ABSTRACT

Studies in cell culture systems have demonstrated synergistic cytotoxicity of thymidine and its in vivo metabolite thymine with cisplatin. We have conducted a Phase I trial to assess the toxic effects and tolerable doses of thymidine plus cisplatin in patients with advanced cancer. Twenty such patients were treated with varying doses of thymidine infused continuously during Days 1–5 of a 28-day cycle. Cisplatin at a dose of 100 mg/m² was administered on Day 3 of the cycle. Using this schedule, the maximally tolerated dose of thymidine was 60 g/m²/day. Hematologic toxicity was dose limiting with median granulocyte and platelet nadirs of 1,500/nl and 55,000/nl, respectively. Central nervous system and gastrointestinal toxicity was also prominent. Plasma and urine thymidine and thymine concentrations were determined using a high-performance liquid chromatography assay. At the maximally tolerated thymidine dose, steady state plasma thymidine concentrations approached or exceeded 1 mM in all patients, and thymine levels of 1–2 mM were achievable. These concentrations approach those demonstrated to produce synergistic cytotoxicity with cisplatin in vitro. Further pharmacokinetic analysis revealed that there is a progressive fall in thymidine plasma clearance with increasing dose and that cisplatin administration is followed by a significant fall in plasma thymidine clearance. No clear-cut relationships between platelet nadir and thymidine pharmacokinetics could be found, although nonlinear regression analysis did reveal a significant correlation between steady-state plasma thymidine concentration and platelet nadir. The recommended thymidine dose for Phase II trials of this combination is 60 g/m²/day in patients with little or no prior therapy.

INTRODUCTION

CDDP is a valuable antineoplastic drug with demonstrated clinical activity in the treatment of a broad spectrum of solid tumors. In experimental systems, CDDP has exhibited dramatic synergy with a number of other commonly used antineoplastic agents (1–3). Naturally occurring nucleosides and nucleotide analogues are among those agents which have been shown to enhance the cytotoxicity of CDDP in vitro and in tumor-bearing animals (4–6). Exposure of cultured human colon carcinoma cells to ara-C in concentrations of 0.1–10 μg/ml produces a dose-related 1–2-log increase in cell kill beyond that predicted to result from an additive effect of the drugs (5). In mice bearing L1210 leukemia, the combination of CDDP plus 5-FU results in cure of 60% of the animals, whereas either drug used alone is ineffective in producing cures (4).

Clinically, both ara-C and 5-FU have been studied in combination with CDDP (7, 8), and recent results have shown the 5-FU/CDDP combination to be highly effective in treatment of head and neck cancer (8) and several other diseases (9, 10). dThd is a naturally occurring nucleoside that has potent growth-inhibitory effects on mammalian cells and is capable of modulating the cytotoxic effects of several other antineoplastic drugs of the antimitabolite class (11). It too has been shown to be synergistic with CDDP in vitro (6). In cultured human colon carcinoma cells, dThd alone produces virtually no cytotoxicity, yet when used in high concentration (200–1000 μg/ml) in combination with CDDP, there results a dose-related 2-log increase in cell kill beyond that expected to occur from an additive effect of the drugs. Also of interest is the observation that thymine, an in vivo metabolite of dThd, is markedly synergistic with CDDP in this cell culture system as well (6).

In view of the marked in vitro synergy of dThd plus CDDP and the significant clinical activity of CDDP combined with some pyrimidine nucleosides, particularly 5-FU, we conducted a Phase I study of dThd plus CDDP with the objectives of defining the maximally tolerated dose and associated toxicities of dThd administered in combination with CDDP and of determining if clinically achievable dThd concentrations approach those which produce synergy with CDDP in vitro.

MATERIALS AND METHODS

Patient Selection

Patient characteristics are shown in Table 1. Twenty patients, 13 men and 7 women, ranging from 26 to 69 yr of age were entered in the study. All patients had a pathologically confirmed diagnosis of cancer and, in each case, the disease had proven refractory to standard therapy or was one for which no therapy of proven benefit was available. All but two patients had been previously treated with chemotherapy, radiation therapy, or combined modalities. Each patient had an initial complete medical history and physical examination, chest X-ray, electrocardiogram, urinalysis, complete blood count and platelet count, determination of serum chemistries, and 24-h urine creatinine clearance. Complete blood counts and platelet counts were then obtained weekly during therapy, while all other parameters were repeated on Day 1 of each cycle. Prior to beginning chemotherapy all patients had adequate renal and liver function (serum creatinine, <1.8 mg/100 ml; creatinine clearance, ≥60 ml/min; and total bilirubin, <1.5 mg/100 ml), WBC count, >4,000 cells/mm³; platelet count, ≥100,000/mm³, and hemoglobin, ≥10 g/dl. The protocol was approved by the Institutional Review Board, and all patients provided written informed consent.

