High-Dose Thymidine Infusions in Patients With Leukemia and Lymphoma

By D. W. Kufe, P. Beardsley, D. Karp, L. Parker, A. Rosowsky, G. Canellos, and E. Frei III

We have recently explored the cytokinetic effects of thymidine given by continuous infusion in an animal model and have demonstrated an arrest of cell cycle traverse in rapidly proliferating tissues. Similar biologic effects have been observed in clinical trials upon infusing thymidine at a dose rate of 75 g/sq m/day. Fifteen courses of at least 5 days in duration have been administered to 11 patients with either leukemia or lymphoma. Steady-state serum thymidine levels were achieved in the range of 1–2 mM and the serum half-life of thymidine was approximately 90 min upon completion of the infusion. The associated toxicity included myelosuppression, headache, anorexia, nausea, vomiting, and diarrhea. The antitumor effect was dependent on the disease being treated and the percentage of blasts in the peripheral blood. Three patients with AML and three patients with T-cell leukemia responded to the thymidine infusions with an abrupt decrease in peripheral blast count. In contrast, no response was observed in two patients with poorly differentiated lymphocytic lymphoma of B-cell origin in a leukemic phase. One patient with T-cell leukemia had a marked clinical response with a reduction in peripheral blast count. In contrast, no response was observed in two patients with poorly differentiated lymphocytic lymphoma of B-cell origin in a leukemic phase. One patient with T-cell leukemia had a marked clinical response with a reduction in peripheral blast count.

Thymidine has been extensively studied as a means of synchronization of mammalian cell growth in vitro. The inhibition of growth results from feedback regulation of several enzymes in pyrimidine biosynthesis by thymidine triphosphate (dTTP). Synthesis of dTTP occurs not only through the conversion of deoxyuridine monophosphate (dUMP) to thymidine monophosphate (dTMP), but also through the phosphorylation of thymidine itself to dTMP and subsequently dTTP. An increase in dTTP levels limits the production of deoxycytidine-5′-triphosphate (dCTP) by inhibiting ribonucleotide reductase and thus the conversion of cytidine-5′-diphosphate (CDP) to deoxycytidine-5′-diphosphate (dCDP). This deficiency limits DNA synthesis, and the nucleoside deoxycytidine (CdR) can bypass the block and reinitiate cellular growth.

The inhibition of DNA synthesis restricts S-phase cellular growth and leads to an accumulation of cells in G1, early S phase. After removal of the thymidine, the cell population proceeds in a partially synchronous manner through S phase. This arrest of cell cycle traverse has also been shown to cause cytotoxicity when cells are exposed to the nucleoside for periods of greater than one generation time. This cytotoxicity has been shown to be selective in that malignant cells have demonstrated a greater in vitro sensitivity to the lethal effects of thymidine exposure than appropriate non-neoplastic control lines. More recent reports have indicated that thymidine can cause regression of human tumor xenografts transplanted into nude mice. The in vivo antitumor effects of thymidine have been confirmed in other human tumor xenograft models.

Recent investigations in our laboratory have demonstrated in rats that the continuous infusion of high doses of thymidine resulted in the arrest of cell cycle traverse of leukemic myeloblasts as well as rapidly proliferating normal tissues. The arrest of both normal and malignant cell growth in vivo can lead to selective antitumor effects. Normal cells have been shown to enter a resting or G0 phase under conditions that are not conducive to continued growth, while transformed cells have evidently lost this ability and thus might be more susceptible to the effects of agents such as thymidine.

As a result of the understanding of the effects of thymidine on DNA synthesis as well as the studies performed on human tumor xenografts in nude mice, clinical trials have been initiated using the nucleoside at high doses sufficient to achieve millimolar plasma levels. The studies reported have included patients with a variety of hematologic malignancies and solid tumors. This report provides data on the clinical effects of thymidine in patients with leukemia and lymphoma. The clinical pharmacology, cytokinetic, and biochemical effects of thymidine were studied.
The diagnosis and age of these patients is presented in Table 1. The patients fulfilled the following criteria: (A) estimated life expectancy of at least 1 mo, (B) a performance status of 2 or less on the ECOG scale, (C) a creatinine clearance of greater than 50 ml/min, and (D) a total bilirubin less than 5 mg/dl. Patients with a history of coronary artery disease or congestive heart failure were excluded from the study to avoid problems resulting from fluid overload. Written informed consent was obtained from all patients. The patients in the study were hospitalized at the Sidney Farber Cancer Institute, The Children's Hospital Medical Center, or the Peter Bent Brigham Hospital.

