High Inter- and Intrapatient Variation in 5-Fluorouracil Plasma Concentrations during a Prolonged Drug Infusion

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ABSTRACT

The purpose of the study was to examine inter- and intrapatient variation in 5-fluorouracil (5-FU) plasma concentrations in adult cancer patients receiving a 3-day drug infusion. Fourteen patients received 1266 mg/m² N-(phosphonacetyl)-L-aspartate (PALA) infused i.v. over 15 min on day 1, followed immediately by a loading dose of 500 mg/m² calcium leucovorin over 30 min. Then a prolonged infusion of leucovorin at 500 mg/m²/day and 5-FU at 1750 mg/m²/day was administered as either a constant rate or as a circadian infusion over 72 h. During constant rate infusions, 5-FU concentrations within individuals varied by 1.7-fold, but no uniform time of peak or trough concentration was observed. Transformation of these data by setting the time of peak to 0 h and by expressing concentrations as the percentage of the 24-h mean value revealed a nonrandom distribution of the time from peak to trough with a median time of 12 h (P = 0.027). These transformed data were also successfully fit to a circadian cosinor function (P < 0.001). During multiple constant rate 5-FU infusions, the intrapatient variability was high; the times of peak 5-FU concentration occurred at the same approximate sampling time 43% of the time, and troughs coincided 17% of the time. No difference in clinical toxicity was observed when matched constant rate and circadian infusions of 5-FU were compared. High inter- and intrapatient variability exists in 5-FU plasma concentrations in adult cancer patients during constant rate infusions with no evidence of a consistent circadian rhythm in untransformed data.

INTRODUCTION

Despite recent advances in cancer chemotherapy, 5-FU3 remains the first-line therapy for patients with advanced colorectal cancer. 5-FU has been in clinical use for >30 years; however, the optimal infusion regimen for this drug is not known, and it is presently administered using a wide range of treatment schedules. Prolonged infusions of 5-FU are theoretically attractive because of the very short plasma half-life (10–15 min) of the drug and because the duration of drug exposure can be an important determinant of 5-FU cytotoxicity (1). Continuous infusions of 5-FU have shown promising response rates in clinical studies in colorectal cancer (2–6) and in head and neck cancer (7).

Altering the rate of a prolonged fluoropyrimidine infusion in a circadian pattern (chronomodulation) has been proposed by several investigators as a novel method of improving the overall tolerability and efficacy of fluoropyrimidine chemotherapy (8–12). However, the physiological and pharmacological reasons why circadian infusions of fluoropyrimidines might improve upon the therapeutic index of these agents have not been characterized precisely. One possible explanation is that pharmacodynamic differences in drug sensitivity in host or tumor tissues may change with the time of day (13, 14). Alternatively, time-dependent changes in drug-metabolizing enzymes, such as DPD, the rate-limiting 5-FU catabolic enzyme (15–17), may lead to changes in drug clearance over a 24-h period. Circadian changes in 5-FU plasma levels have been reported in clinical studies of prolonged drug infusions (16, 18). Treatment of seven patients with head and neck cancer with cisplatin at 45–91 mg/m² i.v. on day 1 and 5-FU at 450–966 mg/m²/day as a continuous i.v. infusion over 5 days on days 2–6 resulted in mean steady-state 5-FU plasma concentrations that varied by 2.2-fold (18). Using a cosinor analysis for all seven patients, the peak 5-FU plasma concentrations over a 24-h period occurred at 1 a.m. and the trough at 1 p.m. In a separate study of seven patients with advanced cancer treated at a lower 5-FU dose of 200–300 mg/m²/day, the mean steady-state plasma concentrations varied by 5-fold over 24 h (16). Cosinor analysis for this group revealed a peak at 11 a.m. and a trough at 11 p.m. Both of these studies suggest that uniform circadian patterns exist in the 5-FU plasma concentrations during a prolonged infusion of this agent.

