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A Phase I–II Study of Combination Therapy with Thymidine and Cytosine Arabinoside

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Summary. *Twenty-one patients with leukemia and lymphoma refractory to conventional therapy were treated with thymidine (dThd) at a dose of 75 g/m²/day for 48 h by continuous intravenous (IV) infusion, followed by cytosine arabinoside (ara-C) on an escalating dose schedule of 25–625 mg/m²/day for 48 h by continuous IV infusion. Toxicity included somnolence (1 patient), mucositis (2 patients), and myelosuppression (19 patients). One patient died prior to completion of therapy. Because of the patient population studied (mainly consisting of patients with leukemia refractory to conventional therapy) and the prevalence of myelosuppression prior to therapy, a median toxic dose was not evaluable. Therapeutic responses included a partial remission in a patient with acute myelogenous leukemia (AML) refractory to ara-C. An additional six patients with acute leukemia refractory to ara-C transiently cleared their peripheral blood of blasts, and a decrease in circulating blasts was noted in two other patients. Biochemical studies conducted on the peripheral blasts of two patients confirmed an enhancement by dThd of the incorporation of ara-C into the DNA of circulating blasts.*

Introduction

We have employed cesium sulfate gradient centrifugation to monitor the incorporation of ara-C into cellular nucleic acids [6]. The use of this approach has demonstrated the specific incorporation of ara-C in DNA using the human HL-60 promyelocytic cell line and blasts from a patient with acute myelogenous leukemia [8]. Further, a highly significant relationship has been found between the extent of (ara-C)DNA formation and inhibition of clonogenic survival [6, 8]. The incorporation of ara-C residues results in the formation of faulty DNA [10] and provides a poor primer terminus for further chain elongation [11], thus slowing DNA synthesis and accumulating DNA strand breaks [2].

Several approaches have been employed to enhance the incorporation of ara-C in DNA. These include the use of inhibitors of ribonucleotide reductase and of de novo pyrimidine synthesis to increase the formation of ara-C nucleotide pools [12]. Thymidine reduces deoxycytidine triphosphate (dCTP) pools [3, 4, 12] and enhances incorporation of ara-C in DNA [3, 12]. We have demonstrated that dTdh (10^{-3} and 10^{-4} *M*) enhances formation of (ara-C)DNA and increases loss of clonogenic survival [9]. This effect is also due in part to enhancement of cells traversing through S-phase following dThd exposure.

The present study was undertaken to determine whether dThd pretreatment would enhance the incorporation of ara-C in clinical samples, as well as to assess the clinical efficacy and toxicity of this combination. We previously completed a phase I–II trial of dThd by continuous IV infusion at 75 g/m²/day (10^{-3} *M* plasma dThd levels) and this trial has served as a basis for the present study [7]. The results demonstrate that dThd can be administered clinically with ara-C and that this approach results in enhancement of ara-C incorporation in human leukemic cell DNA.

Materials and Methods

Clinical Studies. From May 1980 through May 1982, twenty-one patients from Sidney Farber Cancer Institute, Brigham and Women's Hospital, and Children's Hospital Medical Center were placed on a phase I–II trial of dThd/ara-C. Eligible patients had histologically confirmed leukemia or malignancy refractory to standard therapy, a performance status of 2 or less on the Eastern Cooperative Oncology Group scale, and adequate hepatic and renal function with bilirubin less than 2.5 mg%, SGOT less than 60 Wacker units/ml, BUN less than 20 mg%, and creatinine less than 2.0 mg%.

Thymidine was provided by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Each 15 g dThd was diluted in 500 ml 0.6% NaCl and stored at 4° C. Ara-C was obtained from the Upjohn Company, Kalamazoo, MI, USA.

