Effect of Very High-Dose Thymidine Infusions on Leukemia and Lymphoma Patients

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Int vivo and in vitro (7, 8, 9, 10, 11). Biochemically oriented studies have demonstrated that high exogenous dThd concentrations produce elevated intracellular levels of dTTP. Significant reduction in cellular dCTP pools also occurs, presumably caused by allosteric inhibition of ribonucleotide reductase by dTTP (1, 2, 6, 12, 13). The inhibition of DNA synthesis observed in the presence of excess dThd is believed to be due to the above-mentioned lowered dCTP pools (12). Protection against cytotoxic effects of dThd is afforded by exogenous deoxycytidine; this apparently results from repletion of the dCTP pool via the salvage pathway. Because of these in vitro and animal experiments, dThd was taken into clinical trial at several centers at drug dosages producing mean plasma dThd concentrations of 1 mm over periods of 10 to 20 days.

MATERIALS AND METHODS

Upon giving informed consent, 6 patients were entered into this study. There were 5 males and one female. The mean age was 29 (range, 19 to 37), and the median Karnofsky performance status was 40%. Because of anticipated high fluid loads during administration of dThd, only patients without cardiopulmonary disease, effusions, or edema were eligible for participation. Patients received a single infusion of dThd, at dosages of 90 to 240 g/sq m/day for 14 to 29 days. Mean plasma dThd concentration ranged from 3.8 to 5.5 mm. Cerebrospinal fluid levels were measured on three occasions and ranged from 2 to 23.5% of simultaneous plasma levels. Diarrhea was dose limiting in one patient. The other side effects included nausea and vomiting in all patients, hepatotoxicity in two patients, electrolyte imbalance in one, progression of a pericardial effusion to tamponade in one, and mild central nervous system toxicity in five. In all cases, this therapy produced bone marrow aplasia. One patient with acute lymphoblastic leukemia, refractory to prior treatment, achieved complete remission which lasted for 16 weeks. Another patient with lymphoblastic lymphoma had a greater than 50% reduction in his mediastinal mass which lasted for less than 1 month.

At multiple points during therapy, the bone marrow S-phase fraction was measured by flow cytometry and autoradiography. In five patients, the proportion of cells in S phase increased during the first few days of the infusion but then returned to baseline, concomitant with an overall reduction in the number of bone marrow blasts.

Cytoreduction was evaluated by the technique of W. Hiddemann, B. D. Clarkson, T. Buchener, M. R. Melamed, and M. Andreeff (Blood, 59: 216–225, 1982). The magnitude of tumor cell kill ranged from 0.7 to 3.6 logs of blasts/cu mm of bone marrow.

The data demonstrate that dThd is able to induce a complete remission in a patient with acute leukemia previously refractory to treatment. However, because of the very large drug quantities, fluid volumes, and the prolonged course required to produce the necessary tumor cell kill, this treatment approach is too impractical to be used extensively.

INTRODUCTION

dThd, a physiological pyrimidine nucleoside, was introduced into clinical trial following reports of selective activity against neoplastic cells in vitro and in vivo (7, 8, 9, 10, 11). Biochemically oriented studies have demonstrated that high exogenous dThd concentrations produce elevated intracellular levels of dTTP. Significant reduction in cellular dCTP pools also occurs, presumably caused by allosteric inhibition of ribonucleotide reductase by dTTP (1, 2, 6, 12, 13). The inhibition of DNA synthesis observed in the presence of excess dThd is believed to be due to the above-mentioned lowered dCTP pools (12). Protection against cytotoxic effects of dThd is afforded by exogenous deoxycytidine; this apparently results from repletion of the dCTP pool via the salvage pathway. Because of these in vitro and animal experiments, dThd was taken into clinical trial at several centers at drug dosages producing mean plasma dThd concentrations of 1 mm over periods of 10 to 20 days.

Trials using this dose and schedule have been reported from the Baltimore Cancer Research Center (4), the Sidney Farber Cancer Institute (7, 8), and Memorial Hospital (3). dThd did not produce significant antineoplastic effects with solid tumors, even at total cumulative doses which produced moderate to severe bone marrow suppression. In leukemia patients, dThd produced transient lowering of the peripheral blast count, but bone marrow aplasia or hypoplasia was not achieved. Other side effects produced by the above-mentioned dose of dThd were nausea and vomiting, dysphoria or headaches, and diarrhea; in our own hands, these were mild, transient, easy to control, and did not constitute dose-limiting toxicity.