Drug Dosage and Schedule
Thymidine is supplied as 15 g in 500 ml of 0.6% NaCl by the National Cancer Institute and was given by continuous infusion using IMED pumps at 75 g/sq m/day. Courses were initially scheduled for 5 days, but were subsequently extended beyond that in 7 of the 15 courses, reaching a maximum duration of 14 days in 1 patient. The interval between successive courses was variable with a minimum of 5 days, depending on patient tolerance and recovery of the peripheral white blood cell counts.

Clinical Laboratory Studies
Complete blood counts were performed prior to treatment and on each day during the period of infusion as well as at least once a week while the thymidine was being given. Liver function tests, including the bilirubin, serum glutamate and oxalate transaminases, alkaline phosphatase and lactic dehydrogenase were evaluated weekly. Serum uric acids were monitored every other day. Bone marrow aspirates were performed on all patients prior to each infusion as well as during each course to evaluate the cytokinetic and biochemical effects of thymidine on bone marrow function.

Cytokinetic Studies
Bone marrow aspirates were immediately anticoagulated with EDTA, and a cell count was performed using a hemocytometer after appropriate dilution. Aliquots of 10^6 cells were then incubated with 50 µCi of either 3HTdR, specific activity 50 Ci/m mole, 3HAdR, or 3HtdR, specific activity 16 Ci/m mole (New England Nuclear, Boston, Mass.) for 1 hr at 37°C. The bone marrow cells were then subjected to Ficoll-Hypaque separation. The cells were washed with cold Dulbecco’s phosphate-buffered saline without calcium (PBS) and then alkali digested by adding 0.5 ml of 0.6 normal NaOH to 2 ml of a cell suspension at 10^6 cells/ml and incubating overnight at 37°C. Aliquots were then precipitated with 50% trichloroacetic acid (TCA), collected on Whatman GF/C filters, and washed with 5% TCA. The filters were washed with absolute alcohol, dried, and placed in scintillation vials containing 5 ml of Omnifluor (New England Nuclear, Boston, Mass.). Vials were counted in a Beckman Model LS-335 liquid scintillation system.

Flow cytometry was performed on similar bone marrow aspirates. The cells were subjected to Ficoll-Hypaque separation and the resulting interphase of white cells collected and washed twice with 5 mL PBS. The final pellet was resuspended in 2.0 ml PBS, and aliquots of 0.25 ml were added to 4.75 ml of 0.05 mg/ml propidium iodide monohydrate (Calbiochem, San Diego, Calif.) solution in 1.1% sodium citrate for flow microfluorometric analysis according to the method of Krishan. After 30 min of staining, samples were monitored for DNA content per cell on a Beckman LS 4801 (Biophysics Systems, Mahopac, N.Y.). DNA distribution histograms were analyzed using a computer program designed to quantify the percentage of cells with G0/G1, S, and G2 DNA content.

Thymidine and Thymine Determinations
Blood samples were collected in heparinized tubes and the plasma collected by centrifugation at 1000 g for 7 min at 4°C. The plasma samples were then stored at –20°C. One-milliliter aliquots were used for analysis by boiling for 20 min in Dupont polypropylene tubes (16 x 80 mm) after adding 20 µl of 10^3 M BUDR. After heat coagulation, the samples were centrifuged at 30,000 rpm for 90 min using a type 40 rotor in a Beckman L-65 ultracentrifuge. The supernatant was then removed and filtered through a 0.2 µm Millipore filter and then analyzed by high pressure chromatography (HPLC) as described previously. Urine samples were not subjected to heat coagulation, but were diluted tenfold with distilled water.
Thymidine Uptake Studies

Bone marrow or peripheral blood cells were separated by Ficoll-Hypaque and then washed twice with PBS. The cell count was adjusted to 2 x 10^6/ml with RPMI 1640 media without serum. Aliquots of 1 ml were run in duplicate by adding 50 μCi of ^3H]Tdr, specific activity 50 Ci/m mole, over a time course from 0 to 60 min. The zero time point was maintained at 0°C, while the subsequent samples were incubated at 37°C. At each time point, 2 ml of cold 2 x 10^-4 M Tdr in serum-free RPMI 1640 media was added to the duplicate samples. The cells were then spun at 1500 rpm for 8 min and the supernatant media decanted. Cells were washed twice in 4 ml of the cold 2 x 10^-4 M Tdr solution, and then 500 μl of ice-cold 0.5 N perchloric acid was added to the cell pellet. The pellet was then resuspended with the blunted end of the glass rod and the mixture incubated for 1 hr on ice. Following incubation, the samples were spun at 2500 rpm for 10 min and the supernatant removed as the acid-soluble fraction and counted after the addition of 4 drops of glacial acetic acid and 10 ml of Aquasol.