The fluctuation in DPD activity over time was investigated further by our laboratory in six volunteer subjects (19). Periph-

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3 The abbreviations used are: 5-FU, 5-fluorouracil; DPD, dihydropyrimidine dehydrogenase; PALA, N-(phosphonacetyl)-L-aspartate; LCV, leucovorin.
eral blood mononuclear cell DPD activity, which correlates with total body DPD activity (20–22), was measured every 3 h over a 24-h time period in six normal volunteers on three separate occasions over a 6-month period. The median intra-subject variation in DPD activity was 2.4-fold (range, 1.2–4.8-fold), but the time of peak DPD activity was randomly distributed over the 24-h time period (P = 0.68; Ref. 19). A simple cosinor analysis of the data failed to give an adequate fit; however, when these data were transformed by expressing the plasma concentration as the percentage of the mean for each individual and reordered as the time from peak rather than as the actual time of day, the variation in DPD activity was no longer random (P = 0.0055). However, the extreme inter- and intraindividual variation made it impossible to define a uniform circadian pattern of DPD enzyme activity for the entire study group. Furthermore, because DPD enzyme activity is the principal determinant of the rate of 5-FU clearance (16, 18), these data suggested that uniform circadian pattern of 5-FU concentrations in plasma during prolonged drug infusion would be very unlikely. To rigorously test this hypothesis, we measured 5-FU plasma concentrations over a 24-h period in advanced cancer patients receiving a 3-day constant rate infusion of 5-FU. These pharmacokinetic data were analyzed to determine whether 5-FU plasma concentrations demonstrate a consistent circadian pattern of variation and whether these patterns are reproducible from cycle to cycle. We also examined whether circadian rates of drug infusion could achieve a predesigned plasma 5-FU pharmacokinetic profile and whether the clinical effects of these various infusion patterns differed in individual patients.

MATERIALS AND METHODS

Patients and Treatment Protocol. All patients were enrolled in a pharmacological trial of PALA administered in combination with 5-FU and LCV in adult patients with solid tumors. PALA is an inhibitor of aspartate carbamoyltransferase, the rate-limiting enzyme in de novo pyrimidine synthesis (23). Previous studies demonstrated that PALA infusions do not alter 5-FU pharmacokinetics (23). A total of 27 patients were enrolled in this trial, the clinical results of which will be reported elsewhere. Fourteen of these 27 patients received at least 1 constant rate infusion cycle of 5-FU. This circadian infusion pattern was originally developed by Levi et al. (24). Patients were hospitalized as inpatients during the sampling period; however, no attempt was made to impose uniform sleep/wake cycles on patient activities.

Pharmacokinetic Sampling Strategy. Blood sampling began at 8 a.m. on the second day of the 5-FU infusion in all patients, and samples were obtained every 3 h for 24 h at approximately the same times of day. Venous samples were collected in heparinized tubes and placed on ice immediately and spun at 800 × g at 4°C for 10 min; the plasma was then separated and frozen at −70°C for later analysis. 5-FU plasma concentrations in the samples were determined by reversed-phase high-performance liquid chromatography as described previously (25). The lower limit of quantitation for 5-FU in plasma was 0.5 μM.

Statistical Analysis. When data for each patient were combined, the times of day were rounded to the nearest hour (8:00 a.m., 11:00 a.m., 2:00 p.m., 5:00 p.m., 8:00 p.m., 11 p.m., 2:00 a.m., and 5:00 a.m.). The Mann-Whitney-Wilcoxon test was used for pairwise comparisons of groups of 5-FU plasma concentrations. By expressing the 5-FU plasma concentration as a percentage of the mean 5-FU level for that patient during that cycle, any serial correlation was removed. Therefore, the Kruskal-Wallis test was used for comparisons across the eight times of day at which samples were obtained. Tests for uniform distributions were derived from the exact multinomial distribution of the χ² statistic. Cosinor analysis was applied using a model that assumed that the peak 5-FU plasma concentrations for all patients for all study dates occurred at the same time of day. Because of unequal variances for different observations (i.e., the larger observed values had greater SDs), one of the assumptions of the cosinor model fitting procedure was violated. Therefore, the actual plasma 5-FU concentrations were logarithmically transformed to substantially reduce that effect, and then the mean value for the study period was subtracted from each 5-FU value according to the actual time of day the samples were drawn. The values were then rescaled to have the same SD on each study cycle. The cosinor analysis was performed using the method of Tong (26), with the mean fixed at 0. Goodness of fit to the cosinor model was assessed using the zero amplitude test (26). The distribution of the residuals was found to be consistent with normality. Paired grades of worst toxicity for the constant rate and circadian 5-FU infusion cycles in individual patients were compared using McNemar’s test. The SAS (version 6.10; SAS Institute, Cary, NC) and StatXact-3 (version 3.0; Cytel Software Corp., Cambridge, MA) statistical software packages were used for all computations.

