Cancer Chemother Pharmacol (1983) 10: 125–128

Cancer Chemotherapy and Pharmacology

Clinical Pharmacology of Arabinofuranosyladenine in Combination with Deoxycoformycin

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Summary. *We have administered five courses of arabinofuranosyladenine (ara-A) and deoxycoformycin (DCF) to two patients and have studied the effects of DCF on the pharmacokinetics of ara-A. Ara-A was given by IV infusion over 3 h at a dose of 300 mg/m²/day for 3 days on the first two courses of treatment for each patient. One patient received a third course at a dose of 300 mg/m²/day every 12 h for 3 days. During the first course of treatment, patients received DCF (10 mg/m²) 60 min prior to the ara-A infusion on days 2 and 3 only. However, during subsequent courses, DCF was administered on each of the 3 days. On day 1 of the first course of treatment ara-A was not detectable in the plasma, while ara-hypoxanthine (ara-hyp) reached levels of up to 12.0 μg/ml. On day 2, DCF pretreatment resulted in detectable ara-A levels and decreased ara-hyp. On day 3, the levels of ara-A increased further and ara-hyp was no longer detectable. On subsequent courses, the administration of DCF on each of the 3 days of treatment resulted in detectable ara-A levels on day 1, with progressive increases on days 2 and 3. During the second course of treatment, increases in the excretion half-life of ara-A were noted during the 3-day treatment schedule, with values increasing from 258.2 min to 546 min and from 77.3 min to 219 min for patients I and II, respectively. Peak CSF ara-A levels were approximately one-third of peak plasma levels. No toxicity was encountered in patients treated at these doses of ara-A and DCF. Abrupt declines in peripheral blast counts were observed in each patient.*

Introduction

Arabinofuranosyladenine (ara-A) is an inhibitor of DNA synthesis [4] with antiviral [17] and antitumor [1] activity. Ara-A is converted to adenine arabinofuranoside-triphosphate (Ara-ATP), an inhibitor of DNA polymerase [6] and of ribonucleotide reductase [12]. Ara-A is also incorporated in viral [14] and mammalian [13] DNA and may slow synthesis by acting as a relative chain terminator [3, 20]. Ara-A-induced cytotoxicity has been shown to be dependent upon total cellular exposure to ara-ATP [15] and we have recently demonstrated a highly significant relationship between the incorporation of ara-A in DNA and loss of clonogenic survival [11]. These findings suggest that competitive inhibition of DNA polymerase by ara-ATP and slowing of chain elongation beyond incorporated ara-A residues are major mechanisms of action.

The activity of ara-A has been limited by its rapid deamination to the inactive metabolite arabinosyl-hypoxanthine (ara-hyp) by adenosine deaminase (ADA; adenosine aminohydrolase, EC 3.5.4.4), an enzyme widely distributed in body tissues and fluids [22]. The deamination of ara-A has resulted in minimal clinical activity, and dose escalations are prevented by the poor solubility of the drug.

The deamination of ara-A can be circumvented through the use of adenosine deaminase inhibitors such as erythro-9-(2-hydroxy-3-nonyl) adenine [18] and DCF [23]. Deoxycoformycin enhances the cytotoxicity and antitumor activity of ara-A in tissue culture and animal models [2, 9]. Deoxycoformycin has undergone phase I–II clinical evaluation and can be given with limited toxicity at doses that inhibit leukemic cell ADA [10]. In the present study, we have studied the effects of DCF on the pharmacokinetics of ara-A in two patients treated with this combination. The results indicte that pretreatment with DCF significantly prolongs the plasma half-life of ara-A.

Materials and Methods

Patient Description and Treatment Protocol. Patient I was a 26-year-old male (172 cm, 80 kg) with T cell leukemia, who initially entered a complete remission following induction with vincristine (VCR), prednisone (PRED), and adriamycin (ADR) in combination with central nervous system (CNS) prophylaxis. A bone marrow and CNS relapse occurred several months after completion of a 2-year maintenance program and the patient was reinduced with VCR, PRED, and L-asparaginase. Subsequent relapses were treated with high-dose methotrexate, L-asparginase, thymidine/ara-C and single-agent DCF. The patient then started a course of DCF and ara-A.