Thymidine Kinase and Phosphorylase Assays

Thymidine kinase levels were determined in leukemic cells by a radioactive assay based on the conversion of [3H]TdR to [3H]dTMP. The substrate and product have distinct absorption properties on anion-exchange chromatography and thus can be separated to measure thymidine kinase activity as described previously. The thymidine phosphorylase activity was measured spectrophotometrically by determining the conversion of thymidine from thymidime to thymine. The steady-state thymidine levels were in the range of 1–2 mM for those patients tested, while the serum thymidine levels were more variable over a range of 5 x 10^-3 M–10^-2 M. Multiple plasma samples were obtained in several patients to monitor the rise in serum thymidine and thymine levels after starting the infusion. Figure 1 shows representative profiles obtained from patient N.B. for the plasma thymidine and thymine. Steady-state levels for both thymidine and thymine were achieved by 20 hr. Upon completing the infusions, the plasma thymidine concentration rapidly declined with a T1/2 of 1.3 hr as determined by a least squares nonlinear regression computer program. Similar T1/2 values of 1.6 and 1.7 hr were obtained in patients R.F. and L.K. The decline in thymine levels was more prolonged and failed to follow first order kinetics.

Urine specimens collected during the course of the infusion were stored initially at 4°C, causing the precipitation of crystals. Similar crystal formation occurred even at room temperature, and the samples could be redissolved only upon extended periods of incubation at 37°C. HPLC analysis of the crystals revealed primarily thymine with lesser amounts of thymidine. The steady-state concentrations (mean ± standard deviation) of soluble thymidine and thymine and thymine levels listed in Table 1 represent the mean ± the standard deviation for the levels obtained each day during the course of the infusion. The steady-state thymidine levels were in the range of 1–2 mM for those patients tested, while the serum thymidine levels were more variable over a range of 5 x 10^-3 M–10^-2 M. Multiple plasma samples were obtained in several patients to monitor the rise in serum thymidine and thymine levels after starting the infusion. Figure 1 shows representative profiles obtained from patient N.B. for the plasma thymidine and thymine. Steady-state levels for both thymidine and thymine were achieved by 20 hr. Upon completing the infusions, the plasma thymidine concentration rapidly declined with a T1/2 of 1.3 hr as determined by a least squares nonlinear regression computer program. Similar T1/2 values of 1.6 and 1.7 hr were obtained in patients R.F. and L.K. The decline in thymine levels was more prolonged and failed to follow first order kinetics.

RESULTS

Thymidine Pharmacokinetics

Thymidine was administered as a continuous infusion at 75 g/sq m/day to 11 patients for a total of 15 courses, as outlined in Table 1. The courses varied in duration from 2 to 14 days, with the majority of courses being 5 days or longer. The plasma thymidine

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**Fig. 1.** Plasma concentrations of thymidine and thymine during and following the infusion of thymidine at 75 g/sq m/day in patient N.B. The plasma levels were determined by high pressure liquid chromatography.
in the urine for all patients were $23.8 \pm 8.3$ and $15.5 \pm 5.6$ mM, respectively. The renal clearance of thymidine during such conditions was about 50% of the total body clearance.

**Toxicity**

The toxicity associated with the high-dose thymidine infusions was primarily related to the central nervous system, gastrointestinal tract, and bone marrow. The major toxicities not related to myelosuppression consisted of headache, anorexia, nausea, vomiting, and diarrhea. Anorexia was the most common side effect (9/15 courses) and was associated with nausea and vomiting in 2 patients. The vomiting was relatively well controlled by antiemetics, but persisted throughout the course of the infusion. Diarrhea was generally slight and occurred in 5 of the 15 courses. Headache (6/15 cases) was moderate to severe in 2 patients and was minimally responsive to analgesics. All of these side effects occurred within the first 24 hr after starting the thymidine infusion and resolved rapidly within several hours after stopping the thymidine.