RESULTS

Patient Demographics and Data Set. Fourteen total patients with solid tumors participated in this pharmacokinetic study. Patient demographics are summarized in Table 1. All 14 patients received at least 1 constant rate infusion cycle of 5-FU. Nine of these 14 patients received more than one constant rate infusion cycle of 5-FU. This included three patients who received three constant rate infusion cycles and six patients who
received two constant rate infusion cycles. Twelve patients also received at least one cycle of 5-FU administered using a chronomodulated circadian infusion. Overall, pharmacokinetic data were collected in 36 cycles of chemotherapy including 26 constant rate infusion cycles and 10 circadian infusion cycles. The actual time of day of blood sampling in all monitored cycles was (mean ± SD) 8:05 a.m. ± 0:53, 11:03 a.m. ± 0:09, 2:01 p.m. ± 0:11, 5:07 p.m. ± 0:18, 8:01 p.m. ± 0:03, 11:00 p.m. ± 0:03, 2:11 a.m. ± 0:25, and 5:10 a.m. ± 0:29.

Variability in Plasma 5-FU Concentrations during Constant Rate Infusions. Because of the asymmetry in data collection, each patient who received multiple constant rate infusions was analyzed on the basis of average values for all of the cycles received (range, 1–3). For the 14 total patients studied, plasma 5-FU concentrations varied over time with a mean peak concentration of 7.3 ± 2.3 μM (mean ± SD; range, 3.0–10.5 μM) and a mean trough concentration of 4.6 ± 1.8 μM (range, 1.4–8.3 μM). The average peak:trough ratio for all patients was 1.7 ± 0.6 (range, 1.2–3.3). The mean plasma peak and trough concentrations were significantly different (P = 0.003; Wilcoxon rank sum test) showing that there was little overlap between these distributions.

Analysis from Time of Peak 5-FU Concentration. When the measured absolute 5-FU plasma concentrations from all subjects were pooled and analyzed together, no significant differences in the mean values at specific times of the day were observed (P = 0.57, Kruskal-Wallis test; Fig. 1A). Thus, there was no single time of day that all patients demonstrated a common peak or trough 5-FU plasma concentration during constant rate drug infusions.

Because individual patient’s peak 5-FU concentrations appeared to occur at different times of the day, the time of the peak 5-FU level was arbitrarily set to 0 h, and all subsequent data were reordered and expressed as the time from peak concentration (Fig. 1B). Furthermore, the actual plasma concentrations in each individual patient were expressed as the percentage of the mean 24 h value to allow for better graphical representation of common patterns of 5-FU variability in the study population (Fig. 1B). By following this degree of standardization, the individual times from peak to trough were not randomly distributed (P = 0.027, Kruskal-Wallis test), rejecting the null hypothesis that all concentrations were equal from 3 h after the peaks onward were equal. The variation in 5-FU plasma concentrations was asymmetric with peaks at least 25% above the mean and troughs only about 10% below the mean (Fig. 1B). The median time from peak to trough in the 14 patients was 12 h; however, the range of peak to trough times included all possible values from 3 to 21 h (data not shown).

