Pharmacokinetic and Pharmacodynamic Studies of Fludarabine and Cytosine Arabinoside Administered as Loading Boluses followed by Continuous Infusions after a Phase I/II Study in Pediatric Patients with Relapsed Leukemias

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ABSTRACT

The sequential administration of fludarabine followed by cytosine arabinoside (ara-C) has demonstrated significant synergistic effects against the CEM human leukemic cell line. This in vitro synergism was investigated in a Phase I trial in pediatric patients with relapsed acute leukemia. The optimum concentrations of 9-β-d-arabinofuranosyl 2-fluoroadenine and ara-C necessary to achieve significant drug synergism from in vitro studies were between 10 and 20 μM. Fludarabine was infused at a dose to attain a target plasma concentration of 10 μM for 48 h, followed by a continuous infusion of escalated ara-C doses to maintain plasma ara-C concentrations of 10, 12.5, 15, or 17.5 μM for 72 h. Thirteen patients with acute lymphocytic leukemia and 18 with acute myelocytic leukemia were entered into the study, 30 of whom were clinically evaluable for toxicity. Pharmacokinetic and pharmacodynamic studies were performed on specimens from 20 patients. The optimal 9-β-d-arabinofuranosyl 2-fluoroadenine and ara-C concentrations in plasma were easily achieved after continuous infusion regimens of both drugs. Cellular ara-CTP was augmented 5- to 8-fold in leukemic cells from patients receiving fludarabine phosphate treatment followed by ara-C. The maximum tolerated plasma concentrations for this combination regimen was 10 μM fludarabine for 48 h followed by 72 h of 15 μM ara-C, which were achieved at dose level 3. A significant number of responses were also seen. Nine of 18 evaluable patients (50%) with acute myelocytic leukemia achieved complete or partial responses, and 3 of 9 evaluable patients with acute lymphocytic leukemia achieved complete or partial responses. Fludarabine and ara-C successfully eradicated bone marrow disease in 16 of 27 patients (59%), 23 patients of which had been treated previously with high-dose ara-C. These results verified the synergistic effect fludarabine exhibited in augmenting ara-CTP concentrations in patients' leukemic blasts, thus improving the clinical response in relapsed pediatric leukemias.

INTRODUCTION

The metabolism of the nucleoside analogue prodrugs F-araAMP and ara-C has been studied extensively in vitro and in vivo. F-araAMP, the soluble anabolite of F-araA, has been chosen for clinical trials because of its aqueous solubility and rapid hydrolysis of F-araAMP in plasma, which is transported into the cells where it is phosphorylated by cytosolic dCK to the pharmacologically active anabolite. This anabolite is then phosphorylated further by other nucleoside kinases, dCMP kinase and NDP kinase, to the pharmacologically active anabolite F-araATP.

In addition to the direct inhibitory effect F-araATP has on DNA polymerases and its subsequent inhibition of DNA replication, it is also a potent inhibitor of RR. RR catalyzes the key conversion step in the production of deoxyri-
bonucleoside diphosphates, which in turn are phosphorylated further to the respective triphosphate (dNTP) pools. The dNTP pools, the building blocks of DNA that are absolutely necessary for DNA replication, also exert significant feedback inhibition on their respective nucleoside kinases, including dCK (15–18). Hence, inhibition of the RR-mediated reduction step in the cascade of production of dNTPs not only reduces the cellular concentrations of the natural substrates for DNA polymerases but also has an indirect effect in increasing dCK-specific activity. Furthermore, recent studies of dNTPs not only reduce the cellular concentrations of the building blocks of DNA that are absolutely necessary for DNA replication, also exert significant feedback inhibition on their respective nucleoside kinases, including dCK (15–18). Incorporation of fludarabine anabolite into DNA causes irreversible DNA damage, thus forcing the leukemic cells to undergo apoptosis (19, 20).