The hematopoietic effects of thymidine on bone marrow not involved with tumor were evaluable in 2 patients (R.Y. and R.L. in Table 2). Patient R.Y. received 2 days of thymidine and had a slight lowering of his WBC by the third day without a decline in platelet count. Patient R.L. received 2 courses of thymidine consisting of 5 and 7 days in duration. Myelosuppression was evident throughout both courses, with a gradual but progressive decline in WBC as well as platelet count. Sequential bone marrow specimens were unchanged.

**Antitumor Effects**

In this preliminary trial, there was suggestive evidence of antitumor activity in 5 of the 11 patients. Three of three patients with T-cell leukemia showed abrupt declines in their peripheral blast count within 1–2 days after starting the thymidine infusion (Table 2). A representative response is shown in Fig. 2. This patient, F.J., had marked hepatosplenomegaly as well as lymphadenopathy prior to therapy, and after 3 days...

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**Table 2. Effect of High-Dose Thymidine on Peripheral Blood and Bone Marrow**

<table>
<thead>
<tr>
<th>Patient</th>
<th>WBC Baseline</th>
<th>Percent Blasts</th>
<th>Nadir</th>
<th>Day of Nadir</th>
<th>Platelets Baseline</th>
<th>Day of Nadir</th>
<th>Bone Marrow</th>
<th>Percent Blasts</th>
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</thead>
<tbody>
<tr>
<td>T.P.</td>
<td>9.1</td>
<td>38</td>
<td>1.5</td>
<td>12</td>
<td>8</td>
<td>231</td>
<td>100</td>
<td>8</td>
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<tr>
<td>F.J.</td>
<td>230.0</td>
<td>99</td>
<td>14.0</td>
<td>91</td>
<td>10</td>
<td>39</td>
<td>25</td>
<td>5</td>
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<tr>
<td>K.J.</td>
<td>265.0</td>
<td>97</td>
<td>16.0</td>
<td>2</td>
<td>14</td>
<td>28</td>
<td>6.5</td>
<td>16</td>
</tr>
<tr>
<td>E.G.</td>
<td>28.5</td>
<td>39</td>
<td>0.4</td>
<td>3</td>
<td>10</td>
<td>120</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>L.K.</td>
<td>10.9</td>
<td>88</td>
<td>0.1</td>
<td>12</td>
<td>7</td>
<td>20</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>M.D.</td>
<td>1.4</td>
<td>0</td>
<td>0.5</td>
<td>2</td>
<td>8</td>
<td>30</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>A.B.</td>
<td>90.0</td>
<td>97</td>
<td>39.0</td>
<td>98</td>
<td>5</td>
<td>106</td>
<td>33</td>
<td>6</td>
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<tr>
<td>N.B.</td>
<td>143.0</td>
<td>99</td>
<td>25.5</td>
<td>98</td>
<td>8</td>
<td>27</td>
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<td>R.F.</td>
<td>4.3</td>
<td>83</td>
<td>1.3</td>
<td>75</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>R.L.*</td>
<td>386.0</td>
<td>100</td>
<td>380.0</td>
<td>100</td>
<td>7</td>
<td>18</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>R.L.†</td>
<td>12.6</td>
<td>96</td>
<td>5.3</td>
<td>100</td>
<td>11</td>
<td>19</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>R.Y.†</td>
<td>6.7</td>
<td>0</td>
<td>2.0</td>
<td>0</td>
<td>7</td>
<td>152</td>
<td>119</td>
<td>4</td>
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<tr>
<td>A.B.</td>
<td>3.1</td>
<td>0</td>
<td>0.8</td>
<td>0</td>
<td>9</td>
<td>72</td>
<td>36</td>
<td>9</td>
</tr>
<tr>
<td>R.Y.†</td>
<td>12.4</td>
<td>0</td>
<td>9.1</td>
<td>0</td>
<td>3</td>
<td>245</td>
<td>269</td>
<td>—</td>
</tr>
</tbody>
</table>

*NR, no response; NC, no change; PR, partial response.  
*No change in pulmonary nodules.  
†No change in mediastinal mass.
on the thymidine infusion, the liver and spleen were no longer palpable. There was a concomitant marked reduction in his adenopathy. A similar clinical response was observed during the second course of thymidine. Further, at the onset of the second course, this patient had diffuse leukemia cutis that essentially resolved after 7 days of therapy. There was, however, no objective change in the bone marrow following the first course. Another patient with T-cell leukemia, K.J., had a similar abrupt decline in peripheral blast count that was accompanied by a reduction in the percent of blasts in the bone marrow with 90% prior to therapy declining to 40% blasts after 7 days on the thymidine. The infusion was continued for 14 days, and peripheral blasts remained below 3% for the ensuing 2 wk. A repeat bone marrow at that time revealed the return of a majority of blasts in the bone marrow and a rapid rise in the appearance of blasts peripherally.