Reproducibility of Times of Peaks and Troughs. The reproducibility of the times of peaks and troughs was examined in 12 paired cycles obtained from nine patients who received multiple constant rate infusion cycles of 5-FU. Patients who received three cycles of constant rate infusion 5-FU were analyzed for comparisons between cycle 2 versus cycle 1 and for cycle 3 versus cycle 1. The peak plasma 5-FU concentrations occurred at times that were significantly different from a random, uniform distribution over the eight possible time points. In the 12 paired cycles, the peak occurred at the same sampling time in 42% of the cycles (P = 0.011, exact binomial test) and at the same time plus or minus 3 h in 83% of the cycles (P = 0.0016). In contrast, the trough concentrations were not significantly different from a random, uniform distribution over the eight possible time points, occurring at the same time in 17% (P = 0.65) and at the same time ± 3 h in 33% (P = 1.0).

These data were also analyzed using a cosinor analysis to...
fit the individual plasma concentrations to a sinusoidal curve with a fixed 24-h periodicity (circadian pattern). However, a successful fit could only be achieved after expressing the 5-FU plasma concentrations as the percentage of the 24-h mean value for each individual patient and after log transformation of the data, because the variances in the measured plasma concentrations were not normally distributed, as is required for cosinor analysis. The cosinor fit of the transformed data was significant ($P < 0.001$) with a rejection of the null hypothesis that the mean 5-FU plasma concentrations were equal over time in favor of the circadian model (zero amplitude test). Using the fitted curves for each patient, there was a significant tendency for the peak plasma 5-FU concentrations to occur during the overnight period, from 11 p.m. to 5 a.m. ($P = 0.030$, test of uniform distribution). In contrast, the trough concentrations tended to occur during the midday period, from 8 a.m. to 2 p.m., but this was not significant for this study population ($P = 0.22$).

Circadian Pattern of 5-FU Infusion. After a constant rate infusion cycle of 5-FU, 12 patients were then treated with a circadian infusion cycle of 5-FU. The Intelliject infusion pumps were programmed to administer the same dose of 5-FU over a 24-h period using a varying infusion rate in a sinusoidal pattern with a peak infusion rate at 4 a.m. and a zero infusion rate at 4 p.m., an infusion schedule developed by Levi et al. (24). This circadian infusion of 5-FU was administered after each patient had previously received at least one constant rate 5-FU infusion cycle. Ten of 12 patients underwent pharmacokinetic monitoring during the circadian drug infusion. The untransformed plasma concentrations for these 10 patients (Fig. 2) showed that a sinusoidal profile of 5-FU plasma pharmacokinetics could be achieved despite the underlying variability in each individual patient.

Clinical Toxicities after Constant Rate and Circadian Infusions. Clinical toxicities were compared in individual patients receiving constant rate infusions and circadian infusion cycles at the same total dose of 5-FU of 1750 mg/m²/day. In all cases, the circadian infusion cycles were administered following the constant rate infusions. The most common toxicities observed were stomatitis, diarrhea, hand and foot syndrome, and nausea/vomiting. Overall toxicities were mild to moderate with no grade 3 or 4 toxicities reported in any patient. No significant differences in clinical toxicities were observed when the circadian infusions were compared with the constant rate infusion cycles (Table 2; $P > 0.5$, McNemar’s test).

DISCUSSION

Constant rate infusion of 5-FU in our adult cancer patients showed substantial interpatient variability in 5-FU plasma concentrations over a 24-h sampling period. Peak 5-FU plasma concentrations occurred at the same sampling time only 42% of the time in 12 paired constant rate infusion cycles. The time of 5-FU trough concentration was even more variable, occurring at the same time only 17% of the time. No uniform times of peak or trough 5-FU plasma concentrations were identified in the pooled untransformed data (Fig. 1A). However, when each

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**Table 2** Constant rate versus circadian infusions of 5-FU: Number of patients with grade 1 or 2 toxicities ($n = 12$ patients)