Because the i.v. administration of F-araAMP as bolus or infusion has been associated with unpredictable neurological toxicity in adult studies (21–24), we investigated the LB followed by a Clinf regimen for 5 days of F-araAMP as a Phase I/I study in pediatric patients with relapsed leukemias in the CCG (6). This regimen clearly showed that we can achieve and maintain plateau plasma concentrations in the therapeutic range of 5–10 μM at similar safe daily dose ranges used in adults (6, 23). The CCG study also showed that this regimen of F-araAMP has potential antileukemic activity in pediatric patients with relapsed ALL (6).

ara-C has been the cornerstone of successful antileukemic regimens for leukemias in relapse in both adult and pediatric patients (7–11, 25, 26). The cytotoxic activity of ara-C is exerted by the phosphorylation/activation and accumulation to its active triphosphate anabolite in leukemic cells (7, 10, 26). Both F-araAMP and ara-C are activated by phosphorylation to their triphosphate anabolites, F-araATP and ara-CTP (2, 4, 7). The rate-limiting step of the anabolic pathways for both drugs is catalyzed by dCK, which phosphorylates the nucleoside to the respective monophosphates (7, 14, 27–29).

Following the demonstration by many investigators that selective combinations of purine and pyrimidine nucleoside analogue drugs achieve drug synergism in vitro and in vivo studies against leukemic cells (17, 18, 30), we proceeded to test this hypothesis ex vivo in leukemic cells obtained from pediatric patients who were receiving fludarabine phosphate in a Phase I/I study and in vitro in leukemic cell lines (6, 28, 31). Drug synergism is defined as a statistically significant improvement in pharmacological or cytotoxic action, when two or more drugs are used in combination, over the effect of either agent alone (28).

Studies in circulating leukemic blast cells from patients who were receiving LB + Clinf F-araAMP for 5 days indicated that the antileukemic effect is probably due to the cellular accumulation of F-araATP, which has a prolonged half-life greater than 12 h in these cells (6). The leukemic cells were not affected for the first 2 days of infusion when the cellular concentrations of F-araATP were relatively low; however, they were killed in a log-linear fashion many days after the end of the Clinf.

In addition, the determination of the minimum concentrations of F-araA and ara-C to obtain drug synergism has been evaluated in vitro by using human leukemia cells CCRF/CEM (31, 32). The combination regimen from these studies showed an approximate 5-fold drug synergism in CEM/0 cells, but it did not appear to be synergistic when the sequence of the drugs was reversed.

Furthermore, when leukemic blast cells from patients treated with F-araAMP were tested ex vivo with ara-C, a significant level of drug synergism was observed that was time dependent, thus warranting clinical testing of a sequence-specific combination of these two drugs (6, 28, 31). We present here the pharmacodynamic results from the CCG-0895 clinical trial in pediatric patients with relapsed leukemias.

MATERIALS AND METHODS

Materials. Fludarabine phosphate as lyophilized powder was provided by the National Cancer Institute for the Phase I/I studies. Cytosar-U (ara-C) was purchased from Upjohn Pharmaceutical Co. (Kalamazoo, MI). All other reagents and chemicals for extraction and HPLC assays were of analytical or HPLC quality.

Patient Characteristics. Thirty-one pediatric patients, 13 with ALL and 18 with AML, were entered onto the CCG Phase I/I study CCG-0895 (33). Thirty patients were eligible; 27 were evaluable for response, and biological specimens from 20 patients permitted the assessment of pharmacodynamics or pharmacokinetics of fludarabine and ara-C. The patients’ ages ranged from 1 to 19 years, with a median study entry age of 8 years. Twenty patients had previously received high-dose ara-C. All patients were treated in concordance with clinical protocols approved by the Institutional Review Boards of the CCG institutions at which patients were treated, and informed consent from the patient and/or patients’ parents were obtained before entry into the study.

Treatment Plan and Protocol Design. The CCG-0895 clinical protocol required that F-araAMP be administered as a LB of 10.5 mg/m² over 15 min, followed immediately by a Clinf regimen at 30.5 mg/m²×h × 48 h. These doses were kept constant in all patients and were the same as those reported previously (6). This dose produced an average F-araA plasma concentration of 8–10 μM. The shorter duration of infusion, two versus five days, was selected because ex vivo and in vitro studies showed that this exposure time was sufficient to maximally augment ara-C anabolism after fludarabine and because of the nontoxicity condition that was seen after 2 days of F-araAMP infusion in the Phase I/I trial (6, 28, 31).

