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Pharmacology of Cytarabine Given as a Continuous Infusion Followed by Mitoxantrone With and Without Amsacrine/Etoposide as Reinduction Chemotherapy for Relapsed or Refractory Pediatric Acute Myeloid Leukemia

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Background. The outcome of patients with acute myeloid leukemia (AML) who relapse or fail to achieve an initial remission has been dismal. ***Procedure.*** Fifteen pediatric patients with AML, 4 relapsed and 11 primary refractory, were reinduced with a loading bolus of 0.5 g/m^2 cytarabine (ara-C) followed immediately by a continuous infusion of ara-C (130 mg/m^2/day) for 72 hours, followed with four daily doses (12 mg/m^2/day) of mitoxantrone. Eight of 15 patients received an additional course of amsacrine and etoposide. ***Results.*** Ten of 15 (66%) achieved complete response (CR) and 3 achieved partial response (PR) (20%), with an objective response rate of 86% after ara-C/mitoxantrone. One patient died before disease assessment, and one had no response after ara-C/mitoxantrone. Pharmacokinetic studies of ara-C and ara-U were performed in 13 of 15 patients. A steady-state (C_{ss}) ara-C concentration was achieved at 2 hours after the bolus ara-C dose and was maintained up to 72 hours. The C_{ss} plasma concentrations of ara-C and ara-U averaged 10.33 ± 0.81 μM and 139.14 ± 17.8 μM, respectively. Also, cellular pharmacokinetic studies of ara-CTP were performed on circulating leukemic cells from 5 patients. Four patients who had a significant increase (P = 0.0041) in their C_{ss} ara-CTP concentrations achieved CR, whereas one patient with an insignificant increase achieved PR. ***Conclusions.*** Continuous infusion of ara-C followed by mitoxantrone is an active reinduction regimen in refractory or relapsed pediatric AML patients. The addition of amsacrine and etoposide did not improve the remission induction rate. Further studies are needed in a larger patient population to confirm these observations. Med. Pediatr. Oncol. 31:475–482, 1998.

Key words: pediatric acute myeloid leukemia; second-line chemotherapy

INTRODUCTION

Success is limited in treating children with acute myeloid leukemia (AML) who relapse or who fail the initial induction therapy. The outcome is somewhat dependent on the initial treatment and the duration of the first remission. Several active relapse regimens exist for adults and children with AML. Second remission rates of 50–60% have been reported after high dose cytarabine (cytosine arabinoside, ara-C) alone or in combination with anthracyclines, L-asparaginase, or amsacrine [1–5]. However, as the frontline AML regimens become more intensive, success of retrieval regimens may decrease.

Mitoxantrone is a synthetic antineoplastic agent in the anthracenedione class [6,7]. Responses in the range of 20–40% have been reported using mitoxantrone as a single agent in adult patients with relapsed or refractory AML [8–10]. Combinations of mitoxantrone and high-dose ara-C have shown significant activity in relapsed or refractory adult patients with AML. Using 1–3 g/m^2 of ara-C q12 hours for 6–8 doses with 5–12 mg/m^2 of mitoxantrone q24 hours for 4–5 doses, complete remission was achieved in 36–66% of adults with refractory or relapsed AML [11–16]. Reports of intermediate-dose ara-C (500 mg/m^2) in combination with anthracycline or amsacrine have demonstrated clinical responses comparable to those achieved with the conventional high-dose ara-C combinations [13–16]. The use of mitoxantrone

Abbreviations: ara-C, cytosine arabinoside; CR, complete response; PR, partial response; C_{ss}, steady state; AML, acute myeloid leukemia; CCG, Children's Cancer Group; BMT, bone marrow transplantation.

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and ara-C together is attractive because they have different mechanisms of action [17]. In vitro studies of leukemic cell lines indicate that ara-C pretreatment enhances the mitoxantrone-induced DNA damage up to 4-fold [18]. There is also evidence that repair of mitoxantrone-induced DNA damage may be inhibited by cellular accumulation of the phosphorylated ara-C active metabolite, ara-CTP [17,18].

Wells et al. [19] reported 73% complete remission with ara-C (1 g/m^2 q 12 hours × 8 doses) and 4 days of mitoxantrone (12 mg/m^2/day) in pediatric patients with AML in relapse or who had failed to achieve remission. Avramis et al. [20] showed that a minibolus of 0.5 g/m^2 cytosine arabinoside followed by a continuous infusion of 130 mg/m^2/day will achieve a steady-state concentration (C_{ss}) of 20 μM of ara-C 95% of the time in pediatric patients. Ara-C is phosphorylated in the cells by deoxycytidine kinase (dCk) to ara-CMP, which is the rate-limiting step in the cascade of activation of ara-C to ara-CTP [21]. In 1991, our group started a regimen consisting of a loading bolus followed immediately by a continuous infusion of cytosine arabinoside for 72 hours, followed with 4 daily doses of mitoxantrone. The regimen was designed to attain and maintain a C_{ss} of 20 μM in order to saturate the activation of the drug to the active metabolite ara-CTP [21]. We attempted to investigate if there is a correlation between the dCk saturation in leukemic cells before treatment and response to ara-C. We also postulated that intensive timing in relapsed or refractory patients with ara-C/mitoxantrone followed by amsacrine/etoposide may improve the remission induction rate.