Patient II is a 20-year-old male (190 cm, 118 kg) with acute myelogenous leukemia, who failed to enter remission when treated with the VAPA-10 [21] induction regimen. He was treated with several experimental agents without response and treatment was subsequently initiated with DCF and ara-A.

Each patient was treated with DCF/ara-A under special exemption protocol E81-640 through the Investigational Drug Branch, DCT, National Cancer Institute, Bethesda, MD, USA. Informed consent was obtained from each patient in accordance with the Sidney Farber Cancer Institute Human Protection Committee guidelines.

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DCF (10 mg/m^2) was administered by IV bolus 60 min prior to the start of the ara-A infusion. On their first course of treatment the patients received DCF on days 2 and 3 only, while in subsequent courses DCF was administered on days 1, 2, and 3. Ara-A (300 mg/m^2) was administered by IV infusion over 3 h on days 1–3 (Table 1). Patient II received a third treatment with ara-A (300 mg/m^2) given every 12 h for 3 consecutive days.

Determination of Plasma Nucleoside Levels. Blood and CSF samples were collected in heparinized tubes containing DCF (final concentration: 5×10^{-6} *M*). Samples were filtered through Amicon CF25 centriflo membranes (Amicon Corp., Lexington, MA, USA). The filtrates were then analyzed by high-pressure liquid chromatography (HPLC) to quantitate levels of ara-A, ara-hyp, adenosine, and deoxyadenosine by techniques described elsewhere (R. P. Agarwal et al., 1982), unpublished work).

The plasma disappearance of ara-A was plotted according to a decreasing exponential function using the Marquardt/ Levenberg curve fitting method of the MALB computer program [8].

Table 1. Treatment protocol

Treatment		Drug doses	
Course	Day	DCF	Ara-A
1	1	None	300 mg/m^2/day by IV infusion over 3 h
	2	10 mg/m^2/day by IV push given 60 min prior to starting the ara-A infusion	300 mg/m^2/day by IV infusion over 3 h
	3	10 mg/m^2/day by IV push given 60 min prior to starting the ara-A infusion	300 mg/m^2/day by IV infusion over 3 h
2	1	10 mg/m^2/day by IV push given 60 min prior to starting the ara-A infusion	300 mg/m^2/day by IV infusion over 3 h
	2	10 mg/m^2/day by IV push given 60 min prior to starting the ara-A infusion	300 mg/m^2/day by IV infusion over 3 h
	3	10 mg/m^2/day by IV push given 60 min prior to starting the ara-A infusion	300 mg/m^2/day by IV infusion over 3 h
3	1	10 mg/m^2/day by IV push given 60 min prior to starting the ara-A infusion	300 mg/m^2 every 12 h by IV infusion over 3 h
	2	10 mg/m^2/day by IV push given 60 min prior to starting the ara-A infusion	300 mg/m^2 every 12 h by IV infusion over 3 h
	3	10 mg/m^2/day by IV push given 60 min prior to starting the ara-A infusion	300 mg/m^2 every 12 h by IV infusion over 3 h

Patient I received two courses of treatment and patient II, three courses

Results

Plasma and CSF Levels of Ara-A and Ara-hyp

Ara-A (300 mg/m^2) was administered alone on the first day of the initial course of treatment. In both patients, plasma ara-A was undetectable at the end of the 3-h infusion. However, plasma ara-hyp was detectable at peak concentrations of 12 μg/ml (patient I) and 4.42 μg/ml (patient II) at the end of the drug infusion. The administration of DCF (10 mg/m^2) prior to the ara-A infusion on the second day of treatment resulted in lower peak concentrations of ara-hyp, with peak values of 7 μg/ml and 1.4 μg/ml in patients I and II, respectively. Further, pretreatment with DCF resulted in detectable plasma ara-A levels in both patients upon completion of the infusion. In contrast, ara-hyp was undetectable on day 3 of treatment in both patients, and plasma ara-A levels were higher than on the initial 2 days of therapy. Except in patient II on the first day of his second and third courses of treatment, ara-hyp was undetectable during subsequent courses of treatment when DCF was administered prior to the ara-A infusions. The plasma ara-A concentration increased during each of the ara-A infusions.