The ara-C dose was also administered as a biochemically optimal LB + Clinf (28, 33–35). The dose was escalated with increasing dose levels as follows: dose level 1, 250 mg/m² of LB for 15 min and 65 mg/m²×h of Clinf for 72 h; dose level 2, 312 mg/m² of LB for 15 min and 81 mg/m²×h of Clinf for 72 h; dose level 3, 390 mg/m² of LB for 15 min and 101 mg/m²×h of Clinf for 72 h; and dose level 4, 487 mg/m² of LB for 15 min and 126 mg/m²×h of Clinf for 72 h.

In earlier studies, these doses were shown to produce plasma concentrations of 10, 12.5, 15, or 17.5 μM ara-C, respectively (33, 34). A fifth dose level was designed to achieve 20 μM ara-C but was not used.

Pharmacology Studies. Because detailed plasma pharmacokinetic studies have been determined for both F-araAMP and ara-C after LB + Clinf, a limited number of plasma speci-
Mens were obtained to verify the achieved plasma concentrations in this study. Plasma specimens were obtained at 24 and 48 h after F-araAMP infusion, and six additional specimens were drawn every 12 h during the ara-C administration, as shown in the protocol diagram depicted in Fig. 1.

Two bone marrow aspirates were obtained, one before F-araAMP treatment (control) and the second one 24 h after ara-C infusion had begun (72 h after the start of F-araAMP infusion; Ref. 33). All blood specimens were heparinized, placed in an ice bath, and centrifuged immediately to separate the plasma. In the test tubes used for the ara-C specimens, 10 μL of sterile THU were added for a final concentration of THU of 10 μM in 5 mL of blood. THU was added to all test tubes for specimens from all participating CCG institutions. This was done to inhibit cytosine deaminase in plasma specimens; thus, the assayed ara-C concentrations reflected realistic determinations of the patients' ara-C plasma levels.

Bone marrow aspirates were obtained under local anesthesia from patients treated at Childrens Hospital Los Angeles and other institutions from the Los Angeles basin (Millers Childrens Hospital-Harbor/UCLA, Long Beach, CA), where they were placed in heparinized tubes and placed in an ice bath. This limitation was applied because the blast cells were separated, extracted, and tested within 1–2 h after the specimens were obtained from the patients, independent of the time the specimens were drawn. This was done to maintain intact enzymatic activities of the leukemic cells. The pretreatment specimens were tested ex vivo with two concentrations of ara-C, 200 μM and 1 mM, for 1 h, and the cells were extracted with perchloric acid for ara-CTP determination. In similar studies, we have shown that, under these conditions of saturation of ara-C activation by cellular dCK, we obtained linearly related (1:1 ratio) intracellular concentrations of ara-CTP during cell accumulation at the same time compared to in vivo (35).

**Evaluation of Response and Clinical Toxicity.** Criteria for response: A CR was considered to have occurred in any of the following circumstances: (a) the patient had an M1 marrow with recovery of peripheral counts (ANC ≥1,000/mm³ and platelet counts ≥100,000/mm³); or (b) the patient had an M1 marrow without the recovery of peripheral counts prior to pretreatment with fludarabine and ara-C.

A PR was considered to have occurred in any of the following circumstances: (a) the patient had an M2 marrow with recovery of peripheral counts (ANC ≥1,000/mm³ and platelet counts ≥100,000/mm³); or (b) the patient had an M1 marrow without the recovery of peripheral counts prior to pretreatment with fludarabine and ara-C. Toxicity was graded according to the Common Toxicity Scale of the Division of Cancer Treatment of the National Cancer Institute. This is a I–IV scale, with IV defined as life-threatening; specific limits for each toxicity grade depend on the organ system.

**HPLC Assay for Nucleosides, F-araA, ara-C, and ara-U.** All nucleoside analogues were separated by HPLC (Waters Associates, Milford, MA) using a μC18 reverse phase column (Waters Associates) and a 5% methanol in 500 mM ammonium acetate (pH 6.5) elution buffer isocratically as reported earlier (2, 6, 11). The results are presented as the arithmetic mean drug concentrations ± SD. The pharmacokinetic evaluation was performed using the computer programs and equations developed for LB + CInf as described earlier (6, 11).