The effect of thymidine on the growth of acute myelogenous leukemia cells was variable with two patients, E.G. and M.D., exhibiting the abrupt decline in counts observed in patients with T-cell leukemia. In both patients, the peripheral blast counts remained suppressed for several days, and following the thymidine infusions, rose to levels above baseline values. Second courses of thymidine resulted in the same pattern of response. Bone marrows performed prior to and during each course showed no evidence of a reduction in blasts. The two other patients with acute myelogenous leukemia (AML) also failed to demonstrate significant marrow responses.

The pattern of abrupt lowering of the peripheral blast counts in certain patients with T-cell leukemia and AML is in contrast with that observed in two patients with B-cell poorly differentiated lymphoma in leukemic phase. Patient N.B. had no significant lowering of his blast count during or following a 5-day infusion of thymidine. During a longer infusion of 10 days, patient R.F. had a slow but gradual decline in his peripheral WBC that was distinctly different from that observed in the patients with T-cell leukemia. This pattern of a slow progressive decline was also observed in patient R.L. with diffuse histiocytic lymphoma without bone marrow involvement who was given 2 sequential courses of 5 days and 7 days.

Cytokinetic Studies

Figure 3 shows the changes in the percentage of cells in different phases of the cell cycle in the bone marrow of patient M.D. before the infusion of thymidine and 3 days after starting the infusion. The baseline profile (Fig. 3A) has 86% of the cell population in phase Go/G1, 10% in S phase, and 4% in G2. The effects of thymidine on the bone marrow after a 3-day exposure to a mean plasma level of approximately 2 mM is clearly seen by comparing the baseline profile with that shown in Fig. 3B. The percentage of cells in G0/G1 have declined to 44%, while the percentage of cells in S phase has increased to over 4 times that prior to therapy. This marked increase in S phase extends into the G2 region, contributing to the percent of cells in that phase while there is no distinct reappearance of the G2 peak. This pattern could result from an arrest of cells throughout S phase without a significant portion progressing into G2. Flow cytometry was performed on Ficoll-Hypaque preparations of bone marrow and peripheral blood in 10 of the 15 courses of thymidine administered. Analysis of the proportion of
cells in the different phases of the cell cycle was performed prior to and during the infusion. The effect of thymidine on the distribution of cells in G0/G1, S, and G2 is presented in Table 3, and the data are expressed as the percent of control or baseline values. In 9 of 10 courses, there was an increase in the proportion of cells in S phase as a result of the thymidine infusion. These values were comparable for two different courses administered to the same patient, despite the fact that samples were obtained at variable durations after starting the infusion. In general, there was also a decline in the proportion of cells in G0/G1 with a concomitant rise in the proportion of cells in G2. The increase in G2 as a result of thymidine again reflected an extension of cells from S phase through the G2 region. G2 peaks were not clearly observed under these circumstances in a manner similar to that illustrated in Fig. 3.

Bone marrow and peripheral blood preparations were also used to determine the effects of thymidine on labeled nucleoside incorporation into DNA. Samples were obtained during the period of infusion and compared to values obtained just prior to therapy. The removal of erythrocytes and polymorphonuclear cells by Ficoll-Hypaque separation resulted in preparations of malignant blasts comprising greater than 90% of the nucleated cells, with the exception of samples from uninvolved bone marrow (patient R.L.). Table 4 lists the incorporation of \(^{3}H\)Tdr, \(^{3}HCdR\), and \(^{3}HAdR\) into cellular DNA as a percent of the pretherapy values. The incorporation of \(^{3}H\)Tdr into DNA is markedly diminished to levels of 0.1%–2.9% during the period of infusion. The low incorporation values are in contrast with those obtained using \(^{3}HCdR\) where a wide dispersion was observed ranging from 17%–795% of baseline incorporation. The inhibition of DNA incorporation using the purine precursor, \(^{3}HAdR\), correlated more directly with the declines observed in the peripheral white blood counts. In 11 of the 12 courses studied, there was a decrease in the incorporation of \(^{3}HAdR\) into cellular DNA as a result of the thymidine infusion.