CSF samples were obtained from patient I through an Omaya reservoir to monitor arabinofuranosyl nucleoside levels. On the first day of the initial course of treatment, the concentration of ara-hyp was 7.48 μg/ml at the end of the infusion, while no ara-A was detectable. On the second day of that course of treatment, ara-A and ara-hyp were both detected in the CFS. On the third day, values as high as 2.2 μg/ml were recorded in the CSF for ara-A, while no ara-hyp was identified.

Pharmacokinetic Parameters. Plasma concentrations of ara-A were fitted to an exponentially decreasing function (Fig. 1).

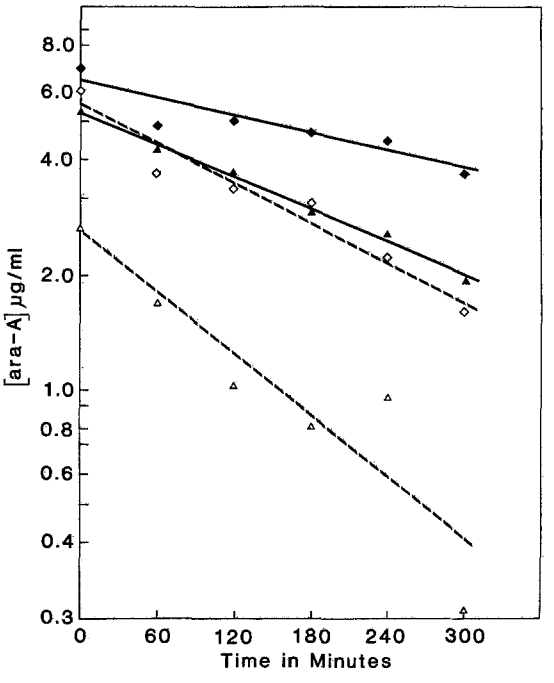

Fig. 1. Plasma pharmacokinetics of ara-A. Plasma samples were drawn at hourly intervals for 6 h following the ara-A infusion on the second course of treatment. Shown are the ara-A levels in patients I (*closed symbols*) and II (*open symbols*) on day 1 (▲ or △) and day 3 (◆ or ◇)

Table 2. Pharmacokinetic parameters

Patient	Course	Day	$t_{1/2}$ (min)	A[a] (μg ara-A/ml)	Sum of squares value	RMS error
I	1	1	–	–	–	–
		2	–	–	–	–
		3	278.0	6.0	0.003	0.02
II	1	1	–	–	–	–
		2	99.3	3.1	0.032	0.07
		3	255.6	5.0	0.008	0.04
I	2	1	258.2	5.2	0.001	0.02
		2	318.6	7.8	0.009	0.05
		3	546.0	6.3	0.008	0.01
II	2	1	77.3	2.6	0.047	0.10
		2	197.9	5.4	0.008	0.05
		3	219.6	5.5	0.011	0.05
II	3	1	106.6	4.4	0.012	0.08
		2	284.2	5.7	0.001	0.04
		3	264.2	6.1	0.001	0.03

Plasma samples were obtained during each ara-A infusion. During each patient's first course of treatment, samples were obtained at 30-min intervals for 6 h after each ara-A infusion; during the second and third courses plasma samples were obtained at the end of the infusion and then at 1-h intervals for 5 h

[a] A, adjusted plasma concentration at the end of the infusion, based on the best fit for the disappearance of ara-A from the plasma

Table 3. Plasma adenosine and deoxyadenosine

Patient	Course	Day	Plasma[a]	
			Adenosine (g/ml)[b]	Deoxyadenosine (g/ml)[b]
I	1	1	–	–
		2	–	–
		3	1	Trace
II	1	1	–	–
		2	Trace	–
		3	0.1	0.1
I	2	1	–	0.2
		2	–	1.0
		3	–	6.6
II	2	1	–	–
		2	Trace	Trace
		3	Trace	Trace
II	3	1	–	Trace
		2	–	Trace
		3	Trace	0.6

[a] Plasma samples were obtained at the same time points as those indicated in Table 2

[b] Trace' indicates that the small amounts of adenosine or deoxyadenosine detected could not be reliably quantitated

The corrected plasma concentration upon completion of the infusion was also computed on the basis of an exponential disappearance of ara-A in the plasma (Table 2). Patient I accumulated more ara-A and had significantly longer plasma half-lives than patient II. Higher ara-A levels were detectable on successive days of treatment in both patients and the plasma decay half-life also increased from day 1 to 3 of each course (Table 2). These observations suggest an accumulation of ara-A in body fluids with each dose of drug.