**DNA Synthetic Capacity.** To determine DSC in leukemic blast cells, the amount of tritiated thymidine incorporation was determined in the cellular DNA via a scintillation counter in cells exposed to one or both of the drugs. The results were expressed as a percentage of control of untreated cells, as reported earlier (2, 6, 11, 34, 35).

**HPLC Assay of Nucleotide Anabolites.** To determine the cellular anabolite concentrations of ara-CTP and F-araATP, nucleotides were extracted from leukemic cells using 0.4 M perchloric acid as described elsewhere (2, 6, 11). ara-CTP eluted in the triphosphate region 2 min after CTP, and F-araATP eluted 3 min after ATP and 3 min before GTP (2, 6, 11).

**RESULTS**

**Pharmacokinetics of F-araA and ara-C.** We have shown that leukemic blast cells from pediatric patients with leukemia treated with a LB followed by a CInf of fludarabine phosphate for at least 18 h and then treated ex vivo with 200 μM ara-C for 1 h accumulated intracellular ara-CTP concentrations up to 10-fold higher than the blast leukemic cells from the same patients just before the treatment with F-araAMP (6, 28, 31). Thus, we combined this mode of administration of F-araAMP

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**Table 1**

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<tr>
<th>Dose level</th>
<th>Total patients</th>
<th>Targeted ara-C, μM</th>
<th>ara-C, μM</th>
<th>ara-U, μM</th>
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<tr>
<td>1</td>
<td>3</td>
<td>10.0</td>
<td>10.37 ± 1.3*</td>
<td>116.1 ± 39.2</td>
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<tr>
<td>2</td>
<td>6</td>
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<td>13.40 ± 4.1</td>
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<tr>
<td>3</td>
<td>7</td>
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<td>94.1 ± 18.9</td>
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<tr>
<td>4b</td>
<td>2</td>
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<td>17.07 ± 1.2</td>
<td>154.2 ± 9.0</td>
</tr>
<tr>
<td>4c</td>
<td>2</td>
<td>17.5</td>
<td>10.73 ± 4.1</td>
<td>182.3 ± 28.5</td>
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<tr>
<td>Total</td>
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</table>

*Mean ± SD.

b These two patients were not treated previously with high-dose ara-C prior to entry into the CCG-0895 study.

These two patients were treated with high-dose ara-C prior to entry into the CCG-0895 study.
Pharmacology of Fludarabine Plus ara-C Infusions

"Steady state" ara-C concentrations are obtained immediately at the end of the LB and at the beginning of the ara-C infusion. These levels are maintained with some variability throughout the 72-h infusion regimen.

**Table 2** Plasma concentrations of F-araA after F-araAMP administration in pediatric patients entered into the CCG-0895 study.

<table>
<thead>
<tr>
<th>No. of Patients</th>
<th>F-araA plasma concentrations, μM</th>
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<tr>
<td>7</td>
<td>10.97 ± 7.0*</td>
</tr>
<tr>
<td>12</td>
<td>11.95 ± 9.5</td>
</tr>
</tbody>
</table>

*Mean ± SD.

with an LB plus CInf of ara-C to treat relapsed pediatric patients with leukemias according to the diagram depicted in Fig. 1.

Table 1 shows the plasma pharmacokinetic data of ara-C and its catabolite ara-U from the recently completed Phase I/II trial of F-araAMP + ara-C in pediatric patients (CCG-0895). The average plasma ara-C levels obtained are remarkably similar to the targeted concentrations. Fig. 2 shows the ara-C and ara-U plasma concentrations in one patient with ALL.

The concentrations of fludarabine and ara-C in patients' plasma were determined by using the dose-to-plasma level relationship and the volume of distribution of ara-C in pediatric patients as determined from previous clinical studies in pediatric patients. The two patients at dose level 4, who show a discrepancy in the obtained plasma ara-C concentrations (Table 1), are patients who had received multiple regimens of ara-C, including the high-dose regimen (3 g/m2 × 8 doses) prior to being entered in this study. It is apparent that these patients had developed an increased capacity to deaminate ara-C to ara-U, as indicated by the lower ara-C and commensurate higher ara-U plasma concentrations as compared to patients who had not been treated previously with this drug. Table 2 shows the similarities between the selected and the obtained plasma concentrations of F-araA, approximately 10 μM, in these patients with acceptable variability.