**Thymidine Sensitivity and Resistance**

Figure 4 shows the patterns of uptake for \(^{3}H\)Tdr into intracellular acid-soluble and insoluble pools for cell populations obtained prior to the infusion of thymidine. Figure 4A shows the patterns obtained with T-cell lymphoblasts from patients F.J. and T.P. The uptake is similar in both cell preparations with the acid-soluble fraction achieving near plateau values after 10–20 min of incubation. Further, the incorporation of the labeled nucleoside into the acid-insoluble fraction is similar in both patients with linear incorporation over the 60-min time period. These patterns are in contrast to those obtained with poorly differentiated lymphoblasts of B-cell origin as illustrated in Fig. 4B. In these cells, the uptake of \(^{3}H\)Tdr into the acid-soluble fraction reaches plateau values between 20 and 40 min. Furthermore, the amount of uptake into the

<table>
<thead>
<tr>
<th>Table 3. The Effect of Thymidine on Cell Cycle Phase Distribution Expressed as Percent of Pretherapy Cytofluorographic Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
</tr>
<tr>
<td>T.P.</td>
</tr>
<tr>
<td>F.J.</td>
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<tr>
<td>E.G.</td>
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<tr>
<td>2</td>
</tr>
<tr>
<td>L.K.</td>
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<tr>
<td>M.D.</td>
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<tr>
<td>2</td>
</tr>
<tr>
<td>N.B.</td>
</tr>
<tr>
<td>R.L.</td>
</tr>
<tr>
<td>2</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent the distribution of cells on the pretherapy cytofluorographic pattern.

| Table 4. Precursor Incorporation into Cellular DNA During Thymidine Infusions Expressed as Percent of Control |
|---|---|---|---|
| Patient | Course | \(^{3}HTdr\) | \(^{3}HCdR\) | \(^{3}HAdR\) |
| T.P. | 1 | 0.1 | 415 | 34 |
| F.J. | 1 | 0.1 | 17 | 18 |
| E.G. | 1 | 0.5 | — | 11 |
| 2 | 0.9 | 193 | 14 |
| L.K. | 1 | 0.1 | 18 | 10 |
| M.D. | 1 | 1.1 | 473 | 11 |
| 2 | 1.3 | 795 | 75 |
| A.B. | 1 | 1.9 | 220 | 402 |
| N.B. | 1 | 1.1 | 30 | 75 |
| R.F. | 1 | 2.9 | 58 | 71 |
| R.L. | 1 | 0.2 | 614 | 88 |
| 2 | 0.6 | 122 | 97 |

Fig. 4. Patterns of uptake for \(^{3}H\)Tdr into intracellular acid-soluble and insoluble pools for preparations of T-cell lymphoblasts (A), B-cell lymphoblasts (B), and myeloblasts (C). The results are expressed as the number of tritium cpm in both pools over a time course of 60 min for \(10^6\) cells per time point.
soluble pool is less than half that seen with the T lymphoblasts. The incorporation into the acid-insoluble fraction is even lower with values 10–100-fold lower than the T-cell counterparts. The most marked uptake of thymidine was observed in the myeloblasts obtained from patient M.D. (Fig. 4C), where the acid-soluble pools plateaued at even higher levels and the incorporation into the acid-insoluble fraction showed relatively higher values than for either of the lymphoblast populations.

The thymidine kinase and phosphorylase activity was also measured in preparations of leukemic cells obtained from these patients. As listed in Table 5, the thymidine kinase activity was up to tenfold higher in the myeloblasts and in the T-lymphoblasts as compared to those values obtained in the B-cell samples. The converse, however, was observed for the thymidine phosphorylase levels in which the B-lymphoblasts had levels tenfold higher than those observed in the myeloblasts or the T cells. A calculation of the ratio of kinase to phosphorylase allows a comparison of the relative synthetic and catabolic activity for thymidine in these cells.

A more definitive approach in defining the intracellular metabolism of thymidine would be to monitor the actual synthesis of nucleotides as well as the catabolism of thymidine. In this regard, we have measured the intracellular conversion of \(^{3}HTdR\) to the respective nucleotides by means of a procedure developed for thin-layer chromatography. As seen in Fig. 5, \(^{3}HTdR\) is phosphorylated to dTMP, dTDP, and dTTP in T-lymphoblasts from patient T.P., while there is no significant nucleotide synthesis observed in B lymphoblasts from patient R.F. Leukemic cells from patients M.D. and F.J. also synthesized significant amounts of dTTP as compared to cells from patient N.B., which failed to phosphorylate \(^{3}HTdR\) (data not shown).