Patient I received his second course of DCF/ara-A 10 days after his first treatment. No ara-hyp was detected in his plasma on day 1 of his second course. This suggests that ADA had not recovered completely from prior treatment and that the first dose of DCF completely inhibited the remaining enzyme activity. Under these conditions enzymatic inactivation would not contribute significantly to drug metablism. Furthermore, plasma ara-A disappearance could be modeled by an exponential decay curve (Fig. 1). Under these conditions, a one-compartment model analysis was performed and the apparent volumes of distribution [7] were calculated to be 119 l and 90 l for patients I and II, respectively. These volumes exceed total body water and indicate that ara-A is sequestered in tissues.

Serum Adenosine and Deoxyadenosine. Micromolar amounts of adenosine and deoxyadenosine were identified in both patients. In patient I, plasma adenosine was not detectable but micromolar amounts of deoxyadenosine were found during the second course of treatment, with a highest value of 6.6 μg/ml obtained on day 3 (Table 3). Adenosine and deoxyadenosine were both identified in the plasma of patient II but at concentrations at the limit of detectability by our assay.

Clinical Response and Toxicity

Abrupt declines (> 90%) in the circulating blast count were observed in both patients following therapy. These effects were transient with reappearance of blast by day 6 following treatment. No toxicity was observed during the five courses of treatment.

Discussion

Ara-A is cytotoxic when human leukemic lymphoblasts are exposed to micromolar concentrations of drug [5]. This

cytotoxic effect depends on total cellular exposure to ara-ATP [15] and correlates with the extent of ara-A residues incorporated in DNA [11]. Ara-A has been administered to patients in doses of up to 1 g/m^2/day for 10 days with limited clinical activity [1]. The plasma levels of ara-A were not monitored in these studies and it is unclear whether these doses achieved therapeutic levels of ara-A. The clinical efficacy of ara-A is limited by intracellular and plasma deamination to the inactive metabolite ara-hyp. Dose escalations have also been limited by drug solubility.

Ara-A (1.8 g/m^2/day × 5 days) has recently been administered to patients in combination with DCF (10 mg/m^2/day × 5 days) [16]. The toxicity with the combination was minimal; however, this investigation did not monitor the effects of DCF on the pharmacokinetics of ara-A. Our studies demonstrate that the administration of DCF (10 mg/m^2) significantly prolongs the half-life of ara-A and results in plasma levels of ara-A that are cytotoxic in vitro. This dose schedule of DCF (10 mg/m^2/day × 3 day) has been shown to be non-toxic [10], and it does not lead to accumulation of plasma deoxyadenosine or adenosine that might compete with ara-A for incorporation in DNA.

Plasma ara-hyp is detectable when the initial dose of DCF is administered 1 h prior to the ara-A infusion. However, the ara-hyp becomes undetectable when DCF is administered on the second day of treatment. This finding suggests that ADA activity is not completely inhibited by this initial dose of DCF and that additional amounts of drug are required to completely inhibit deamination. It will now be necessary to monitor the ratios of intracellular ara-ATP and dATP to determine whether the inhibition of adenosine deaminase results in elevations of dATP pools sufficient to antagonize the effects of ara-ATP [16]. Three daily doses of DCF (10 mg/m^2) result in minimal accumulations of plasma adenosine or deoxyadenosine. This duration of treatment may be preferable to 5-day courses which have resulted in marked plasma elevations of these metabolites [10]. It should now be possible to maximize the therapeutic response of the DCF/ara-A combination by monitoring appropriate biochemical and pharmacokinetic parameters.

Acknowledgements. This work was supported in part by American Cancer Society grant no. CH-199 and by a Faculty Research Award from the American Cancer Society (DWK).

The authors would like to thank Carolyn Klebart for her excellent secretarial assistance.

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Received March 15/Accepted July 6, 1982