**Pharmacodynamic Studies of F-araATP and ara-CTP.** Patients from Childrens Hospital Los Angeles who had circulating blast cells while receiving F-araAMP for 2 days were assayed for intracellular F-araATP concentrations. These concentrations ranged from 40 to 145 μM at 48 h after F-araAMP infusion, with an average of 93.7 ± 49.4 μM (n = 4 ± SD). The cellular ara-CTP concentrations before time zero determined by *ex vivo* determinations averaged 293.32 ± 119.86 μM (n = 6 ± SD) in these patients. The cellular ara-CTP concentrations in circulating and bone marrow blast cells before F-araAMP administration were not statistically different from these values. To obtain these values, the leukemic cells were tested *ex vivo* with two concentrations of ara-C, as described in "Materials and Methods." The cellular ara-CTP concentrations after the initiation of the ara-C infusion showed a significant increase, 3-8-fold (average, 5-fold), over the concentrations obtained before F-araAMP infusion started. The increased range of ara-CTP concentrations was from ~300 to 500 μM and as much as 3800 μM in one patient's bone marrow blast cells (Fig. 3).

Fig. 4 represents the cellular ara-CTP concentrations from two other patients who were treated with this regimen. In both cases, we obtained drug synergism of approximately 3.5- and 5-fold, respectively. In all patients tested, we obtained up to an 8-fold increase in cellular ara-CTP concentrations after F-araAMP LB + CInf administration in comparison with before treatment. Previously, we have shown that there is a linear relationship between the ara-CTP concentrations in peripheral blast cells obtained *ex vivo* and *in vivo*. Hence, this regimen of F-araAMP followed by ara-C infusions can also produce significant biochemical synergism and hence, augmented cytotoxicity, in patients' leukemic cells.

**DSC after F-araAMP and ara-C.** The DSC was slightly affected after F-araAMP infusion, achieving 60–80% of untreated control values. However, DSC determinations after ara-C and after F-araAMP infusion was less than 1% of pretreatment control, indicating the significantly increased effect of both drugs to inhibit thymidine uptake into DNA by the leukemic blast cells. There is a linear relationship between cellular ara-CTP concentrations and inhibition of DSC in these leukemic blast cells.
Clinical Efficacy in Pediatric Patients with Relapsed Leukemia. Although the principal objective of this Phase I study was to determine the maximum tolerated dose and define the dose-limiting toxicity, a significant number of responses were seen, and the study was expanded to include a Phase II evaluation. Thirty-one pediatric patients 1 to 19 years of age were entered into this study. One patient was ineligible due to prior cranial radiation, and three other patients could not be evaluated for clinical response. Two patients died prior to day 14, and the third patient’s family refused a bone marrow examination after study entry and drug treatment. Twelve of the 20 (60%) eligible patients had relapsed ALL, and 20 of 30 (67%) had previously received high dose ara-C. Two patients had central nervous system leukemia at study entry; two others had relapsed after they had undergone bone marrow transplantation, and a third patient relapsed after the patient had undergone autologous bone marrow transplantation.

Hematological Toxicity. All 27 evaluable patients experienced myelosuppression after all dose levels of F-araAMP followed by ara-C. The median time of recovery of the patients ANC to \( \geq 1000/\text{mm}^3 \) was 37 days, and the platelet recovery to at least a \( \geq 100,000/\text{mm}^3 \) was 26 days. Seventy-five % of these patients recovered their ANC and platelet limits (shown above) by days 55 and 81, respectively.