**DISCUSSION**

The administration of thymidine at 75 g/sq m/day by continuous infusion in humans achieves plasma levels in the 1–2 mM range. Previous studies administering thymidine at 8 g/sq m/day produce mean plasma levels at 1.5 μM.\(^3\) In those studies, the T’/2 for thymidine clearance from patient serum was found to be approximately 8–10 min. This finding is in contrast to the half-life values of greater than 1 hr obtained with plasma thymidine in the millimolar range. At the lower dose rate, the renal excretion of unchanged thymidine was calculated to be less than 2% of the administered dose.\(^3\) At millimolar plasma concentrations, renal excretion accounted for approximately 50% of the total body clearance.

Thymidine is catabolized to dihydrothymine and subsequently γ-amino-isobutyric acid and CO\(_2\). The enzyme thymidine phosphorylase, which is responsible in part for this catabolism, is present in plasma as well as a variety of tissues. One major site of catabolism is within the liver, and recent studies have shown that the hepatic extraction becomes saturated as dose rates increase from 16 to 32 g/sq m/day.\(^3\) Other organs probably play a role in this catabolism, but once other sites become saturated, then the renal mechanism must account for the total body clearance. The result of thymidine catabolism is the production of thymine, which in these patients ranges from \(10^{-5}\) to \(10^{-3}\)M in the plasma. The plasma T’/2 of thymine is somewhat
longer than that obtained with thymidine and probably reflects the continued catabolism of the nucleoside.

The maintenance of millimolar plasma thymidine steady-state levels for up to 14 days resulted in minimal toxicity. The most common side effect experienced in the 15 courses was that of anorexia, associated with nausea and vomiting in 2 of the patients. Bilateral frontal headache also occurred and was moderate to severe in two patients. Both of these patients had relatively higher thymine levels. It is also of interest to note that the onset of the side effects began approximately 12 hr after starting the thymidine infusion and resolved completely several hours after stopping the thymidine. This corresponds quite closely to the patterns of achieving millimolar levels and then a rapid decline in these levels within several hours after completing the course.

Myelosuppression and bone marrow hypoplasia have been documented in animal studies as a result of thymidine infusions. This effect was found to be dependent on dose and duration of exposure. One of our patients, R.L., had no tumor involvement of the marrow, and during the course of two thymidine infusions, showed a slow but progressive decline in his peripheral white blood count and platelet count.

The effect on peripheral counts was more marked in several of the patients with T-cell leukemia and AML. These patients responded to thymidine with an abrupt decline in their peripheral blast counts, which in one patient (F.J.) was associated with resolution of hepatosplenomegaly and leukemia cutis as well as partial resolution of lymphadenopathy. Despite the declines in peripheral blast counts, there was only one instance of partial clearing of the blasts in the bone marrow. It is also of interest to note that one patient with B-cell leukemia failed to respond, and another patient with the same disorder had the slow progressive decline in peripheral blood counts. This suggested different patterns of sensitivity to thymidine for B cells as compared to T cells or myeloblasts.

Although certain patients had sharp declines in peripheral blast counts while on thymidine, the return of the blasts was equally as rapid after completing the thymidine infusion. In several patients, the return of blasts after thymidine reached levels higher than that observed prior to therapy. A similar rebound effect was observed in the animal studies and was shown to be secondary to a synchronous pattern of release following thymidine exposure. The pattern of rapid recovery and rebound suggested that these cells were actively progressing through S phase and, therefore, should be susceptible to the effects of cell cycle specific agents.

The cytokinetic effects of thymidine on the bone marrow leukemic cells probably account for the patterns of clinical response. Analysis of bone marrow preparations by flow cytometry prior to and during ten courses of thymidine revealed an accumulation of cells in S phase in all but one patient. The profiles obtained were consistent with an arrest of cells throughout the S phase, thus suggesting inhibition of DNA synthesis, probably by expanded pools of dTTP.

