Relationship between Dihydropyrimidine Dehydrogenase Activity and Plasma 5-Fluorouracil Levels with Evidence for Circadian Variation of Enzyme Activity and Plasma Drug Levels in Cancer Patients Receiving 5-Fluorouracil by Protracted Continuous Infusion

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ABSTRACT

The activity of dihydropyrimidine dehydrogenase (DPD) in peripheral blood mononuclear cells and plasma concentration of 5-fluorouracil (FUra) were simultaneously determined in cancer patients receiving FUra by protracted continuous infusion (300 mg/m²/day). Blood samples were drawn every 3 h over 24-h period and the resulting DPD and FUra values analyzed for circadian periodicity. In the seven patients studied, a circadian rhythm of DPD activity was observed (P < 0.00001, Cosinor analysis) with the peak of activity at 1 a.m. (0.197 ± 0.007 nmol/min/mg) and the trough at 1 p.m. (0.113 ± 0.007 nmol/min/mg). In addition, a circadian rhythm was observed for the plasma concentrations of FUra obtained over a 24-h period (P < 0.00001, Cosinor analysis) with peak values (27.4 ± 1.3 ng/ml) occurring at 11 a.m. and trough values (5.6 ± 1.3 ng/ml) occurring at 11 p.m. The ratio of the maximum concentration of FUra to the minimum concentration observed was almost 5-fold. This study demonstrates a circadian variation of DPD activity in human peripheral blood mononuclear cells and a circadian variation of FUra plasma levels in patients receiving FUra by protracted continuous infusion. An inverse relationship between the circadian patterns of DPD activity and FUra plasma levels was also noted, suggesting that an association may exist between DPD activity and FUra plasma concentration. Further evidence of an association between DPD activity in peripheral blood mononuclear cells and plasma FUra concentration was demonstrated by a linear relationship between the two parameters in all patients (r² = 0.627) and within individual patients (r = -0.976 < r < -0.742). With the recent advent of programmable pumps, information on the circadian pattern of FUra and/or DPD may be useful in planning continuous infusion schedules in order that optimal plasma drug concentration may be maintained over a 24-h cycle, thereby enhancing the therapeutic efficacy of FUra administered by continuous infusion.

INTRODUCTION

Several studies have shown that plasma drug levels vary significantly during continuous infusion of FUra (1-4). In addition, Petit et al. (3) reported that the variability followed a predictable pattern, suggesting that there was a circadian variation in plasma FUra levels during continuous infusion at a constant rate. The biochemical mechanism for the observed variability and/or circadian periodicity of FUra during continuous infusion has not been determined.

DPD is the initial enzyme of pyrimidine catabolism with an estimated 80% or greater of an administered dose of FUra being rapidly degrades by this route (5). The importance of catabolism and, particularly, DPD in FUra chemotherapy has been demonstrated in studies with competitive inhibitors (6, 7) and in a recent report of a patient with a complete deficiency of DPD activity (8). These studies indicate that catabolism has an important role in determining the availability of FUra for anabolism which, in turn, determines the cytotoxic effects of FUra. We have previously demonstrated that DPD exhibits a circadian periodicity both in rat liver (9) and human peripheral blood mononuclear cells (10).

The purpose of this study was to simultaneously determine DPD activity in peripheral blood mononuclear cells and plasma FUra concentrations in patients receiving low dose "flat" continuous infusion of FUra to determine if either exhibited a circadian pattern and if any relationship existed between DPD activity and plasma FUra levels.

MATERIALS AND METHODS

Patient Characteristics. Seven patients (five men, two women) participated in this study. The age of the patients ranged from 48 to 69 years. All seven patients had biopsy-proven gastrointestinal malignancies (e.g., gastric, colorectal, or pancreatic) and were receiving protracted continuous infusion of FUra. Characteristics of the patients including hepatic and renal function are listed in Table 1.

Assay of DPD Activity. Blood samples (25 ml) were drawn from the hepar-in-lock into a 30-ml syringe containing 5 ml of heparin (1000 units/ml) and immediately added to 15 ml of Hanks’ balanced salt solution in a 50-ml centrifuge tube. Plasma was removed by centrifugation and the pellet cells were loaded onto a Ficoll-Hypaque discontinuous gradient to separate mature erythroid blood cells from peripheral blood mononuclear cells. The peripheral blood mononuclear cell fraction was removed and mixed with 1 ml of 135 mM sodium phosphate buffer (pH 7.5). The cells were placed in an ice bath and then lysed by sonicating five times for periods of 10 s. After removal of debris by centrifugation (20,000 x g for 3 min) the supernatant was assayed for DPD using a modification (8) of a method originally described by Marsh and Perry (11). The supernatant was incubated at 37°C for varying intervals up to 30 min in the presence of 250 μM

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3 The abbreviations used are: FUra, 5-fluorouracil; DPD, dihydropyrimidine dehydrogenase (EC 1.3.1.2); HPLC, high-performance liquid chromatography.
NADPH and 20 μM [6-3H]FUra (3.5 μCi/μmol). At the end of the incubation time an aliquot (300 μl) was added to an equal volume of ice-cold ethanol and then (within 3 h) analyzed for the presence of FUra and FUra catabolites by a HPLC method described previously (12). Protein concentration was determined by the method of Lowry et al. (13). The enzyme activity was expressed as nanomoles of total catabolites formed/min/mg protein.