Thymidine was administered on days 1 and 2 at 75 g/m²/day, as a continuous IV infusion and ara-C at 25–625 mg/m²/day by IV infusion on days 3 and 4. Dose escalations were conducted in individual patients as well as between patients. Any escalations in dose required that at least two patients had received the prior ara-C dose with acceptable toxicity. Therapy was generally repeated every 3–4 weeks, depending on the patient's clinical status. Informed consent was obtained from all patients prior to therapy, in accordance with the regulations of the human protection committee at the Sidney Farber Cancer Institute, Brigham and Women's Hospital, and Children's Hospital Medical Center.

All patients had a complete history and physical examination. Laboratory evaluation included CBC with differential, platelet count, BUN, creatinine, and liver function tests. These tests were repeated twice a week during therapy and weekly

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after completion of therapy. A complete resolution of measurable disease constituted a complete remission, while a 50% or greater decrease in the products of the perpendicular diameters of measurable lesions was considered a partial remission. In patients with leukemia, a complete remission required that the bone marrow aspirate show less than 5% blasts with a normal percentage and maturation of all cell lines, and also a granulocyte count greater than 1,000/mm^3, platelet count greater than 100,000/mm^3, and hemoglobin greater than 12 g%. A partial remission was defined as a marrow with less than 5% blasts and the absence of any circulating blasts. A disappearance or reduction in circulating blasts was also documented.

Patients with acute leukemia were considered resistant or refractory to ara-C if their disease recurred during therapy or during the first 3 months after therapy with ara-C. In assessing toxicity, we defined myelosuppression as thrombocytopenia with a platelet count less than 100,000 or leukopenia with a WBC less than 3,000.

Pharmacological Studies

Effect of dThd on the Incorporation of Ara-C into Nucleic Acids. In experiments assessing the effect of dThd on the incorporation of [^{3}H]ara-C into human leukemic cell DNA, dThd (Sigma Chemical Company, St Louis, MO, USA) was freshly prepared in RPMI 1640 medium with 10% heat-inactivated dialyzed fetal calf serum at a concentration of 10^{-2} *M*. The stock solution was sterilized by millipore filtration.

HL-60 promyelocytes were incubated with 10^{-3} *M* dThd for 0, 2, and 16 h, washed twice in phosphate-buffered saline (PBS), and then incubated with 10^{-6} *M* [^{3}H]ara-C (26 Ci/mmole; New England Nuclear, Boston, MS, USA) for 1, 2, 4, and 6 h. Cells were then washed twice in PBS and resuspended at 1×10^7 cells/ml in PBS. The cells were lysed by the addition of 2.5 mg pronase B (Calbiochem-Behring Corporation, LaJolla, CA, USA) in 2 ml 0.01 *M* Tris (pH 7.4), 0.01 *M* EDTA, and 0.5% sodium dodecyl sulfate. Procedures used in the purification and Cs_2SO_4 gradient analysis of the DNA have been previously described [6, 8].

In evaluating the clinical effect of dThd, circulating blasts were obtained from the peripheral blood of two leukemic patients prior to and at 24 and 48 h during therapy with dThd. The blasts were isolated from Ficoll-Hypaque gradients, washed twice with PBS, and then resuspended in RPMI 1640 medium. All these procedures were performed in the presence of 10^{-3} *M* dThd. Cells were then exposed in vitro to [^{3}H]ara-C at 10^{-6} *M* (26 Ci/mmole) for 6 h at 37° C in a 5% CO_2 incubator and washed twice inPBS; the nucleic acids were then analyzed by Cs_2SO_4 gradient centrifugation.

Results

Clinical Studies

Twenty-one patients were eligible for the study. A mean of two courses of therapy per patient was administered (range: 1–6). Early death occurred in one patient with AML, who expired from sepsis in the absence of detectable cytoreduction 2 days after the completion of therapy. The clinical features of the patients are outlined in Table 1. Of the 21 patients, 17 had acute leukemia and 15 were refractory to a variety of agents including ara-C.

Although seven patients had persistent pancytopenia prior to therapy, myelosuppression was noted in all 20 evaluable patients. The median platelet and white blood counts at nadir were 15,000 and 1,400, respectively, while the median times to nadir were 10 and 9 days, respectively. Other toxicities included mild mucositis (2 patients), somnolence (1 patient), nausea (1 patient), and frontal headache (1 patient). A mild elevation in liver function tests occurred in one patient who had a history of non-A, non-B hepatitis.