PATIENTS AND METHODS

Patients

The study opened in May 1991 to patients <21 years old with AML who either failed to achieve remission (refractory) or relapsed after one or more complete remissions. Patients with extramedullary leukemia, except central nervous system (CNS) leukemia, were eligible. Patients who had previously been entered into a phase I or II study, or who had received bone marrow transplantation, were ineligible. Patients had to be off chemotherapy for at least 3 weeks and had to have recovered from the toxicity of prior therapy. Patients had to be free of active infection at the time of study entry.

At the time of study entry, all patients had normal cardiac, renal, liver, and pulmonary function and had received <350 mg/m^2 of anthracycline therapy in the past. The study was approved by the Institutional Review Board of Childrens Hospital of Los Angeles and the patient and/or parent or guardian was required to sign an approved consent form.

Treatment Protocol

All patients received a loading bolus of ara-C 0.5 g/m^2 intravenously (iv) over 1 hour. Immediately upon completion of the loading dose, continuous infusion of ara-C 130 mg/m^2/hr was initiated to achieve a steady-state concentration of 20 μM and continued for 72 hours (days 1–3). The ara-C dose was fixed as 130 mg/m^2/hour for 72 hours and was not changed based on pharmacokinetic test results. After the cessation of ara-C infusion, mitoxantrone 12 mg/m^2/day was given iv as a 30-minute infusion for 4 consecutive days (days 3–6). Intrathecal ara-C was administered on day 1 at an age-adjusted dose (<1 year, 20 mg; 1–2 years, 30 mg; 2–3 years, 50 mg; >3 years, 70 mg).

Initial intent was to treat all the patients with ara-C/mitoxantrone followed by amsacrine/etoposide. The study was initially implemented with a day-14 bone marrow examination, followed with amsacrine and etoposide starting on day 15 for 5 consecutive days. The amsarcine dose was 75 mg/m^2/day iv over 60 minutes, and etoposide 100 mg/m^2/day iv as a continuous infusion for 5 days. Granulocyte colony-stimulating factor (G-CSF) 10 μg/kg/day as a 2-hour iv infusion or subcutaneously (sc) was started on day 20 and continued until an absolute granulocyte count (AGC) of >500 was reached. Bone marrow examination was repeated on day 28 and weekly thereafter until a response could be assigned.

The first 8 patients were treated according to the above protocol; however, because of prolonged and profound neutropenia complicated by severe infections, amsacrine and etoposide were discontinued thereafter. In this modified treatment plan, G-CSF 10 μg/kg/day as a 2-hour iv infusion or sc was started on day 7. Bone marrow examination was performed on day 28 and weekly thereafter until a response could be assigned. Seven patients were treated according to the modified treatment plan.

Plasma pharmacokinetic and cellular pharmacokinetic studies were required as part of the protocol for each patient enrolled. Amsacrine was supplied by the National Cancer Institute (Bethesda, MD).

Criteria for Response and Toxicity

Complete remission (CR) was defined as an M1 bone marrow (<5% blasts) with cellularity ranging from moderately hypocellular to hypercellular and with no evidence of circulating blasts or extramedullary disease, and with recovery of peripheral counts (AGC >750/mm^3 and platelet count >75,000/mm^3). Partial remission (PR) was defined as complete disappearance of circulating blasts and achievement of M2 bone marrow status (M2A, 6–15% blasts; M2B, 16–39% blasts), with recovery of peripheral counts. Toxicity was graded by the Cancer Clinical Trials Common Toxicity Scale (Division of Cancer Treatment, National Cancer Institute, Bethesda, MD).

This is a I–IV scale, with grade IV defined as life-threatening; specific limits for each toxicity grade depend on the organ system.

Pharmacokinetics of Ara-C

Ara-C was administered as a loading bolus, followed immediately by a continuous infusion of the drug as described earlier. Adequate pharmacokinetic studies were obtained on 13 of 15 patients. Planned sampling was at 2, 24, 48, and 72 hours postbolus ara-C dose (on continuous ara-C infusion). However, in 4/13 patients, samples were obtained only at 2 and 24 hours postbolus dose of ara-C.

Tetrahydrouridine (THU) was added to the heparinized tubes to achieve a final concentration of 10 μM in whole blood. The specimens were centrifuged and the plasma was separated, labeled, and frozen at −20°C or −80°C until analysis for ara-C and ara-U by high-performance liquid chromatography (HPLC) reverse phase chromatography, as described earlier [21]. The plots of average plasma ara-C and ara-U concentrations over time were then made.