Assay of FUra Plasma Concentration. In addition, 10–12 ml of blood were drawn from the heparin lock at each of the time points into heparinized vacutainer tubes and quickly centrifuged. Plasma was removed and stored at −20°C until FUra concentration was determined by HPLC (within 7 days following sampling). Quantitation of FUra by HPLC followed the technique of Buckpitt and Boyd (14) with a limit of sensitivity of approximately 2 ng/ml. A separate set of FUra plasma standards was used for each set of assays with minimal interpatient variation.

Statistical Analysis. The data obtained from the DPD and FUra assays were analyzed by the “Cosinor” method (15). Due to the large interpatient variability of observed values for both DPD activity and FUra plasma concentration, comparisons between patients were carried out by expressing the data as a percentage of the 24-h mean for that patient. The values obtained were fitted to a cosine wave by regression analysis utilizing the method of least squares (16). Three parameters were quantitated in this analysis. These parameters include the mesor (i.e., the rhythm-adjusted mean), the amplitude (i.e., maximum or minimum value from the mean), and the acrophase (i.e., time of maximum or minimum value from a given phase of reference). In addition, relationship of DPD activities and FUra concentrations was evaluated by linear regression analysis (17).

RESULTS

Variation of DPD Activity. In the seven patients studied, there was a circadian variation in DPD activity of each patient (Table 2). Fig. 1A illustrates the pattern of DPD activity from one of the seven patients (patient 6, B. B.) studied with DPD activity expressed as nmole catabolites formed per minute per milligram protein. Most of the patients had a peak of DPD activity between 10 p.m. and 4 a.m., while one patient (patient 3, W. J.) had a peak at 11 a.m. (Table 2). Due to the interpatient variability in DPD activity and the time of maximum or minimum activity, the data for each patient was normalized in order to establish an overall pattern for all patients. The data was normalized in two ways: (a) DPD activity was expressed as a percentage of the 24-h mean (i.e., mesor) and (b) the data for each patient was adjusted along the time scale to a common reference point (i.e., the DPD activity peak). The normalized data was analyzed by Cosinor analysis and the overall values for all patients are shown in Table 3. The peak of DPD activity for the entire group of patients was at approximately 1 a.m. (0.197 ± 0.007 nmol/min/mg) and the trough at approximately 1 p.m. (0.113 ± 0.007 nmol/min/mg). For the normalized data (% 24-h mean) maximum DPD activity exceeded minimum activity by approximately twofold (Table 3).

Variation of Plasma FUra Concentration. The plasma concentration of FUra (ng/ml) varied significantly both within a single patient and between patients. All seven patients exhibited a significant circadian rhythm with respect to plasma FUra concentration over a 24-h period (Table 2). Fig. 1B represents the plasma FUra concentration (ng/ml) in one of the seven patients (patient 6, B. B.) in this study. As with the DPD activity, FUra plasma concentrations for each patient were normalized to a percentage of the 24-h mean (i.e., mesor) and adjusted along the time scale to a common reference point, the DPD activity peak for that patient. The Cosinor analysis of these values is shown in Table 3. The peak FUra concentration for the entire group of seven patients was at approximately 11 a.m. (27.4 ± 1.3 ng/ml) and the trough at approximately 11 p.m. (5.6 ± 1.3 ng/ml). For the normalized data (% 24-h mean) maximum FUra concentration exceeded minimum concentration by almost 5-fold (Table 3).

Correlation between DPD Activity and FUra Concentration. As shown in Fig. 2, as DPD activity decreases, FUra plasma concentration appears to increase and vice versa. In support of this observation, comparison of DPD activity with FUra concentration for any sample from any patient yielded an overall correlation between the two parameters (Fig. 3, r = −0.627). It is interesting to note that if consideration was given to individual patients only, the correlation of DPD activity and FUra plasma concentrations would be much better (−0.978 < r < −0.742) than the overall correlation for all patients combined (r = −0.627). Since it is clear that interpatient variability is greater than intrapatient variability, the mean DPD activity for each patient was examined with the corresponding mean value for the FUra concentration in the same patient. The correlation of these two parameters (Fig. 3, inset) further suggests that there is an inverse relationship (r = −0.973) between DPD activity in peripheral blood mononuclear cells and plasma FUra concentrations in patients receiving FUra by protracted continuous infusion.