Response to therapy was evaluable in 20 patients. A partial remission occurred in one patient with AML (patient 18, Table 1) who had previously failed to respond to therapy with ara-C at both conventional (200 mg/m^2/day) and higher

Table 1. Clinical features of patients

Patient	Age	Sex	Diagnosis	Prior therapy	Dose of ara-C following dThd		Hematologic toxicity	Other toxicity	Response
					mg/m^2	(# of cycles)			
1	1	M	AMML	VCR, ADR, Pred, Ara-C, VP-16, AZA	25 75 125	(1) (1) (1)	None	None	Progressive disease
2	19	M	AML	ADR, Ara-C, VCR, Pred, MTX, AZA, 6-TG, VP-16, DAD	50 75 125	(1) (1) (1)	T	None	No response
3	32	M	Non-Hodgkin's lymphoma	M-BACOD, VP-16	75 125 175 225 275 325	(1) (1) (1) (1) (1) (1)	T at > 275 mg/m^2 ara-C	Mucositis	Progressive disease
4	48	M	CML (blast crisis)	HU, MEL, VCR, Pred, ADR	125 175 225 275 325	(1) (1) (1) (1) (1)	L, T	None	No response

Table 1 (continued)

Patient	Age	Sex	Diagnosis	Prior therapy	Dose of ara-C following dThd		Hematologic toxicity	Other toxicity	Response
					mg/m^2	(# of cycles)			
5	5	M	Non-Hodgkin's lymphoma	M-BACOD	225	(1)	L, T	None	Transient clearing of peripheral lymphosarcoma cells
6	3	F	ALL	ADR, VCR, Pred, 6-MP, L-ASP, MTX, Ara-C, CTX, ACT VM-26	225	(2)	Pancytopenia[a]	None	No response
7	9	F	ALL	ADR, VCR, Pred, Ara-C, 6-MP, L-ASP, VP-16, AZA, M-AMSA	275	(1)	Pancytopenia[a]	None	Transiently cleared peripheral blasts
8	33	F	AMML	ADR, VCR, Pred, Ara-C, M-AMSA	275	(1)	Not evaluable	Death early in course of therapy from sepsis	Not evaluable
9	18	M	AML	ADR, Ara-C, 6-TG, VCR, AZA, L-ASP, MTX	75 125 175 225	(1) (1) (1) (1)	L[a], T	Mucositis	No response
10	34	F	AML	ADR, VCR, Pred, Ara-C, L-ASP, 6-MP, MTX, DCF	75 125	(1) (1)	L, T	None	Reduction in peripheral blasts
11	32	M	Non-Hodgkin's lymphoma	BACOP, C-MOPP, MTX, VP-16, VBL	125 175	(1) (1)	L, T	None	No response
12	35	M	AML	VP-16, Ara-C, ADR, 6-TG	175	(1)	L, T	None	No response
13	21	M	ALL T cell	CTX, ADR, VCR, Pred, MTX, L-ASP	325	(1)	L, T	None	Reduction in peripheral blasts
14	22	F	ALL	ADR, Pred, VCR, 6-MP, L-ASP, VM-26, Ara-C, M-AMSA	375 425	(1) (1)	L, T	None	Transient clearing of peripheral blasts
15	19	M	ALL	ADR, VCR, Pred, L-ASP, MTX, Ara-C, 6-MP, VM-26	425	(1)	L, T	Frontal headache	Transient clearing of pheripheral blasts
16	54	F	AML	Ara-C, Dauno, 6-TG, AZA	475	(1)	T	Somnolence	No response
17	20	M	AML	Ara-C, Dauno, 6-TG VP-16, AZA, M-AMSA, DCF, Ara-A	475	(1)	Pancytopenia[a]	None	No response
18	44	M	AML	Ara-C, Dauno, VP-16, AZA	500 525	(1) (1)	L, T	None	Partial remission with clearing of blasts from marrow
19	45	M	AML	Ara-C, Dauno	575	(1)	L, T	None	Transient clearing of peripheral blasts
20	46	F	AML	Ara-C, ADR	575	(1)	L, T	None	No response
21	23	F	AML	Dauno, Ara-C, 6-TG, VP-16, AZA	575 625	(1) (1)	T[a]	Nausea	Transient clearing of peripheral blasts