With drugs that are myelosuppressive but lack dose-limiting toxic effects on other organs, remission induction in acute leukemia may sometimes be achieved by further escalation of the dose that is myelosuppressive in solid-tumor patients. Many effective chemotherapeutic regimens do not induce complete remissions without a preceding period of myelosuppression. Therefore, in an effort to rigorously assess the capacity of dThd to produce remissions in acute leukemia, we progressively increased both the plasma concentration and the duration of the infusion, until marrow hypoplasia was reached. Concomitantly, we have measured dThd levels achieved in plasma and spinal fluid, as well as the kinetic alterations induced and the magnitude of cytoreduction in the bone marrow. This paper reports the results of that study.
entry into this trial. The patients included the following diagnostic categories: one, acute lymphocytic leukemia; 3, acute nonlymphocytic leukemia (acute myelogenous leukemia, acute myelomonocytic leukemia, and chronic myelogenous leukemia in blastic crisis, one each); one, lymphoblastic lymphoma; and one, diffuse histiocytic lymphoma (each of the last 2 patients with marrow and blood involvement). All had been heavily pretreated and were in frank relapse before initiating therapy with dThd.

**Response Criteria.** In acute leukemia, for complete remission, criteria included normal marrow cellularity and differential count with less than 5% blasts, normal peripheral blood count with greater than 3000 WBC/cu mm with normal differential, and greater than 100,000 platelets/cu mm, all lasting for a minimum of 4 weeks. For partial remission, criteria included normal bone marrow that persists for less than 4 weeks or more than 5% but less than 10% blasts with an otherwise normal cellularity and normal peripheral blood counts, lasting for at least 4 weeks. Response criteria for measurable mass lesions were: complete remission, disappearance of all evidence of cancer; partial remission, greater than 50% decrease in the sum of the products of the diameters of all measurable lesions lasting more than 5% blasts, normal peripheral blood count with greater than 3000 WBC/cu mm with normal differential, and normal peripheral blood counts, lasting for at least 1 month.

**dThd Administration.** dThd for clinical use was supplied by the National Cancer Institute, Bethesda, MD, in bottles containing 15 g of dThd in 500 ml of 0.6% NaCl solution. The solution was transferred to plastic collapsible bags under sterile conditions and administered as constant i.v. infusion using a central venous catheter. When possible, double lumen catheters were used. Infusion rates were controlled using perfusate pumps (IVAC Model 530). In some cases, a single pump was unable to deliver the large volumes of fluid required, and 2 pumps had to be used simultaneously, connected in parallel, both feeding into the central venous line. Potassium chloride was added to the dThd solution in adequate quantities to replace urinary loss. When necessary, magnesium sulfate was also added.

Plasma levels of dThd were determined daily for the duration of the infusion. Five-ml blood samples were drawn from peripheral veins into heparinized tubes and promptly centrifuged, and the plasma was stored at -5° until analysis. The chromatographic method described previously (15) was modified to use isocratic elution, which shortened analysis time. Doses of dThd were increased, as tolerated, every few days until doses of 180 to 240 g/sq m/day (740 to 990 mmHg/m2/day) were reached. This required the infusion of as much as 14 liters of fluid per 24 hr at the higher doses. The infusions were given for a mean of 20 days (range, 14 to 29 days). dThd plasma levels correlated linearly with the dose given, as shown in Chart 1. At the highest tolerated dose level, 3.8 to 5.5 mm (919 to 1330 μg/ml), plasma concentrations were achieved. Within a given patient, the plasma dThd concentration varied by as much as 20%, despite careful attention to pump setting and central venous catheter care.

**RESULTS**

**Pharmacological Data.** dThd infusion was started in doses ranging from 90 to 200 g/sq m/day and escalated gradually every few days until doses of 180 to 240 g/sq m/day (740 to 990 mmHg/m2/day) were reached. This required the infusion of as much as 14 liters of fluid per 24 hr at the higher doses. The infusions were given for a mean of 20 days (range, 14 to 29 days). dThd plasma levels correlated linearly with the dose given, as shown in Chart 1. At the highest tolerated dose level, 3.8 to 5.5 mm (919 to 1330 μg/ml), plasma concentrations were achieved. Within a given patient, the plasma dThd concentration varied by as much as 20%, despite careful attention to pump setting and central venous catheter care.