Cellular Anabolism

To determine cellular anabolism, bone marrow and/or peripheral blasts obtained before treatment were isolated by Ficoll-Hypaque (Sigma, St. Louis, MO) gradient centrifugation, and counted by a Coulter counter (Coulter Electronics Inc., Hialeah, FL), and their relative cell size was determined with the help of a Coulter Channelyzer. The cell size was used to calculate the intracellular concentrations of ara-CTP from nanomoles of ara-CTP per 10^7 blast cells. Aliquots of 1×10^7 cells were incubated in RPMI enriched with 10% fetal calf serum (FCS) in the presence of both 200 and 1,000 μM concentrations of ara-C for 1 hour at 37°C. After perchloric acid (PCA) extraction, the nucleotide and nucleoside extracts were then analyzed by HPLC, with strong anion exchange (SAX-10) column chromatography to separate the different species of phosphorylated anabolites, as described earlier [21]. This ex vivo test of sensitivity to ara-C was designed to simulate the clinical high-dose ara-C regimen administered over 1 hour. The calculated intracellular concentration of ara-CTP in micromoles was accomplished as described elsewhere [21,23–25].

HPLC Assay of Nucleotide Anabolites

The neutralized PCA extracts were assayed using a Waters Associates (Milford, MA) HPLC system, described earlier [21]. Briefly, the elution buffers were solvent A (H_2PO_4, 0.005 M, pH 2.8) and solvent B (H_2PO_4, 0.75 M, pH 3.50) at a combined flow rate of 2 ml/minute. Under these conditions, efficient separation of nucleosides/bases, and mono-, di-, and triphosphate anabolites, has been reported [21,23]. Using this technique, ara-CTP eluted in the triphosphate region 2 minutes after CTP.

RESULTS

Patients

Between May 1991–February 1996, 15 pediatric patients with AML, with age ranging from 1–17 years, who had relapsed or who had failed to achieve remission with the initial therapy (refractory), were entered onto this study. All patients were treated at the Childrens Hospital of Los Angeles. Four of 15 had relapsed AML, while the remaining 11 had refractory AML. Two of the 11 refractory patients had AML as a second malignancy (Table I). One had a peripheral primitive neuroectodermal tumor of the mandible, which was treated according to the Children's Cancer Group (CCG) study 7881 regimen B, which consisted of alternating cycles of vincristine, doxorubicin, cyclophosphamide, and ifosfamide with etoposide. Six months into this chemotherapy, he was diagnosed with AML (M4). Cytogenetics studies revealed 7q−. The second patient was initially diagnosed with a choroid plexus carcinoma of the lateral ventricle. He received chemotherapy as per CCG-9008 (''Baby POG'') following partial resection of tumor. Chemotherapy consisted of five cycles of induction chemotherapy with cyclophosphamide, cisplatin, etoposide, and vincristine, followed by eight cycles of maintenance with alternating courses of carboplatin, etoposide, vincristine, and cyclophosphomide with etoposide. Eleven months after the end of chemotherapy, pancytopenia was noted. Bone marrow aspirate and biopsies revealed myelodysplastic syndrome (MDS) with 5q− and abnormalities of chromosomes 9, 15, 19, and 22. MDS progressed to AML (M7) over a period of 2 months. Both patients failed to achieve remission with the CCG frontline regimen for AML (CCG-2891) [22]. Patients on CCG-2891 were randomized at diagnosis to one of two induction approaches involving a 4-day cycle of five drugs, including dexamethasone, cytarabine, thioguanine, etoposide, and rubidomycin (DCTER), with a second cycle administered either 10 days after the first cycle despite low or dropping blood counts, or 14 days or later from the beginning of the first cycle, depending on bone marrow status. Patients achieving remission then received consolidation treatment. Patients with an HLA-identical sibling underwent allogeneic bone marrow transplantation (BMT), and patients without a match were randomly assigned to either autologous BMT or continuing conventional chemotherapy. All 11 refractory patients failed to achieve a remission with the CCG-2891 induction, i.e., two cycles of DCTER. One of 11 refractory patients received a cycle of reinduction with fludarabine phosphate and ara-C after failing to achieve a remission with CCG-2891 induction. However, he remained refractory.

All 4 relapsed patients were initially treated according to CCG-2891 protocol. Of the 4 relapsed patients, one completed the conventional chemotherapy, and relapsed

TABLE I. Patient Characteristics, Remission Induction, and Nonhematologic Toxicity