Abbreviations: ACT: actinomycin-D; ADR: doxorubucin; Ara-A: adenine arabinoside; Ara-C: cytosine arabinoside; AZA: 5-azacytidine; CTX: cytoxan; DAD: dihydroxyanthracenedione; DCF: deoxycoformycin; HU: hydroxyurea; L-ASP: L-asparaginase; MEL: melphalan; MTX: methotrexate; Pred: prednisone; 6-TG: 6-thioguanine; VCR: vincristine; VBL: vinblastine; Bleo: bleomycin; Dauno: daunorubicin; DEX: dexamethasone; M-BACOD: high-dose MTX, Bleo, ADR, CTX, VCR, DEX; BACOP: Bleo, ADR, CTX, VCR, Pred; C-MOPP: CTX, MTX, VCR, Pred, procarbazine; T: thrombocytopenia; L: leukopenia

[a] Evidence of limited marrow reserve prior to therapy

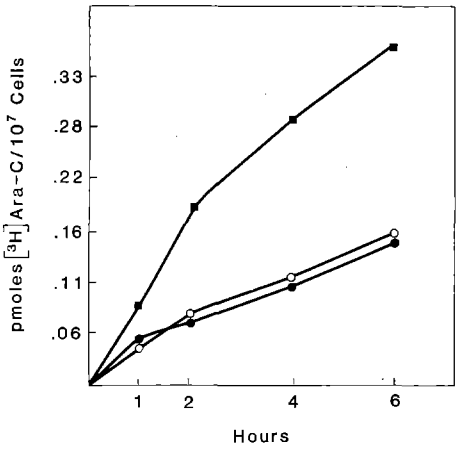

Fig. 1. Incorporation of [^{3}H]ara-C into HL-60 DNA following exposure to thymidine. HL-60 promyelocytes were incubated with thymidine at 1×10^{-3} *M* for 0 (●), 2 (○), and 16 (□) h, washed twice, and then exposed to 10^{-6} *M* [^{3}H]Ara-C for 1, 2, 4, and 6 h. The extent of ara-C incorporation in DNA was monitored by Cs_2SO_4 gradient analysis. Values represent the mean of two determinations. Standard deviations were less than 10% of the mean

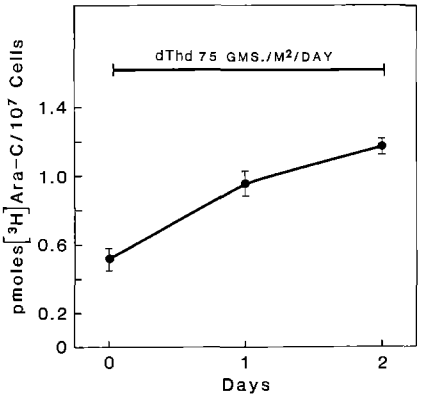

Fig. 2. Incorporation of [^{3}H]Ara-C into T cell ALL DNA. After in vivo exposure to thymidine, circulating blasts were isolated at either 24 or 48 h, after which they were exposed to [^{3}H]ara-C at 10^{-6} *M* for 6 h and the extent of incorporation was assessed by Cs_2SO_4 gradient analysis. Values represent the mean and standard deviation of two determinations

doses (500 mg/m^2/day). While this patient continued to be thrombocytopenic after therapy, the bone marrow was cleared of myeloblasts and the peripheral blood remained free of myeloblasts for several months. An additional six patients with acute leukemia refractory to ara-C transiently cleared their peripheral blood of blasts and a reduction in the circulating blasts was noted in an additional two patients.