DISCUSSION

The present study demonstrates that there was a significant variation in the plasma level of FUra during protracted continuous infusion in cancer patients. This observation has also been made by others (1–4) although no apparent mechanism has been elucidated. In an attempt to determine the biochemical mechanism, we examined FUra catabolism in patients receiving FUra by continuous infusion and observed a significant variation of DPD activity in peripheral blood mononuclear cells. Although it has been apparent for some time that metabolism is very important in determining the effectiveness of the fluo-
Table 2 Summary of DPD activity in peripheral blood mononuclear cells and FUra plasma concentrations in patients receiving FUra by protracted continuous infusion (300 mg/m²/day)

<table>
<thead>
<tr>
<th>Patient</th>
<th>DPD activity (nmol/min/mg)</th>
<th>Maximum</th>
<th>Minimum</th>
<th>Time of maximum</th>
<th>Time of minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mesor ± St. (h)</td>
<td>(h)</td>
<td>(h)</td>
<td>R²</td>
<td>P</td>
</tr>
<tr>
<td>1</td>
<td>0.212±0.021</td>
<td>0.278</td>
<td>0.146</td>
<td>22.6</td>
<td>10.6</td>
</tr>
<tr>
<td>2</td>
<td>0.362±0.008</td>
<td>0.414</td>
<td>0.310</td>
<td>2.2</td>
<td>14.2</td>
</tr>
<tr>
<td>3</td>
<td>0.074±0.004</td>
<td>0.108</td>
<td>0.040</td>
<td>11.3</td>
<td>23.3</td>
</tr>
<tr>
<td>4</td>
<td>0.064±0.003</td>
<td>0.083</td>
<td>0.045</td>
<td>4.4</td>
<td>16.4</td>
</tr>
<tr>
<td>5</td>
<td>0.108±0.003</td>
<td>0.167</td>
<td>0.049</td>
<td>1.0</td>
<td>13.0</td>
</tr>
<tr>
<td>6</td>
<td>0.128±0.004</td>
<td>0.166</td>
<td>0.090</td>
<td>0.3</td>
<td>12.3</td>
</tr>
<tr>
<td>7</td>
<td>0.135±0.005</td>
<td>0.182</td>
<td>0.088</td>
<td>21.8</td>
<td>9.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FUra plasma concentration (ng/ml)</th>
<th>Maximum</th>
<th>Minimum</th>
<th>Time of maximum</th>
<th>Time of minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.3±1.5</td>
<td>25.9</td>
<td>12.0</td>
<td>24.0</td>
</tr>
<tr>
<td>2</td>
<td>8.5±0.7</td>
<td>13.2</td>
<td>12.5</td>
<td>3.8</td>
</tr>
<tr>
<td>3</td>
<td>18.6±1.0</td>
<td>35.5</td>
<td>4.4</td>
<td>1.6</td>
</tr>
<tr>
<td>4</td>
<td>21.4±2.7</td>
<td>29.8</td>
<td>14.2</td>
<td>12.9</td>
</tr>
<tr>
<td>5</td>
<td>21.4±1.9</td>
<td>38.1</td>
<td>13.2</td>
<td>4.6</td>
</tr>
<tr>
<td>6</td>
<td>15.9±1.3</td>
<td>27.0</td>
<td>12.3</td>
<td>4.7</td>
</tr>
<tr>
<td>7</td>
<td>15.5±1.3</td>
<td>26.4</td>
<td>9.2</td>
<td>4.6</td>
</tr>
</tbody>
</table>

* Mesor = rhythm-adjusted mean.

Max/Min = mesor ± amplitude (not shown).

* Time is expressed on a 24-h scale.

Dihydropyrimidine dehydrogenase (DPD) and plasma FUra levels

In the present study, we report the simultaneous analysis of DPD activity and plasma FUra concentration and demonstrate a circadian variation of DPD activity (P < 0.00001, Cosinor analysis) in human peripheral blood mononuclear cells and a circadian variation in the plasma FUra level (P < 0.00001, Cosinor analysis) in patients receiving FUra by protracted continuous infusion at 300 mg/m²/day. Although a circadian pattern was evident for both DPD activity and plasma FUra levels, a general trend that is characteristic of all patients is not demonstrated. The time of maximum or minimum values for each patient varied as much as 7 h for six of the seven patients (9:30 p.m. to 4:30 a.m.) with one patient (patient 3, W. J.) demonstrating times of maximum and minimum values that were up to 12 h out of phase with the other patients. It is interesting to note that this patient (patient 3, W. J.) had worked a night shift for several years. While a circadian pattern was evident for each of the seven patients, the striking interpatient variation in the time of maximum/minimum values for DPD and FUra suggests that caution should be used in developing generalized time-programmed infusion schedules as previously suggested (19). However, for individual patients, monitoring DPD activity and/or plasma FUra levels of individual...
Table 3  Rhythmic summary of single Cosinor analysis of DPD activity and FUrA concentration in seven patients receiving protracted continuous infusion of FUrA (300 mg/m²/day)