Nonhematological Toxicity. The dose-limiting toxicity was cholestasis at dose level 4 at an estimated average plasma concentration of 17.5 \( \mu M \) ara-C. This was achieved after an LB of 487 mg/m\(^2\), followed by a CInf rate of 126 mg/m\(^2\)/h of ara-C. Grade IV hyperbilirubinemia was seen in two of five patients treated at this level, starting on days 9 and 13 after the second course of treatment, respectively. The average duration of cholestasis was 12.5 days. The dose levels 1, 2, and 3 of this drug regimen were considered tolerable, according to the definition of maximum tolerated dose, with nausea and vomiting and were well controlled with antiemetic therapy. However, there were patients treated at these dose levels with other reversible host toxicities (Table 3). One patient treated at dose level 2 experienced a tumor lysis syndrome. The protocol was subsequently amended to provide supportive care guidelines for the management of tumor lysis, and no subsequent incident was experienced. No neurological or pulmonary toxicity was seen with this dose regimen, although each of these drugs have been known to cause these types of host toxicities.

Remission Induction. Twelve of 27 patients (44%; 95% CI, 25–65%) evaluable for response achieved a CR or PR with this regimen. Nine of 18 patients with acute non-lymphocytic leukemia (50%; 95% CI, 20%–65%) achieved a CR or PR: 3 of 9 patients with ALL (33%; 95% CI, 7.5%–70%) achieved a CR or PR. CRs were observed at every escalating dose level of ara-C. Eighteen of 27 pediatric patients had prior high-dose ara-C and either failed induction or relapsed. Six of 18 (33%) evaluable patients who had previously been treated with high-dose ara-C achieved a CR (4 patients) or PR (2 patients) with this regimen. Three others (16%) achieved a clearance of bone marrow of blasts without return of normal marrow or peripheral counts; six others (33%) had “stable disease,” and only three (16%) other patients had progressive disease. Of the two patients who had central nervous system disease, one cleared the leukemia blasts present in the cerebrospinal fluid and achieved a CR by day 16 of the second course of therapy with this regimen.

DISCUSSION

This multiinstitutional CCG study showed that the LB + CInf of F-araAMP for 2 days followed by a LB + CInf of ara-C increased the accumulation of ara-CTP in leukemic cells in vivo. This protocol was designed to produce maximally tolerated plasma and intracellular concentrations of F-araA and F-araATP, respectively. A F-araAMP LB of 10.5 mg/m\(^2\), followed by a CInf regimen of 30.5 mg/m\(^2\)/day over 2 days, achieved the desired plasma concentrations (36). The study demonstrated that a steady-state plasma concentration (C\(_{ss}\)) of approximately 10 \( \mu M \) F-araA could be attained and maintained for the duration of the 2-day treatment period as shown in Table 2. This study reproduced the plasma concentrations of F-araA seen after the same dose was administered in pediatric patients with cancer in an earlier Phase I/II clinical study (6).

F-araAMP is rapidly dephosphorylated in plasma to F-araA, which circulates and is taken up by leukemic cells, where it is anabolized to the triphosphate anabolite, F-araATP (4, 6, 12, 13). Because F-araA achieves a high plasma concentration range when the ara-C CInf is started and because we achieved the same plasma levels of ara-C as in pediatric patients when the same doses were administered alone (6, 11), there appears to be no nucleoside drug-drug interaction in the plasma of pediatric patients. This was further examined by NONMEM population analyses, and no drug-drug interaction was determined (37).
Other studies have reported that intracellular concentrations of 75 μM F-araATP or greater in leukemic blast cells produced the highest drug synergism between F-araAMP and ara-C (30). We clearly achieved 75 μM or higher cellular concentrations after 48 h of F-araAMP infusion, which were similar to those reported earlier in our Phase I/II trial of F-araAMP (6).