Biochemical Studies

The effect of dThd pretreatment on the incorporation of ara-C in human leukemic cell DNA was initially monitored in HL-60 promyeloblasts. Figure 1 illustrates the formation of HL-60 (ara-C)DNA following exposure to 10^{-3} *M* dThd for 2 h and 16 h. The 2-h dThd pretreatment resulted in little, if any, enhancement of (ara-C)DNA formation over that seen with HL-60 cells not previously exposed to dThd. In contrast, a 16-h

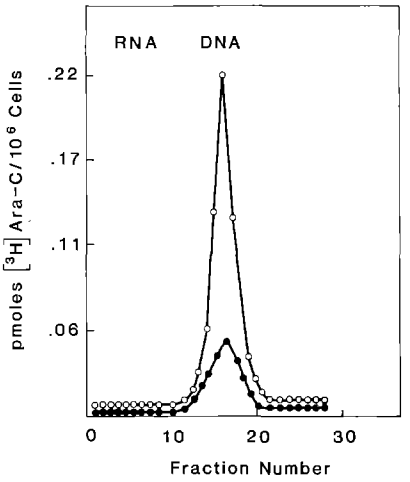

Fig. 3. Incorporation of [^{3}H]Ara-C into AML DNA after in vivo exposure to thymidine. Circulating blasts were isolated prior to (●) and after 48 h (○) of an IV thymidine infusion at 75 g/m^2/day and then exposed to 10^{-6} *M* [^{3}H]ara-C for 6 h; the extent of incorporation was then assessed by Cs_2SO_4 gradient analysis

dThd pretreatment increased (ara-C)DNA formation by nearly 3-fold.

The effect of dThd on the incorporation of ara-C into the DNA of human leukemic blasts was also monitored by isolating circulating blasts prior to and at 24 or 48 h after dThd therapy. The blasts were then exposed to 10^{-6} *M* [^{3}H]ara-C for 6 h and the extent of incorporation compared with that obtained in blasts from the same patient prior to dThd therapy. After 48 h of dThd therapy, at least a 2-fold enhancement in the incorporation of ara-C was achieved in lymphoblasts from a patient with T cell ALL (Fig. 2). Similarly, in a patient with AML, a 4-fold enhancement of the formation of (ara-C)DNA was observed under the same experimental conditions (Fig. 3).

Discussion

We have demonstrated a significant correlation between the incorporation of ara-C in DNA and inhibition of clonogenic survival in HL-60 promyelocytes and in circulating blasts obtained from a patient with acute leukemia [8]. Thymidine enhances incorporation of ara-C in murine leukemic cell DNA and increases loss of clonogenic survival [3, 9]. The present report extends these findings by demonstrating that dThd can be administered clinically in combination with ara-C and results in an enhancement of ara-C incorporation in human leukemic cell DNA.

While the therapeutic efficacy of this regimen was limited to a partial remission in one patient with AML refractory to prior therapy with ara-C, cytoreduction and transient clearing of peripheral blasts was evident in eight patients with leukemia refractory to prior ara-C treatment. The potential therapeutic efficacy of combining dThd and ara-C is further suggested by a recent phase I trial in which a more intensive scheduling of this drug combination resulted in complete remissions in three of five patients with CML in blasts crisis, one of five patients with resistant or relapsing AML, and a single patient with previously untreated AML [14]. These responses may be a result of the effect of dThd on depletion of dCTP pools and enhancing formation of (ara-C)DNA.

Our studies indicate that dThd increases ara-C incorporation in leukemic cell DNA by approximately 2- to 3-fold. A similar effect might be acheived, however, by simply administering higher doses of ara-C, since incorporation follows a concentration × time effect. In this regard, other regimens using high doses of ara-C have demonstrated more significant response rates in the treatment of patients with refractory leukemia [1, 5, 13] and may ultimately prove more effective.

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