Patient no.	Age (years)	Type of AML	Cytogenetics	Remission status after Ara-C/ mitoxantr	Remission status after amsacrine/ etop	Toxicity after Ara-C/mitoxantrone	Toxicity after amsacrine/etoposide
1[a]	3	M1	t(8;21)(q22:q22)	CR[b]	CR	Grade III infection (fever/neutropenia)	Grade IV infection, *Pseudomonas putida*
2[c]	14	M2	t(13q;15q),t(7;16)	PR[d]	NE[e]	Grade III infection (fever/neutropenia) Grade II mucositis; grade II nausea and vomiting; grade I transaminitis	Grade IV infection, alpha-strep: sepsis followed by death secondary to disseminated candidiasis
3[a]	1	M4	NA[f]	CR	PD[g]	Grade IV infection, group D enterococcus sepsis	Grade III infection (fever/neutropenia)
4[c]	13	M2	t(1;10;11)(p36; p13;q21)	No response	PD	Grade III infection (fever/neutropenia)	Grade IV bilirubin Grade IV infection, died of disseminated aspergillosis
5[c]	17	M5a	No clonal abnormality	CR	CR	Focal seizure	Grade IV infection, died of group D enterococcus sepsis and meningitis
6[c]	4	M7	NA	PR	PR	Grade III transaminitis Grade II diarrhea	None
7[c]	14	M1	No clonal abnormality identified	CR	NA[h]	Grade IV infection, disseminated candida tropicalis; grade IV azotemia; grade III pericardial effusion; grade II sensory impairment; died of pulmonary aspergillosis	Not applicable
8[c]	7	MI	t(8;10;21)	CR	CR	Grade IV stomatitis (herpes simplex detected); grade III hyperglycemia	Asymptomatic central venous catheter colonization with Gram(−) rods (no ID)
9[c]	13	M2	t(8;21)(q22;q22)	CR	NA	Grade III infection (fever/neutropenia)	Not applicable
10[c]	1	M5a	NA	CR	NA	Grade IV transaminitis; grade IV stomatitis; grade IV infection-alpha strep. meningitis	Not applicable
11[i]	2	M4	del(18)t(11;18) (q13;q21)	PR	NA	Grade III cardiac-congestive heart failure, which responded to digoxin	Not applicable
12[i]	7	M7	del5(q12;q33), t(9;19)(q11;p13), t(9;15)(q11,p11)	CR	NA	Grade III infection (fever/neutropenia); grade III emeses; grade IV diarrhea	Not applicable
13[a]	11	M4	t(6;21)(p22;q22)	CR	NA	Grade IV infection, alpha strep. sepsis	Not applicable
14[c]	5	M4	del(9q)(q22;q34)	CR	NA	Grade IV infection, *E. coli* sepsis	Not applicable
15[a]	14	M1	−7	NE	NA	Grade IV infection, Gram (−) rods (no ID); died of filamentous fungus (*Acremonium*) in the lungs	Not applicable

[a]Relapsed AML.
[b]CR, complete remission.
[c]Refractory AML.
[d]PR, partial remission.
[e]NE, not evaluable, died before disease assessment.
[f]NA, cytogenetic result not available.
[g]PD, progressive disease.
[h]NA, not applicable, did not receive amsacrine/etoposide.
[i]Patient initially had primitive neuroectodermal tumor (PNET) of the mandible, developed AML 6 months into PNET chemotherapy (see text).
[j]Patient initially diagnosed with choroid plexus carcinoma, developed MDS 11 months off chemotherapy for brain tumor, which evolved to AML within 2 months (see text).

2 months off therapy. The second patient relapsed while on therapy 4 months after diagnosis. The third patient relapsed following the intensification (consolidation) phase of CCG-2891, and failed to achieve remission with fludarabine phosphate and ara-C reinduction. The fourth patient achieved a remission on CCG-2891 after four cycles of induction chemotherapy. However, therapy was discontinued because of fungal sepsis with *Candida tropicalis.* He relapsed 3 months later and failed to respond after two courses of fludarabine and ara-C. He then received two courses of ifosfamide and etoposide, and achieved a PR which lasted only 1 month. Therefore, all patients had had intensive prior treatments including ara-C.

The first 8 patients were entered into the study with the intention of treatment with ara-C and mitoxantrone, followed by amsacrine and etoposide. However, one patient (patient identification no. 7) expired of infection after ara-C and mitoxantrone. Only 2 of 7 patients (2 and 4) received amsacrine and etoposide on time, i.e., on day 15 as planned. In 5 of 7 patients, the amsacrine/etoposide course was delayed because of intercurrent complications. The delays were 3, 4, 19, 24, and 25 days.

Nonhematologic Toxicity

The major toxicities seen during this therapy were infection and myelosuppression. Eleven of 15 patients (73%) had documented life-threatening (grade IV) infections. Five patients died of infection, 2 of disseminated aspergillus, 1 of disseminated candidiasis, 1 of filamentous fungus (*Acremonium*) of the lungs, and 1 of enterococcus sepsis/meningitis. Four of 11 life-threatening infections were caused by streptococci. One patient developed congestive heart failure. This patient had a cumulative dose of 330 mg/m^2 anthracycline prior to entering the study. He quickly responded to digoxin. Another patients developed grade III pericardial effusion, which resolved without intervention. There were three instances of grade III or IV hepatic toxicities, three gastrointestinal tract, one pulmonary, one renal toxicities, and one hyperglycemia (Table I).