**dThd concentration in cerebrospinal fluid was determined 3 times in 2 patients, reaching 3, 22, and 23.5% of the simultaneously measured plasma levels. dThd levels in pericardial fluid in one instance were 92% of the plasma level.**
Toxicity. Side effects are shown on Table 1. Nausea and vomiting were universal, starting a few hr after the beginning of the infusion. The number of vomiting episodes was variable but not severe in any case. Prochlorperazine was avoided as much as possible for fear of synergistic liver damage. Diarrhea occurred in 5 patients. In one patient, on 240 g/sq m/day, it was so severe that it required complete discontinuance of the infusion; the symptoms subsided after a few days. Two patients manifested significant liver function abnormalities. One patient experienced elevation in bilirubin, serum transaminases, and alkaline phosphatase which returned to normal 2 to 3 weeks after treatment. A second patient became jaundiced a few days before death in a setting of sepsis (see below).

Central nervous system toxicity was characterized by lethargy or insomnia, dysphoria, and, in one case, pleasurable visual hallucinations. Moderately severe hypokalemia and hypomagnesemia occurred in one patient, despite vigorous potassium and magnesium replacement via the infusion. All other patients had increased urinary losses of potassium and magnesium that could be successfully replaced with i.v. supplements.

Alopecia occurred in all 4 patients who had hair at the beginning of treatment.

One patient had a small, hemodynamically insignificant pericardial effusion when the dThd infusion was begun. It progressed to pericardial tamponade within 24 hr, requiring the placement of a drainage catheter. dThd was then restarted and rapidly escalated to tolerance without further cardiovascular trouble. As mentioned above, this patient had elevation of bilirubin, serum transaminases, and alkaline phosphatase on Day 24 of therapy and died on Day 27, probably of sepsis. At autopsy, no histological abnormalities could be demonstrated in the liver. An extensive search for leukemic cells failed to reveal any. A second patient died of sepsis 2 days after the infusion was completed. An autopsy was not performed.

Antitumor Effects. In all patients, circulating blasts disappeared during the dThd infusion; moreover, profound bone marrow hypopcellularity or aplasia was observed in each, as determined by bone marrow aspirates and microscopic examination of standard histological preparations of bone marrow biopsies.

A minor regression was documented in the patient with lymphoblastic lymphoma. The mediastinal mass regressed by more than 50% with regression evident by the fourth day of therapy. The infusion was discontinued because of the mentioned dose-limiting diarrhea and liver function abnormalities. Although the magnitude of regression of the mediastinal mass exceeded 50%, it was not sustained following discontinuation of the infusion; therefore, the patient is considered to have had only a minor response.

One patient achieved a complete remission. (Chart 2). Because of the unique nature of a dThd-induced complete remission, the case is described briefly.