Treatment Plan

dThd was obtained from the Investigational Drug Branch, Division of Cancer Treatment, National Cancer Institute, and was supplied as 15 g in 500 ml of 0.6% NaCl solution for direct i.v. administration. All patients received dThd as a continuous i.v. infusion for 120 h (5 days). CDDP, at a dose of 100 mg/m², was administered i.v. in 250 ml of normal saline over 1–2 h between 48 and 72 h from the start of the dThd infusion (Day 3). Prior to CDDP administration, patients received 12.5 g of mannitol by rapid i.v. injection. Administration of i.v. fluid in addition to that required for dThd administration was not routinely done as dThd administration alone generally required administration of 1.5–4 liters of fluid daily. Urine output and serum electrolytes were carefully monitored following CDDP administration, and fluid and electrolytes were replaced if necessary.

The initial dose of dThd was 24 g/m²/day infused continuously for 5 days. Dosage escalation was carried out according to a modified
Fibonacci scheme after at least 3 patients had been treated at each dThd dose chosen for evaluation. There was no dose escalation in individual patients. Cycles of chemotherapy were repeated every 28 days. Patients were treated for at least 2 cycles of therapy unless rapid disease progression or unacceptable toxicity occurred.

Pharmacological Studies

Sample Collection. Plasma and urine dThd and thymine levels were determined in selected patients at each dosage level studied. At the highest dThd doses (50 g/m²/day and 60 g/m²/day), patients were admitted to the Clinical Research Center at the University of Chicago for treatment coupled with frequent blood and urine sampling. In these individuals, 10 ml of heparinized blood were obtained prior to the start of chemotherapy and then twice daily during the dThd infusion on Days 1 and 2 of treatment. On the day of CDDP administration (Day 3), 10 ml of heparinized blood were obtained prior to mannitol administration, after 30 and 60 min of the CDDP infusion, at the completion of the CDDP infusion, and at 30, 60, 120, 360, and 480 min following completion of CDDP administration. Twice daily blood sampling was again done on Days 4 and 5 of the dThd infusion. Blood was immediately centrifuged, and the plasma was decanted and frozen at −20°C until the time of analysis. During chemotherapy administration, all urine was collected in 24-h aliquots for determination of creatinine clearance and dThd and thymine concentrations. Samples were refrigerated during collection and, at the completion of each 24-h period, total volume was measured, and aliquots were frozen at −20°C for subsequent analysis.

HPLC Assay. dThd and thymine concentrations were determined using a modification of a previously described HPLC assay (12). Plasma samples were prepared by addition of 50 μl of a 1-mg/ml solution of ara-C to 200 μl of plasma. Following addition of the ara-C internal standard, plasma proteins were immediately precipitated by addition of 5 ml of methanol. The samples were then centrifuged, frozen over night, and recentrifuged, and 10 μl of the supernatant were injected on to a Waters C18-Bondapak column, and elution was carried out at room temperature at a flow rate of 2 ml/min using Curve 8 in the Waters gradient system. The gradient was run from initial conditions of 0% Pump B to 20% Pump B over 10 min, following which elution was continued at 20% Pump B for an additional 5 min. UV absorbance was monitored at 280 nm. Retention times for authentic standards were: ara-C (internal standard), 2.45 min; thymine, 3.40 min; dThd, 6.50 min.

Renal clearance of dThd and thymine was determined using the formula

\[
\text{Clearance (ml/min/m²) = \frac{\text{Infusion rate (mg/min) \times Cpss (mg/ml)}}{\text{Cpss (mg/ml)}}}
\]

Statistical Analysis. Statistical analysis of the data was done using the RS/1 software package (Bolt, Beranek, and Newman, Inc., Cambridge, MA).

RESULTS

Clinical Study. Twenty patients began chemotherapy with dThd plus CDDP during this study. Two patients withdrew consent midway through the first treatment cycle and failed to return for follow-up visits. The remaining 18 patients received 28 cycles of chemotherapy.