Hematologic Toxicity

All patients developed grade 3–4 myelosuppression. For the 8 patients who received ara-C and mitoxantrone followed by amsacrine and etoposide, the mean and median times of duration of white cell count <1,000/mm^3 were 35.6 and 35 days, respectively (range, 24–50 days). The mean and median times of duration of absolute neutrophil count of <500/mm^3 were 47 and 44.5 days, respectively (range, 30–62 days). The mean and median times of duration of platelet count of <50,000/mm^3 were 38.8 and 38.5 days, respectively (range, 23–62). For the 7 patients who received only ara-C and mitoxantrone, the mean and median times of duration of white cell count <1,000/mm^3 were 25.3 and 26 days, respectively (range, 17–37 days). The mean and median times of duration of absolute neutrophil count of <500/mm^3 were 28 and 29 days, respectively (range, 17–37 days). The mean and median times of duration of platelet count of <50,000/mm^3 were 43 and 52 days, respectively (range, 13–never >50,000/mm^3 during the study period).

Remission Induction

Of the first 8 patients who were intended to be treated with ara-C and mitoxantrone followed by amsacrine and etoposide, 5 achieved CR, 2 achieved PR, and 1 showed no response after ara-C/mitoxantrone (Table I). Of the 7 patients who received only ara-C and mitoxantrone, 5 achieved CR, 1 achieved PR, and 1 died of infection before disease assessment. Therefore, of the 15 patients, 10 (66%) achieved CR, and 3 PR (20%), after ara-C/mitoxantrone. Hence, the objective response rate (CR plus PR) of ara-C/mitoxantrone was 86%.

Of the 10 patients who achieved CR after ara-C/mitoxantrone, one progressed after amsacrine/etoposide (patient 3). The patient developed group D enterococcus sepsis after ara-C/mitoxantrone and could not receive amsacrine/etoposide as planned. He received amsacrine/etoposide on day 38 when he was in CR. Of the remaining 9 CR patients, 1 died of infection before BMT, 4 developed progressive disease while waiting for BMT, and 4 underwent BMT. Three of 4 who underwent BMT relapsed after BMT. Therefore, only 1 of 15 patients (patient 8) became a long-term leukemia-free survivor following an allogeneic bone marrow transplantation.

Pharmacokinetic (PK) Studies of Ara-C and Ara-U

Adequate pharmacokinetic specimens for PK studies were obtained from 13 of 15 patients. The average data of ara-C and ara-U are shown in Figure 1. This graph depicts the means of ara-C and ara-U concentrations of these 13 patients ± standard deviations (SD) for the 2- and 24-hour time points, and of 9 patients ± SD for the 48- and 72-hour time points. Steady-state concentrations (C_{ss}) of ara-C were achieved by 2 hours after the loading bolus plus continuous infusion, and this C_{ss} was maintained on average for up to 72 hours (Fig. 1). The C_{ss} plasma concentrations of ara-C and ara-U averaged 10.3 ± 0.8 μM (mean of means) and 139.1 ± 17.8 μM, respectively. One individual patient's plasma levels at 12, 36, and 60 hours were slightly lower than these averages (data not shown).

Cellular Pharmacokinetic Studies of Ara-CTP

The pretreatment (control-sensitivity evaluation to ara-C) cellular concentrations of ara-CTP were determined in leukemic blasts in 10 of 15 patients. The formation of cellular concentrations of ara-CTP by peripheral blast cells at 24 hours after initiation of ara-C infu-

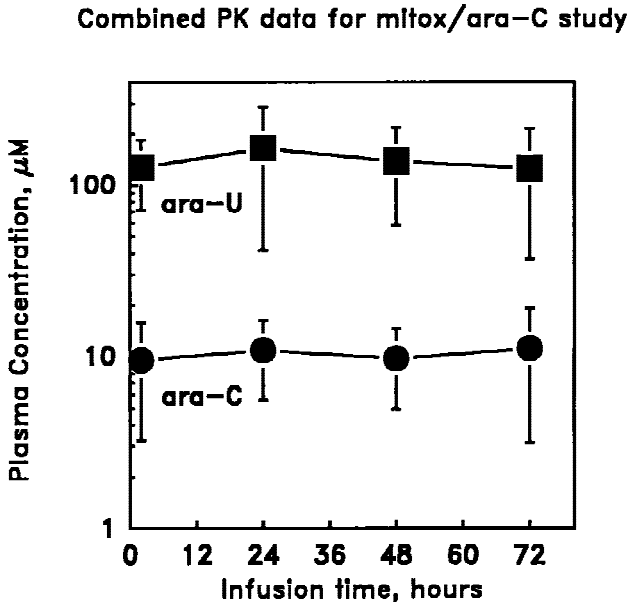

Fig. 1. Mean ara-C and ara-U concentrations from 13 patients with standard deviations.

