (6S)-CH3-THF (66 ± 16% at 250 mg/m2/day and 53 ± 3% at 1000 mg/m2/day).

No significant differences were found in the CLr of free (6S)-folinic acid, the CLr of free (6S)-CH3-THF, or the CLwar between patients receiving 250 mg/m2/day of (6S)-folinic acid and patients receiving 1000 mg/m2/day. When the data from both dose levels were combined, the CLp of free (6S)-folinic acid (56 ± 6 ml/min/m2) was not significantly different from the CLwar (58 ± 6 ml/min/m2), while the difference between the CLr of free (6S)-CH3-THF (51 ± 5 ml/min/m2) and the CLwar was small but statistically significant (P = 0.050 by paired t test).

DISCUSSION

During continuous i.v. infusion of (6R,S)-folinic acid, (6R)-folinic acid accumulates to a high steady-state plasma concentration, 13-fold higher than the concentration of the natural isomer, (6S)-folinic acid, and 7-fold higher than the concentration of the primary metabolite, (6S)-CH3-THF (18). The high concentration of the unnatural isomer is of concern because it can interfere with the transport and metabolism of (6S)-folinic acid or its metabolites (22–25), although recent evidence suggests that (6R)-folinic acid may not interfere with the enhance-
Several studies have taken advantage of this selectivity to reduce the plasma concentration of (6S)-folinic acid, as such, is even lower than that of (6R)-folinic acid (28-30). Oral administration, however, introduces theoretical problems of its own. The bioavailability of (6S)-folinic acid, as such, is even lower than that of (6R)-folinic acid; either through presystemic or first-pass metabolism, nearly all of the bioactive isomer appears in the blood as (6S)-CH$_2$-THF. This is not a problem if, as in many model systems, (6S)-folinic acid and (6S)-CH$_2$-THF have equivalent activities. However, this is a serious problem if (6S)-CH$_2$-THF does not augment intracellular folate pools in human colon tumors as efficiently as (6R)-folinic acid, as has been shown in a colorectal xenograft model (10). Other problems with oral administration include the known saturation of absorption (17, 27, 28). Administration of pure (6S)-folinic acid i.v. resolves the pharmacokinetics of (6S)-folinic acid. However, at the lowest dose used in the current study. The presence of (6S)-CH$_2$-THF in the plasma and the appearance of (6S)-CH$_2$-THF in the plasma. Plasma pharmacokinetics alone cannot identify the step at which saturation occurred. Quantitation of intracellular metabolites or minor metabolites in the plasma would be of benefit in identifying the site of saturation. Plasma pharmacokinetics alone cannot identify the step at which saturation occurred. Quantitation of intracellular metabolites or minor metabolites in the plasma would be of benefit in identifying the site of saturation.

Initially, the $C_p$ versus time data were fit to a one-compartment linear model as previously described (18). Although the data for individual patients usually fit a single exponential well, the CL$_P$ and the apparent half-lives were different at the two doses tested. This phenomenon has been described previously for the pharmacokinetics of 5-FUra (33). To render the pharmacokinetic parameters independent of a linear model, the CL$_P$ for each patient was recalculated from the dose rate and the $C_p$. At steady state, the parameters are constant, although not predictive of the $C_p$ at other times. Because the patients treated with the lower dose and the higher dose had significantly different $C_p$ of (6S)-folinic acid, the change in the CL$_P$ as a function of $C_p$ could be observed without the need to model the rising and falling portions of the $C_p$ curves. The CL$_P$ is significantly lower at the higher $C_p$. The lower relative concentration of (6S)-CH$_2$-THF at the higher dose of (6S)-folinic acid suggests that saturation of the metabolism of (6S)-folinic acid is responsible for the decrease in its CL$_P$. Transport of the substrate, several enzymatic steps, and transport of the product intervene between the disappearance of (6S)-folinic acid from the plasma and the appearance of (6S)-CH$_2$-THF in the plasma. Plasma pharmacokinetics alone cannot identify the step at which saturation occurred. Quantitation of intracellular metabolites or minor metabolites in the plasma would be of benefit in identifying the site of saturation.

Table 3 Pharmacokinetic parameters

<table>
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<tr>
<th>Folinic acid dose (mg/m$^2$/d)</th>
<th>Patient no.</th>
<th>(6S)-Folinic acid $C_w$ (µM)</th>
<th>(6S)-CH$_2$-THF $C_w$ (µM)</th>
<th>Ratio</th>
<th>$CL_P$ (ml/min/m$^2$)</th>
<th>$CL_N$ (ml/min/m$^2$)</th>
<th>$CL_{nr}$ (ml/min/m$^2$)</th>
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<td>2</td>
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* $C_w$ of (6S)-folinic acid$/C_w$ of (6S)-CH$_2$-THF.

* Two-tailed $P$ of 250 mg/m$^2$/day versus 1000 mg/m$^2$/day of (6S)-folinic acid.

* Data from 6 patients, adapted from Ref. 18.
in any in vitro study (1–4), higher dose rates are unlikely to be of greater therapeutic benefit.

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REFERENCES


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