The sequence of F-araA followed by ara-C is very specific to elicit drug synergism in vitro in CEM cells via cellular apoptosis (19, 20, 38). The time of exposure of leukemic cells to F-araA(MP) is also important, a minimum of 18 h to a maximum of 48 h. This was necessary for cellular F-araATP concentrations to accumulate in the leukemic cells and achieve cytotoxic levels (6). However, the difference between 24 and 48 h exposure to F-araA was not statistically different in augmenting cellular ara-CTP. Less than 18 h of incubation with F-araA negated the drug synergism, probably due to lack of F-araATP accumulation. Also, in vitro studies showed the reversal of the sequence produced an antagonistic pharmacological effect in CEM cells (28, 31, 32, 39). This phenomenon may be due to the severe cytotoxicity of ara-C exerted on CEM cells, thus inhibiting the production of intracellular F-araATP by the cellular kinases: thus, no drug synergism and hence, reduced cellular apoptosis (19, 20, 38, 39). As a result of these data and because we did not wish to possibly obtain a similar antagonistic effect in vivo, the protocol design did not allow for a study of F-araAMP to the patients. In addition, such studies could not have been performed, because in our protocol, ara-C was administered as a LB, followed by ClInf for 72 h. However, when we compare the rate of ara-CTP accumulation before and after F-araAMP administration, the data show the augmentation of ara-C anabolism. Measurement of the AUC of cellular ara-CTP was not possible in all patients because not all patients had circulating blast cells. However, in the few patients that was measured, the AUC had an extremely large value when compared to the AUC from short-term infusions (3 h, 3 g/m²), thus demonstrating the pharmacodynamic superiority of the LB followed by ClInf of this drug in pediatric patients (11, 28, 34, 35).

The cellular ara-CTP concentrations in these cells were much higher (5–8-fold) after 48 h of fludarabine treatment as compared to control values. High cellular F-araATP concentrations are necessary for cytotoxicity via cellular apoptosis (19, 20). We conclude from these results that the biochemical effect seen in other studies with similar protocol designs of F-araAMP and ara-C in adult patients are reproducible (17, 18, 30). These augmented concentrations of ara-CTP had been seen in CEM/0 leukemic cells, where they generated a 5-fold synergism, as compared to ara-C alone, in vitro or ex vivo (6, 28, 32, 35).

Studies with other ara-CTP-augmenting agents, such as 5-azacytidine, arabinosyl-5-azacytidine (Fazarabine), thymidine, deazauridine, hydroxyurea, and other drugs have also been shown increases in ara-CTP levels (28, 30, 35, 40–44). 5-Azacytidine enhanced ara-CTP anabolism and commensurate decrease in DSC but not to less than 1%, as seen with F-araAMP and ara-C (34, 35). Thus, these results may indicate that F-araATP, which is present in the leukemic cells long after F-araAMP infusion is discontinued, may exert a direct synergistic effect with ara-CTP on inhibiting the DNA polymerase activity. This may be an additional and separate potentiating factor of drug synergism, in addition to the reported effect on the inhibition of RR and on the direct potentiation on dCK (15–18, 30).

CR in relapsed ALL and acute non-lymphocytic leukemia was achieved in significant percentages in both forms of leukemia in pediatric patients entered into CCG-0895. These complete responses were achieved with tolerable toxicity using this combination regimen (36). As a result, similarly designed clinical studies are planned in other cooperative groups and in adult patients. These studies will examine whether the improved clinical response observed in this cohort is limited to pediatric patients or is due to an improved protocol design. Similar biochemical modulation studies are being tested with various drugs in combination with ara-C, producing various results. One such regimen is an expansion of the CCG-0895 study, where dose level 3 of fludarabine plus ara-C (10 μM F-araA and 15 μM ara-C in plasma) has been combined with an anthracycline (idarubicin; CCG-0922). This combination has been shown to be tolerated in pediatric patients with relapsed AML (44). A Phase III trial will follow to examine the long-term efficacy of the idarubicin/fludarabine/ara-C combination regimen.

We conclude from these studies that: (a) the optimum concentrations to elicit drug synergism between F-araA and ara-C are 10 and 15 μM, respectively. These concentrations can easily be achieved after LB followed by ClInf regimens of both drugs; (b) cellular ara-CTP is augmented 5-fold in leukemia cells from pediatric patients receiving F-araAMP prior to ara-C treatment; (c) the achieved plasma concentrations of F-araA and/or ara-C in pediatric patients were similar to those obtained after identical regimens of both drugs, i.e., 10 μM F-araA and 15 μM ara-C (6, 34, 35); and (d) no drug interaction of F-araA and ara-C in plasma was determined, and the increased intracellular ara-CTP concentrations are most likely responsible for the improved clinical response rates in relapsed pediatric patients.
APPENDIX

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<th>Institution</th>
<th>Investigator</th>
<th>Grant no.</th>
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