sion in 5 of 10 patients is shown in Figure 2. Five additional patients from whom we had obtained pretreatment leukemic blast cells had an insufficient number of circulating blast cells at 24 hours for intracellular ara-CTP determination. The average pretreatment ara-CTP concentration (control sensitivity to ara-C) was 160.8 ± 82.4 μM (mean, n = 10 ± SD), which measures the initial rate for ara-C phosphorylation to its active anabolite, ara-CTP, which is approximately the peak ara-CTP concentration after the high-dose ara-C regimen of 3 g/m^2 infused over 1 hour. The average 24-hour post-ara-C administration ara-CTP concentration was 474.7 ± 270.1 μM (mean, n = 5 ± SD), which was statistically significant ($P = 0.0041$). The increased cellular ara-CTP concentration at 24 hours post-ara-C infusion could be attributed to the C_{ss} of ara-C and the continuous ara-CTP formation and accumulation by the leukemic blasts.

Patients 1, 3, 5, and 12 achieved CR after the ara-C/mitoxantrone regimen, and they had a significant increase in their steady-state ara-CTP cellular concentrations when compared to control-sensitivity values (Fig. 2). Patient 6, who had only an insignificant increase of ara-CTP over the control parameter, achieved PR. Among the 5 additional patients from whom pretreatment leukemic blasts were obtained but who had an insufficient number of peripheral blasts at 24 hours, 3 achieved a CR, 1 achieved PR, and 1 died before disease assessment (patient 15).

DISCUSSION

The survival of patients with AML who relapse or fail to achieve an initial remission has been very poor. Woods et al. [22] recently reported the success rate of time-sequential induction therapy for newly diagnosed pediatric AML patients (CCG-2891). Remission induction was achieved in 75% and 70% of patients randomized to intensive timing and standard timing, respectively. Fourteen percent and 26% of patients on intensive and standard timing induction failed to achieve remission (refractory), respectively. Eleven percent and 4% of patients on intensive and standard timing died during the induction treatment, respectively. Although the CCG has not conducted a formal study of refractory or relapsed patients on CCG-frontline regimens, a review of the last 10 years shows a dismal outcome. Following relapse, a 2-year survival of 7–17% was reported among patients who were treated on CCG front-line protocols (Wells and Lee, personal communication). Our study describes the outcome of patients who were either refractory or who relapsed following the initial treatment with CCG-2891.

Most published reinduction regimens have a CR rate of about 50% for relapsed or refractory patients. So far, the best reinduction CR rate has been that reported by Wells et al. [19] of 73%, using an ara-C/mitoxantrone regimen in relapsed or refractory pediatric AML patients. They used ara-C 1 g/m^2 q 12 hours for 8 doses, followed by 4 days of mitoxantrone (12 mg/m^2/day), whereas we chose to administer a minibolus followed by 72 hours of continuous infusion of ara-C before mitoxantrone. In Wells et al. [19], most patients received two courses of the regimen, although very few patients who failed to

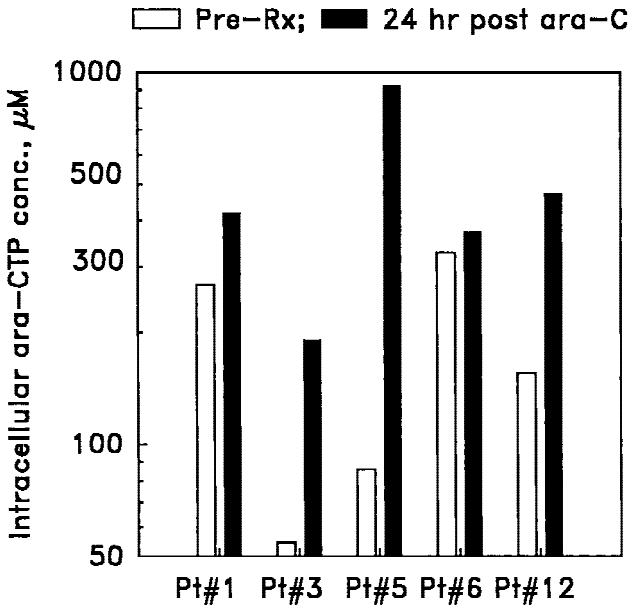

Fig. 2. Intracellular concentrations of ara-CTP in leukemic blasts before and at 24 hours of ara-C treatment in 5 patients. Pretreatment ara-CTP concentration stands for an ex vivo peak ara-CTP concentration in patients' leukemic cells after an exposure to simulate the 3 g/m^2 ara-C infusion over 1 hour, whereas the 24-hour values refer to the actual ara-CTP accumulation in blasts in vivo.

achieve CR after the first course achieved CR after the second course. However, our results in a smaller group of patients, compared to those of Wells et al. [19], represent a significant improvement compared to the historical data. These two reports on pediatric patients have clearly established ara-C/mitoxantrone as a very effective retrieval regimen in refractory or relapsed AML patients.