<table>
<thead>
<tr>
<th></th>
<th>Mesor ± SE (%)</th>
<th>Amplitude ± SE (%)</th>
<th>Maximum ± SE (%)</th>
<th>Time of maximum (h)</th>
<th>Minimum ± SE (%)</th>
<th>Time of minimum (h)</th>
<th>Maximum/minimum</th>
<th>R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPD activity</td>
<td>100.1 ± 2.6</td>
<td>30.4 ± 3.7</td>
<td>130.6 ± 4.5</td>
<td>1.0 ± 0.7</td>
<td>69.7 ± 4.5</td>
<td>13.0 ± 0.7</td>
<td>1.9</td>
<td>0.930</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>FUrA plasma concentration</td>
<td>99.1 ± 4.4</td>
<td>65.0 ± 6.3</td>
<td>164.1 ± 7.7</td>
<td>11.4 ± 0.5</td>
<td>34.0 ± 7.7</td>
<td>23.4 ± 0.5</td>
<td>4.8</td>
<td>0.955</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

* Cosinor analysis was carried out on normalized data expressed as %24-h mean.
* Mesor = Rhythm-adjusted mean.
* Maximum/minimum = mesor ± amplitude.
* Time is expressed on a 24-h scale.

patients may be useful in planning FUrA continuous infusion chemotherapy.

The present study also demonstrates an apparent relationship between DPD activity in peripheral blood mononuclear cells and plasma FUrA levels. Comparison of DPD activity and FUrA plasma concentrations for all patients at all sampling times yielded a moderate correlation (r = −0.627, Fig. 3). However, if the comparison of DPD activity and FUrA plasma levels was limited to those values within a single patient the correlation was much better (−0.978 < r < −0.974, Fig. 3) indicating that interpatient variation was responsible for the weaker overall correlation and not intrapatient variation. Therefore, although drug concentrations cannot be accurately predicted for all patients treated with a specific regimen, FUrA plasma concentrations potentially can be predicted within individual patients from representative samples taken at various times of the day. Further support for the relationship of these two parameters is suggested by the correlation between mean DPD activity and mean FUrA plasma concentration in the seven patients in this study (Fig. 3, inset).

Clearance of FUrA primarily results from catabolism within the liver (20), although extrahepatic sites may also be involved (21). DPD has previously been demonstrated to have the highest activity in liver and peripheral blood mononuclear cells (22) with minimal activity in kidney, spleen, lung, colon, pancreas, breast tissue, and bone marrow cells (23). It has been suggested that the activity of numerous liver enzymes in drug metabolism exhibits circadian rhythms (24) leading to the hypothesis proposed here that a circadian variation in DPD might be responsible for the variation in plasma drug levels during constant-rate continuous infusion of FUrA. The importance of DPD in determining the clinical pharmacokinetics of FUrA has previously been demonstrated in a patient with a complete deficiency of DPD activity (8).

Although the liver is the primary site of pyrimidine catabolism leading to the degradation of FUrA, it is not feasible to directly study DPD activity in liver tissue of patients. Therefore, in the present study, DPD activity was examined in peripheral
blood mononuclear cells with the assumption that the DPD patterns and/or activity in these cells may be related to that of DPD in liver. This assumption is supported by previous studies from our laboratory which demonstrate that DPD exhibits a circadian pattern in rat liver (9) as well as in human peripheral blood mononuclear cells (10).

In summary, the present study demonstrates a highly significant circadian rhythm in both DPD activity in peripheral blood mononuclear cells and plasma FUra levels during protracted continuous infusion of FUra with an apparent inverse relationship between these two parameters. Since FUra plasma levels are associated with drug-induced side effects (25), knowledge of the circadian variation of plasma drug levels in individual patients may allow more precise planning of time-modified schedules of drug delivery which, in turn, may result in increased effectiveness of continuous infusion regimens. The recent availability of programmable infusion pumps which permit modulation of the infusion rate on a 24-h scale has greatly increased the ease with which this information may be utilized in planning infusion schedules. Although further evaluation of both host and tumor tissues is needed, this study suggests that DPD may be a major determinant of the periodicity of FUra plasma levels when this drug is administered by protracted continuous infusion and should be considered in planning infusion schedules.

REFERENCES

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