The effects on DNA synthesis can also be determined by monitoring incorporation of labeled precursors. However, in these studies using millimolar concentrations of thymidine, the incorporation of labeled nucleosides is perturbed by thymidine-induced alteration of deoxyribonucleotide pools. We have explored the incorporation of labeled thymidine, deoxyctydine, and deoxyadenosine into cellular DNA prior to and during the exposure to thymidine. Labeled thymidine incorporation into DNA was uniformly low and clearly represents the expanded thymidine pool in the bone marrow preparations. The incorporation of labeled deoxyctydine was enhanced during 7 of the 11 courses studied, and this increase was, in certain instances, over 7 times that of the pretherapy incorporation. The increases observed for this labeled nucleoside are most likely to be due to declines in the dCTP pools as has been described for other human white blood cells exposed to thymidine and is consistent with the fact that CdR can reverse the thymidine effect.

The incorporation of the labeled purine precursor, deoxyadenosine, into DNA probably represents more closely the effect of thymidine on DNA synthesis. Thymidine has been shown to reduce dATP pools in human cells and thus should increase the incorporation of this labeled precursor under conditions of similar rates of DNA synthesis. In 11 of the 12 courses studied, however, there was a decrease in the incorporation of 3HAdR into cellular DNA as a result of the thymidine infusion. Patients showing the most marked decline in peripheral counts had the lowest incorporation of this labeled precursor, while patients with the more gradual decline in peripheral counts had values more closely approaching that of the control. In this regard, two circumstances in particular are worth mentioning. During the second course administered to patient M.D., the bone marrow sample was harvested at a time when the peripheral counts had plateaued and the incorporation of the purine precursor was significantly higher at this point than during the first course when the sample was obtained with the counts continuing to decline. Further, the incorporation of 3HAdR was elevated to four times the control value in patient A.B., and this probably reflects the unexpected rise in this patient’s peripheral counts at that time from 1400 to 4600.

We have also attempted to determine whether the
pattern of the clinical response could be correlated with the sensitivity of the leukemic cell to thymidine. Various factors must be taken into consideration, such as the ability of these cells to transport thymidine as well as the relative intracellular concentrations of thymidine kinase and phosphorylase. Further, it is even more important to demonstrate the metabolism of thymidine within the cells in terms of whether it is phosphorylated to dTTP or degraded to thymine. The results indicate that the AML and T-cell preparations take up and incorporate into DNA more labeled thymidine when compared with leukemic B cells. These findings are strengthened by measuring the thymidine kinase and thymidine phosphorylase in similar samples. The AML and T-cell preparations had up to tenfold higher levels of thymidine kinase and up to 30 times lower levels of thymidine phosphorylase in contrast to that for the leukemic B cells. Thus, there is a differential of over 100-fold when comparing the ratio of thymidine kinase to phosphorylase in these two groups. The relative ratio of thymidine kinase to phosphorylase provides an indirect measure for predicting the conversion of thymidine to dTTP, which is obviously crucial when considering cellular sensitivity.

This approach is taken one step further by monitoring the intracellular phosphorylation of thymidine to dTTP as well as the degradation to thymine. The patterns obtained correlate closely with that predicted from the measurements of kinase and phosphorylase activity. Furthermore, the ability of the leukemic cells used in these studies to form dTTP correlate directly with the pattern of clinical response. Thus, one patient with AML and two patients with T-cell ALL had high intracellular ratios of thymidine kinase to phosphorylase and these patients all responded with either abrupt declines in blast count or with more significant cytoreduction in terms of resolution of hepatosplenomegaly or partial clearing of the marrow. In contrast, however, the two patients with B-cell ALL had low ratios of kinase to phosphorylase and failed to demonstrate significant cytoreduction. T-cell lymphoblasts have also been shown to have relatively lower intracellular 5'-nucleotidase activity, and this factor may also have a significant role in maintaining the higher levels of dTTP.

The infusion of high doses of thymidine has provided evidence of leukemic cytoreduction in certain patients. It could well be that thymidine infusions would be capable of producing complete remissions if continued for periods sufficient to empty the marrow and allow for recovery of normal cells. Another more effective approach might result from the sequential combination of thymidine with cell-cycle-specific agents. The arrest of cell populations in S phase and the abrupt return of peripheral blast counts following thymidine exposure suggest a period of enhanced DNA synthesis and S-phase traverse during which time these cells should be more susceptible to a cycle-specific agent such as ara-C. The therapeutic efficacy of such an approach remains to be determined.

ACKNOWLEDGMENT

The authors appreciate the excellent technical assistance of E. Michael Egan, Laila Sargenti, Nick Paphathanasopoulos, and Carolyn Klebart.

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