Our attempt to deliver two regimens in an intensive timing fashion, i.e., ara-C/mitoxantrone followed by amsacrine/etoposide on day 15, was not successful because of the toxicity encountered. We were successful in delivering amsacrine/etoposide on day 15, as planned, in only 2 of 8 patients. One patient who achieved PR after ara-C/mitoxantrone remained as PR after amsacrine/etoposide, and another who achieved CR after ara-C/mitoxantrone progressed on the amsacrine/etoposide regimen. Single-agent studies of amsacrine in recurrent AML have demonstrated response rates of 25–30% [26,27]. In addition, complete responses were seen in 18–47% of refractory or recurrent AML patients who were treated with amsacrine and etoposide combination [28,29]. Based on these results, we postulated that intensive timing of amsacrine/etoposide following ara-C/mitoxantrone may improve the remission induction rate. However, we found out that the scheduling is not feasible in this setting. Even if we were successful in delivering amsacrine/etoposide as planned, it would have been difficult to sort out the contribution of amsacrine/etoposide, given the relatively high objective response rate observed with the combination of ara-C/mitoxantrone, which was obviously not known when the study was initiated.

The importance of a loading bolus followed by continuous ara-C infusion is that we can attain and maintain a steady-state concentration of ara-C in these pretreated AML patients [20]. This may be of paramount significance in that we avoid the peak and trough levels of ara-C over time as it occurs with the intermittent schedule of high-dose ara-C, a fluctuation of the prodrug that can adversely influence the production and accumulation of the active anabolite, ara-CTP, in the leukemic cells [21]. The ex vivo control sensitivity of ara-C to ara-CTP test attempted to provide us with a measure of the cellular concentrations of ara-CTP that would have been accumulated in these blast cells after a classical high-dose ara-C regimen of 3 g/m^2 infused over 1 hour. Under these conditions, the plasma ara-C would be in the range of 120–200 μM, as we have shown in earlier studies in pediatric patients treated with this regimen [20,21,23]. The advantage of the loading bolus followed by continuous infusion is that it eliminates the high peak and low troughs of this rapidly eliminated prodrug from the plasma [20,21], and it provides a constant C_{ss} concentration so that all unsynchronized leukemic cells would be exposed to a sufficient concentration of the drug at a level at or near the saturation concentration for optimal activation [20]. Although our target level for C_{ss} of ara-C was 20 μM, the achieved level averaged 10.3 μM. This is most likely because of previous exposure to ara-C. Patients who are heavily pretreated with regimens, including ara-C, have an upregulation of the liver and body cytidine/deoxycytidine deaminase(s) [20,25].

The control cellular ara-CTP concentration, which is the concentration observed after a high-dose ara-C regimen of 3 g/m^2, averaged 160.8 ± 82.4 μM, and 24 hours after the loading bolus plus continuous infusion it averaged 474.7 ± 270.1 μM, a value which was statistically significant ($P = 0.0041$). Thus, the data suggest that, given a constant supply of the substrate of ara-C at C_{ss} in the plasma, the leukemic blast cells accumulate a significantly higher concentration by 24 hours. We have determined in other clinical studies that a strong association exists between high cellular concentrations of ara-CTP and the rapid elimination of leukemic blasts from blood. This, in turn, has been correlated with improved clinical response [21,23].

Further studies are needed to verify these encouraging clinical and cellular pharmacokinetic results. The combination of ara-C/mitoxantrone should be incorporated and tested in not only relapsed and refractory patients, but also in children with newly diagnosed AML.

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REFERENCES

1. Champlin R, Gale RP: Acute myelogenous leukemia: Recent advances in therapy. Blood 69:1551–1562, 1987.
2. Wells RJ, Feusner J, Devney R, et al.: Sequential high-dose cytosine arabinoside-asparaginase treatment in advanced childhood leukemia. J Clin Oncol 3:998–1004, 1985.
3. Herzig RH, Wolff SN, Lazarus HM, et al.: High-dose cytosine arabinoside therapy for refractory leukemia. Blood 62:361–369, 1983.
4. Herzig RH, Lazarus HM, Wolff SN, et al.: High-dose cytosine arabinoside therapy with and without anthracycline antibiotics for remission reinduction of acute nonlymphoblastic leukemia. J Clin Oncol 3:992–997, 1985.
5. Hines JD, Oken MM, Mazza JJ, et al.: High-dose cytosine arabinoside and m-AMSA is effective therapy in relapsed acute nonlymphocytic leukemia. J Clin Oncol 2:545–549, 1984.
6. Shenkenberg TD, VonHoff DD: Mitoxantrone: A new anticancer drug with significant clinical activity. Ann Intern Med 105:67–81, 1986.
7. Le Maistre CF, Herzig R: Mitoxantrone: Potential for use in intensive therapy. Semin Oncol [Suppl] 17:43–48, 1990.
8. Estey EH, Keating MJ, McCredie KB, et al.: Phase II trial of mitoxantrone in refractory acute leukemia. Cancer Treat Rep 67: 389–390, 1983.