Case Report. A 37-year-old male presented to an outside hospital with a history of cough for a few weeks. A chest X-ray revealed an upper mediastinal mass. The WBC count was 180 x 10^3/cu mm, almost all blasts. The bone marrow was hypercellular, with over 90% blasts. Terminal deoxynucleotidyl transferase was positive; no cell surface markers were detected. Philadelphia chromosome was not present. A diagnosis of acute lymphocytic leukemia was made. Therapy was begun with cyclophosphamide, vincristine, and prednisone. Remission was not achieved. Doxorubicin was then given, followed by doxorubicin and 1-β-D-arabinofuranosylcytosine, without benefit. On transfer to Memorial Hospital, the WBC count was 66 x 10^3/cu mm with over 90% blasts; hemoglobin was 10.9 g/dl, and the platelet...
count was $93 \times 10^3$/cu mm. The bone marrow was hypercellular with almost 90% lymphoblasts. A continuous i.v. infusion of 5-dThd was started at 105 g/sq m/24 hr. The total duration of the infusion was 29 days. The 5-dThd dose was escalated to 120, 140, 160, 180, 200, and 220 g/sq m/24 hr on Days 4, 7, 15, 17, 21, and 24, respectively. Blasts could not be found on peripheral blood after Day 14. The WBC count at that time was $1.1 \times 10^3$/cu mm, predominantly lymphocytes. Serial bone marrow aspirates showed gradual diminution in cellularity with 66% blasts on Day 13, 8% on Day 24, and 2% on Day 29, at which time the marrow was profoundly hypocellular. The mediastinal mass was not detectable after Day 14. The following side effects were observed. Polyuria, secondary to the large volumes of fluid given, ranged from 6 to 13.4 liters/day. No fluid overload resulted; diuretics were not required. Urinary potassium and magnesium losses were easily replaced. Nausea was present since Day 6, vomiting and diarrhea, since Day 20. Nocturnal visual hallucinations were noted. Two weeks after completion of the infusion, WBC and platelet counts started to rise and were normal after 6 weeks. Nineteen days after completion of the infusion, the marrow had a normal cellularity; there were 51.5% myeloid precursors, 17% erythroid precursors, 26% lymphocytes, 4% monocytes, 1.5% blasts, and a normal amount of megakaryocytes. Two additional 3-day courses of 5-dThd at 220 g/sq m/24 hr were given as maintenance, with a slight decrease in marrow cellularity. Intrathecal 1-β-d-arabinofuranosylcytosine was administered for central nervous system prophylaxis at the end of these short infusions. The remission lasted 16 weeks, with relapse in the central nervous system followed shortly by bone marrow involvement. Plasma 5-dThd levels in this patient ranged between 1.9 and 5.1 mm, with a mean of 3.3 mw for the entire course. Integrated 5-dThd exposure for this patient was estimated at 95.7 mw-days. The S-phase fraction increased during 5-dThd infusion from a base-line value of 10 to 36% on Day 13 and then dropped to 8.1% on Day 24 and 6.1% at the end of the infusion. Therapy induced a cell kill of $3.6 \log_{10} (0.13 \log/day)$. Cytokinetics. Flow cytometry and autoradiography yielded similar results, correlating on most occasions. Changes in the S-phase fraction, as measured by flow cytometry, are shown on Chart 3. In 5 of 6 cases, the S-phase fraction increased gradually for as long as 2 weeks, followed by a decrease in 4 patients despite the continuation of the infusion and increase of the dose rate of 5-dThd in some cases. Synchronous with the drop in the S-phase fraction, a decrease in the proportion of blasts in the bone marrow was seen in these 4 cases. Cytoreduction. This was measured in 5 patients; results are shown in Chart 4. The 5-dThd infusion induced a cell kill of more than 3 logs in 3 cases. The remaining had reductions of 1.7 logs and 0.7 log. However, this last patient had marked bone marrow hypocellularity on standard histological preparations at the end of the infusion, confirmed during autopsy. DISCUSSION Phase II studies of a new chemotherapeutic agent should be carried out at the highest tolerated dose. An exposure to 12 mw-days in patients with solid tumors and normal bone marrow would function will produce dose-limiting hematological toxicity. In patients with leukemia and lymphoma with bone marrow and peripheral blood involvement, increased rates of remission induction can be achieved with some cytotoxic agents by giving dosages considerably greater than those that would produce unacceptable bone marrow depression in solid tumor patients. 1-β-d-Arabinofuranosylcytosine, daunorubicin, and amsacrine are typically used in this manner in the treatment of leukemia. Based on this consideration and the observation that 1 mw 5-dThd plasma concentration was subjectively well tolerated by our patients and produced a decrease in circulating leukemic cells, we proceeded to escalate the dose of 5-dThd, seeking whether...
remission could be induced at any dose that did not produce dose-limiting toxicity.

Plasma concentrations up to 5 mw dThd were relatively well tolerated; side effects were seen, but they were not serious and were controllable. As dThd is a cycle-specific agent, it was predictable that those tissues with a high growth fraction would be predominantly affected. Discounting hematopoietic depression in leukemic patients, gastrointestinal toxicity became the dose-limiting toxicity, but not before plasma concentration was increased to 5 to 6 mw. Alopecia was also noted.

In the first days of the dThd infusion, bone marrow cells were arrested in S phase. This effect per se could account for the peripheral blast count to fall but might not be sufficient to kill a significant number of leukemic cells. As the duration of the infusion was prolonged, a significant cell loss became apparent. The bone marrow became aplastic or markedly hypocellular in all patients. There were a decrease in the proportion of blast cells and a relative increase in numbers of lymphocytes, plasma cells, and macrophages.

The patient population studied was heavily pretreated and resistant to other forms of therapy, conventional and experimental. In spite of this, in all cases, bone marrow aplasia was achieved, and in one case refractory to therapy, a complete remission was obtained.

Therapy that requires a month of continuous infusion of such massive fluid volumes is cumbersome and impractical. We would not advocate its use save for exceptional circumstances. Its cell kill rate is much slower than that of other antineoplastic agents. However, these characteristics may potentially make it useful as a modulating agent, by inducing either cytokinetic or nucleotide pool alterations.

ACKNOWLEDGMENTS

The authors thank Alvin Donner, R. Ph., for technical assistance.

REFERENCES

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