<table>
<thead>
<tr>
<th>Infusion pattern</th>
<th>Stomatitis$^a$ Grade 1/2</th>
<th>Diarrhea$^a$ Grade 1/2</th>
<th>Hand and Foot syndrome$^a$ Grade 1/2</th>
<th>Nausea$^a$ Grade 1/2</th>
<th>Vomiting$^a$ Grade 1/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant rate</td>
<td>6/5</td>
<td>6/2</td>
<td>2/3</td>
<td>3/2</td>
<td>0/1</td>
</tr>
<tr>
<td>Circadian</td>
<td>7/5</td>
<td>4/2</td>
<td>0/3</td>
<td>3/2</td>
<td>0/1</td>
</tr>
</tbody>
</table>

$^a$ No significant difference in paired grades of worst toxicity for the constant rate and circadian 5-FU infusion cycles, $P > 0.50$, McNemar’s test. No grade 3 or 4 toxicities were observed in any patient.
patient’s time of peak 5-FU plasma concentration was arbitrarily set to 0 h to synchronize individual patients and the drug plasma concentrations were expressed as the percentage of the 24 h mean value for each subject, nonrandom variability in the time from peak to trough was observed with a median time of 12 h ($P = 0.027$). Transformation of the data also allowed for analysis of the 5-FU plasma concentrations using a circadian (24 h) cosinor function and resulted in a significantly better fit of the data set ($P < 0.001$) than a straight line function (zero amplitude test). However, the obvious asymmetry in the 5-FU plasma concentrations about their mean values with higher peaks and shallower troughs (Fig. 1B) suggests that a perfectly symmetrical cosine function may not be the optimal model to fit these data. Our findings do support the existence of nonrandom variation in 5-FU plasma concentrations for individual patients; however, the degree of data transformation necessary to define a uniform pattern for the entire group of 14 patients suggests that a consistent circadian variation in 5-FU plasma concentrations does not exist for this population. Furthermore, in three patients, we tried to achieve a constant steady 5-FU plasma concentration over 24 h by varying the rate of 5-FU infusion to match the time-varying clearance observed in prior cycles. Unfortunately, this attempt was unsuccessful (data not shown), which again was consistent with high cycle to cycle variability in individual patients. This finding complements our earlier study on the high individual variability in the circadian pattern of DPD enzyme activity in peripheral blood mononuclear cells of normal volunteers (19).

As might be predicted, chronomodulated infusions of 5-FU administered in a circadian pattern using programmable infusion pumps did result in a sinusoidal 5-FU pharmacokinetic profile in individual patients (Fig. 2). Thus, it may be possible to target specific 5-FU pharmacokinetic profiles in plasma to test the pharmacodynamic efficacy and tolerability of chronomodulated 5-FU on normal or tumor tissues. However, we found little evidence to support a substantial difference in clinical tolerability when circadian infusion cycles were compared with constant rate infusions. Still, this comparison suffers from several limitations. Because we planned on administering multiple cycles of chemotherapy, the initial dose of 5-FU, LCV, and PALA were purposely selected to produce modest side effects to avoid the need for dose reductions. Consequently, the observed toxicities were of mild to moderate severity, and no grade 3 or 4 toxicities occurred in any patient. Furthermore, patients were not randomized, and all initially received the fixed constant rate infusion prior to their circadian patterned infusion. Finally, the time of the peak rate of infusion of 5-FU at 4 a.m. may not have been optimal for the maximal benefit of chronomodulation, as suggested by more recent studies (11).

Nonetheless, our major goal was to examine the consistency and reproducibility of the patterns of variation in 5-FU plasma concentrations in individual patients receiving continuous drug infusions. High inter- and intrapatient variability in 5-FU plasma concentrations was observed with no uniform pattern present in the untransformed data. Our findings are in close agreement with an earlier abstract by Fleming et al. which failed to find uniform peak and trough time for 5-FU plasma concentrations during a 24-h weekly drug infusions in 28 patients (27). On the basis of these studies, we conclude that consistent circadian patterns in 5-FU pharmacokinetics are not present in a heterogeneous group of adult cancer patients receiving prolonged drug infusions.

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