The major clinical toxic effect observed during this study was bone marrow suppression. As shown in Table 2, dThd, in combination with CDDP, produced marrow suppression at doses of only 24 g/m²/day in some patients. Escalation of the dThd dose was associated with more severe myelosuppression with thrombocytopenia being most prominent. At the maximally tolerated dThd dose of 60 g/m²/day, the median platelet nadir was 55,000/mm³, and the median granulocyte nadir was 1500 cells/mm³. The median nadir day for granulocytes was Day 12 (range, Day 9–Day 17) and for platelets was Day 13 (range, Day 10–Day 17). Complete hematological recovery occurred by Day 28 in virtually all patients. There was no evidence for the development of cumulative bone marrow suppression, even in one patient who received 4 cycles of chemotherapy at a dThd dose of 50 g/m²/day.

Another noteworthy toxic effect observed during this study was central nervous system toxicity which occurred in essentially all patients receiving dThd doses in excess of 24 g/m²/day. This was manifest primarily as somnolence, dizziness, headache, personality change, agitation or, in one patient, visual hallucinations. In all but one patient, these adverse effects were completely reversible within a few hours after discontinuing the dThd infusion. One patient, however, treated at 60 g/m²/day,

Table 2 Hematological toxicity

<table>
<thead>
<tr>
<th>dThd dose (g/m²/day)</th>
<th>No. of patients/ no. of cycles</th>
<th>WBC (×10³/mm³)</th>
<th>PMN* (×10³/mm³)</th>
<th>Platelet (×10³/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>4/8</td>
<td>6.5 (1.7–12.4)</td>
<td>5.3 (0.9–9.3)</td>
<td>174 (23–307)</td>
</tr>
<tr>
<td>40</td>
<td>5/6</td>
<td>6.4 (5.9–12.6)</td>
<td>4.0 (2.1–10.0)</td>
<td>180 (33–573)</td>
</tr>
<tr>
<td>50</td>
<td>4/9</td>
<td>3.7 (1.8–8.6)</td>
<td>2.9 (1.5–6.2)</td>
<td>119 (45–339)</td>
</tr>
<tr>
<td>60</td>
<td>5/5</td>
<td>2.9 (0.4–7.9)</td>
<td>1.5 (0.30–7.2)</td>
<td>55 (20–72)</td>
</tr>
</tbody>
</table>

* PMN, polymorphonuclear leukocyte count.
Numbers in parentheses, range.
died of apparent central nervous system toxicity. The patient was a 53-yr-old woman with widely metastatic squamous cell carcinoma of the lung. Brain metastases had been documented by computerized tomography scan approximately 2 mo prior to the start of chemotherapy, and she had received whole-brain radiation therapy. During the first 2 days of the dThd infusion she became somnolent but was easily arousable, alert, and oriented. Prior to CDDP administration on Day 3 of the treatment cycle she received prophylactic antiemetic therapy consisting of prochlorperazine, flurazepam, and hydroxyzine. Following CDDP administration she was noted to be obtunded and unresponsive but with stable vital signs and without focal neurological defects. A repeat brain computerized tomography scan was normal, cerebrospinal fluid cytology was negative, and electroencephalogram displayed findings consistent with a metabolic encephalopathy. Despite aggressive supportive therapy, she remained obtunded and expired approximately 2 wk from initiation of chemotherapy. Permission for postmortem examination was refused.

Other toxic effects observed during this study were characteristic of CDDP and included gastrointestinal upset and altered renal function. Most patients experienced mild nausea during the first 2 days of the dThd infusion which became more severe following CDDP administration. In addition, mild to moderate diarrhea occurred in 1 of 4 patients treated at 24 g/m²/day, 0 of 5 at 40 g/m²/day, 3 of 4 at 50 g/m²/day, and 2 of 5 patients treated at 60 g/m²/day. In all cases, the diarrhea resolved soon after completion of chemotherapy. Clinically significant nephrotoxicity was not prominent in this study. Median pretreatment creatinine clearance for all patients was 86 ml/min (range, 73–135 ml/min). This fell to a median of 53 ml/min (range, 30–122 ml/min) during therapy. The average decrease in creatinine clearance was 36% of the pretreatment value.