9. Arlin ZA, Silver R, Cassileth P, et al.: Phase I–II trial of mitoxantrone in acute leukemia. Cancer Treat Rep 69:61–64, 1984.
10. Pauciucci PA, Cuttner J, Holland JF: Mitoxantrone in patients with refractory acute leukemia. Semin Oncol [Suppl] 11:36–40, 1984.
11. Hiddemann W, Kreutzmann H, Straif K, et al.: High-dose cytosine arabinoside and mitoxantrone: A highly effective regimen in refractory acute myeloid leukemia. Blood 69:744–749, 1987.
12. Walters RS, Kantarjian HM, Keating MJ, et al.: Mitoxantrone and high-dose cytosine arabinoside in refractory acute myelogenous leukemia. Cancer 62:677–682, 1988.
13. Sanz MA, Martinez D, Borrego D, et al.: High-dose cytosine arabinoside and mitoxantrone in high-risk acute nonlymphoblastic leukemia. Semin Oncol [Suppl] 14:18–20, 1987.
14. Brito-Babapulle F, Catovsky D, Newland AC, et al.: Treatment of acute myeloid leukemia with intermediate-dose cytosine arabinoside and mitoxantrone. Semin Oncol [Suppl] 14:51–52, 1987.
15. Lejeune C, Tubiana N, Gastaut JA, et al.: High-dose cytosine arabinoside and mitoxantrone in previously treated acute leukemia patients. Eur J Haematol 44:240–243, 1990.
16. Amadori S, Meloni G, Petti MC, et al.: Phase II trial of intermediate dose ara-c with sequential mitoxantrone in acute myelogenous leukemia. Leukemia 3:112–114, 1989.
17. Ritter J, Creutzig U, Henze G, et al.: Hochdosiertes ARA-C in Kombination mit Mitoxantrone bei der Therapies der AML im Kindersalter. Erste Ergebnisse der AML-Rezidivstudie BFM-85. Onkologie 10:24–27, 1987.
18. Heinemann V, Murray D, Adams T, et al.: Mitoxantrone-induced DNA damage in leukemic cells is enhanced by treatment with high-dose arabinosylcytosine. Proc Am Soc Clin Oncol 6:113, 1987 [abstract].
19. Wells RJ, Gold SH, Krill CE, et al.: Cytosine arabinoside and mitoxantrone induction chemotherapy followed by bone marrow transplantation or chemotherapy for relapsed or refractory pediatric acute myeloid leukemia. Leukemia 8:1626–1630, 1994.
20. Avramis VI, Weinberg KI, Sato JK, et al.: Pharmacology of cytosine arabinoside (ara-C) in pediatric patients with leukemia and lymphoma after a biochemically optimal region of loading bolus plus continuous infusion of ara-C. Cancer Res 49:241–247, 1989.
21. Avramis VI, Biener R, Krailo M, et al.: Biochemical pharmacology of high-dose cytosine arabinoside (HDara-C) in childhood acute leukemia. Cancer Res 47:6786–6792, 1987.
22. Woods WG, Kobrinsky N, Buckley JD, et al.: Timed-sequential induction therapy improves postremission outcome in acute myeloid leukemia: A report from the Children's Cancer Group. Blood 87:4979–4989, 1996.
23. Ramilo-Torno LV, Avramis VI: Continuous infusion cytosine arabinoside compound by high dose and conventional dose therapy in pediatric patients with relapsed leukemias. J Infusional Chemother 4:9–18, 1994.
24. Ramilo-Torno L, Avramis VI: Intracellular pharmacodynamic studies of the synergistic combination of 6-mercaptopurine (6-MP) and cytosine arabinoside (ara-C) in human leukemia cell lines (CCRF/CEM). Cancer Chemother Pharmacol 35:191–199, 1995.
25. Avramis VI, Mecum RA, Nyce J, et al.: Pharmacodynamics, DNA methylation studies and clinical response of high-dose 1-beta-D-arabinofuranosylcytosine before and after in vivo 5-azacytidine treatment in pediatric patients with refarctory acute leukemia. Cancer Chemother Pharmacol 24:203–210, 1989.
26. Louie AC, Issell BF: Amsacrine (AMSA)—A clinical review. J Clin Oncol 3:562–592, 1985.
27. Krischer J, Land V, Civin C, et al.: Evaluation of AMSA in children with acute leukemia: A Pediatric Oncology Group study. Cancer 54:207–219, 1984.
28. Bishop JF: Etoposide in the treatment of leukemia. Semin Oncol 19:33–38, 1992.
29. Steuber CP, Krischer J, Holbrook T, et al.: Therapy of refractory or recurrent childhood acute myeloid leukemia using amsacrine and etoposide with or without azacytidine: A Pediatric Oncology Group randomized phase II study. J Clin Oncol 14:1521–1525, 1996.