Response Data. Although the primary objective of this study was to determine the nature and magnitude of dThd plus CDDP toxicity, patients with clearly measurable lesions were evaluated for response to treatment using standard response criteria. No complete or partial responses were seen. One patient with colon cancer had stable disease during 4 mo of therapy, and a patient with non-Hodgkin’s lymphoma had a minor response with less than a 50% decrease in the size of enlarged lymph nodes.

Pharmacological Studies. Plasma levels of dThd and thymine were determined in 10 of the 18 evaluable patients on-study. Table 3 displays the steady-state plasma concentrations of dThd and thymine achieved in patients receiving varying dThd doses. At the maximally tolerated dThd dose of 60 g/m²/day, plasma dThd concentrations ranged from nearly 700 μM to greater than 2 mM, and plasma thymine levels varied from nearly 1 mM to in excess of 2 mM.

Data on plasma and renal clearance of dThd and thymine are shown in Table 4. Renal clearance of dThd ranged from 31 to 87 ml/min/m² and accounted for approximately one-third of dThd plasma clearance. Renal thymine clearance was substantially less than that of dThd, falling in the range of only 11–20 ml/min/m². Fig. 1 depicts the relationship between dThd plasma clearance and administered dose. There is a progressive and significant fall in dThd plasma clearance with increasing dose, such that at a dThd dose of 24 g/m²/day plasma dThd clearance is greater than 400 ml/min/m² but falls to a mean of 170 ml/min/m² at the highest dose level studied.

The design of the treatment plan used in this study provided a unique opportunity to examine the effects of CDDP administration on dThd and thymine pharmacokinetics. This analysis was limited to the seven patients studied at dThd doses of 50 or 60 g/m²/day. As shown in Fig. 2, plasma dThd clearance fell significantly following CDDP administration from a mean of 187.8 ± 53 ml/min/m² to a mean of 159.4 ± 44 ml/min/m². Although renal clearances of dThd, thymine, and creatinine all tended to fall following CDDP administration, no statistically significant differences could be found between pre- and post-CDDP values.

Since the most prominent toxic effect observed in this study was thrombocytopenia, we next examined the relationship between dThd pharmacokinetics and nadir platelet counts. Linear regression analysis of the platelet nadir versus steady-state dThd concentration failed to reveal a significant correlation. We then reexamined this relationship using nonlinear regression analysis. To control for the greater absolute variance in platelet counts that occurs at the higher values, an attempt was made to weight the data by a factor of 1/variance. We assumed that the variance in platelet counts was proportional to the square of the platelet count and therefore weighted the data by a factor of 1/(platelets)². As shown in Fig. 3, this analysis revealed a statistically significant correlation. The data is shown in Table 4.

### Table 3 Steady-state plasma levels

<table>
<thead>
<tr>
<th>Thymidine dose (g/m²/day)</th>
<th>Patient</th>
<th>[Thymidine] (mM)</th>
<th>[Thymidine] (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>1</td>
<td>0.160 ± 0.01*</td>
<td>0.406 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.158 ± 0.05</td>
<td>0.874 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.381 ± 0.08</td>
<td>0.699 ± 0.23</td>
</tr>
<tr>
<td>50</td>
<td>4</td>
<td>0.767 ± 0.18</td>
<td>1.59 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.722 ± 0.19</td>
<td>1.71 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.884 ± 0.15</td>
<td>1.66 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.697 ± 0.12</td>
<td>0.985 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.882 ± 0.11</td>
<td>1.30 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>1.19 ± 0.21</td>
<td>2.13 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.21 ± 0.26</td>
<td>2.06 ± 0.20</td>
</tr>
</tbody>
</table>

* Mean ± SD.
In combination with CDDP, the maximally tolerated dThd dose is considerably less than that reported for dThd alone administered as a 5-day continuous infusion. Previous studies have shown that dThd doses of at least 75 g/m²/day can be administered with only moderate bone marrow suppression and that platelet nadirs less than 100,000/mm³ are uncommon (14). While it is not yet possible to draw firm conclusions regarding dThd-CDDP interactions, it does appear that the combination of the two drugs may produce more bone marrow toxicity than would be expected to occur from either drug used alone. Clearly, though, more patients with little or no prior therapy must be treated with this combination before such conclusions can be drawn with certainty. By contrast, the central nervous system and gastrointestinal toxicities observed in the present study do not appear to be substantially different from those known to result from administration of dThd or CDDP as single agents (14, 15).

One of our goals in measuring plasma dThd and thymine concentrations was to determine whether it is possible to achieve drug levels in the range of those reported to produce synergy with CDDP in vitro. In cultured human colon carcinoma cells, a 1-h exposure to dThd at a concentration of 4 mM produces maximal synergy with CDDP, although enhanced cytotoxicity is observed at dThd concentrations as low as 1 mM (6). For thymine, there is no evidence of synergy in vitro unless concentrations of at least 3 mM are used with maximal cell kill occurring at thymine concentrations of nearly 8 mM (6). At the maximally tolerated dThd dose used in the present study, steady-state plasma dThd concentrations approached or exceeded 1 mM in all patients, and thymine levels of 1–2 mM were achievable. Thus, using a 5-day continuous infusion of dThd it was clearly not possible to achieve nucleoside concentrations which produce maximal synergy in vitro although, at the highest doses, plasma dThd levels were in the range of those shown to produce some enhancement of cisplatin cytotoxicity. It might therefore be reasonable to begin Phase II trials of this combination at dThd doses of 60 g/m²/day with further dose escalation in good performance status patients with minimal prior therapy.

Few prior studies have examined dThd pharmacokinetics at varying dose levels. We have observed that plasma dThd clearance falls significantly as the administered dose is increased from 24 to 60 g/m²/day and may begin to plateau at doses of 50–60 g/m²/day. Unfortunately, we did not have an opportunity to evaluate renal dThd clearance at the lowest dose levels and therefore cannot exclude the possibility that declining renal clearance contributes to the fall in plasma clearance. However, our findings are consistent with previous observations by Ensminger and Frei (16) that the hepatic dThd extraction ratio falls progressively as dThd doses are increased from 16 to 128 g/m²/day, suggesting saturability of hepatic pyrimidine clearance mechanisms. As pyrimidine catabolic pathways become saturated, accumulation of thymine in plasma would be expected to result in diminished net clearance of dThd by nucleoside phosphorylases in plasma and tissues. A similar interaction has been proposed to explain the delay in 5-FU clearance that occurs following dThd administration (17).

CDDP administration has previously been shown to result in delayed clearance of methotrexate (18) and the epipodophyllotoxins (19). Administration of CDDP on the third day of a 5-day continuous dThd infusion provided a unique opportunity to examine the impact of an acute dose of CDDP on dThd pharmacokinetics. Our results demonstrate a significant fall in plasma dThd clearance following CDDP administration.
Though renal clearance of dThd, thymine, and creatinine fell following CDDP administration, no statistically significant alterations in renal clearance were observed, raising the possibility that CDDP administration may in some way perturb extrarenal dThd clearance mechanisms.

Although the dose-limiting toxicity in this study was clearly thrombocytopenia, no clear-cut relationships between platelet nadir and dThd pharmacokinetics could be found. Nonlinear regression analysis did, however, indicate a significant correlation between steady-state plasma dThd concentration and log platelet nadir. This type of analysis suggests that, while a relationship can be found between platelet nadir and dThd concentrations, other less easily quantifiable factors, such as extent of prior therapy, have a significant impact on the hematological toxicity of this treatment regimen. Indeed, as part of our data analysis, we performed multiple regression analysis of a number of patient characteristics including dThd dose, pretreatment platelet count, and pretreatment creatinine clearance and found that, of these, only pretreatment platelet count was significantly correlated with posttreatment platelet nadir ($P = 0.01$). This observation raises the possibility that an unsuspected bias may have been introduced into this trial by the enrollment of patients at the highest dose levels who had the most severely diminished bone marrow reserve. Analysis such as this is not ordinarily done in Phase I trials and again underscores the great variability of the patients enrolled in these studies and the difficulties of using the results to predict clinical outcomes in subsequent trials.

Further clinical development of nucleoside-cisplatin combinations will be difficult without a better understanding of the mechanism by which these drugs interact at the cellular level. Additional laboratory studies are clearly needed from which new, rationally designed clinical studies can emerge.

ACKNOWLEDGMENTS

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Phase I Clinical and Pharmacological Study of Thymidine (NSC 21548) and cis-Diamminedichloroplatinum(II) in Patients with Advanced Cancer

Richard L. Schilsky, Kathleen O'Laughlin and Mark J. Ratain


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