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Phase I clinical trial of fludarabine phosphate (F-ara-AMP)*

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Summary. F-Ara-AMP (fludarabine phosphate) is an adenosine analogue that is resistant to deamination; it is a more potent cytotoxic compound than ara-A in experimental tumor systems. F-Ara-AMP was given by continuous IV infusion over 5 days once every 4 weeks to 27 evaluable adult patients with advanced cancer. The median Karnofsky performance status was 70% (range 50%–90%), and the median age was 58 years (range 41–74). In addition to adequate blood counts, a creatinine clearance of at least 60 ml/min was required. The initial dose level was 35 mg/m^2/day. Dose-limiting myelosuppression was seen in the first patient. Subsequent patients were treated at lower doses. Myelosuppression was the only major toxicity. Leukopenia was generally more prominent than thrombocytopenia, but 2 patients experienced prolonged thrombocytopenia which prevented further therapy. Nausea was minimal, and neither renal nor neurologic toxicity was encountered. In patients with good renal function a dose of 25 mg/m^2/day can be safely administered. However, because of apparent cumulative myelosuppressive effects a lower dose is more appropriate for patients who have had extensive prior chemotherapy or radiotherapy.

Introduction

Ara-A (9-β-D-arabinofuranosyladenine) is one of several adenosine analogue possessing antiviral and antitumor activity [1, 9, 11]. The clinical use of this drug as an antineoplastic agent is limited, however, by its rapid hydrolysis to ara-H (9-β-D-arabinofuranosylhypoxanthine) via adenosine deaminase [8]. In experimental systems, the antitumor effects of ara-A are enhanced by concomitant use of 2'-deoxycoformycin, an inhibitor of adenosine deaminase [4].

The observation that 2-fluoroadenine is a poor substrate for this enzyme has stimulated interest in fluorinated analogue of ara-A. A number of analogues have been synthesized and screened for antitumor activity; F-ara-A (9-β-arabinofuranosyl-2-fluoroadenine, fludarabine) is one such compound. It is resistant to deamination, and is a more potent antitumor agent than ara-A in sensitive systems [2], but is not sufficiently soluble for clinical use. Fludarabine phosphate (F-ara-AMP, Fig. 1) was synthesized as a water-soluble form of F-ara-A.

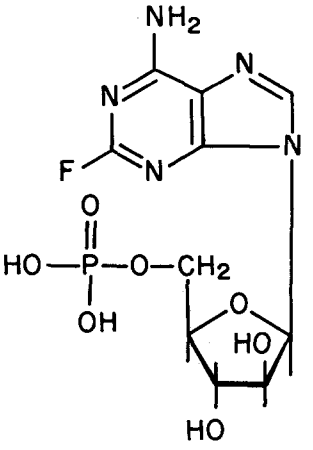

Fig. 1. Structure of F-ara-AMP (fludarabine phosphate)

Phosphorylation of F-ara-A to F-ara-ATP is required for antitumor activity. F-ara-ATP is a potent inhibitor of ribonucleotide reductase and of DNA α-polymerase [13]; it appears to selectively inhibit DNA synthesis, with little effect on the synthesis of RNA or protein. A "self-potentiation" mechanism has been suggested for F-ara-A; this might occur by its inhibition of the synthesis of dATP, a compound which competes with F-ara-ATP for DNA α-polymerase [12].

F-Ara-AMP is active in L1210 leukemia and the LX-1 human lung tumor xenograft; it has limited activity in the CD8F$_1$ mammary tumor and in P388 leukemia [6]. In schedule-dependency testing, optimal activity was seen with a schedule involving 5 doses daily. Toxicologic evaluation with this schedule identified the mouse LD$_{10}$ of F-ara-AMP phosphate as 1118 mg/m^2/day. Mice demonstrated decreased activity, dehydration, and myelosuppression at toxic doses. No toxic signs were noted in dogs at one-tenth of this dose. At higher doses, dogs exhibited emesis, diarrhea, myelosuppression, hepatic dysfunction, and renal injury [6].

This clinical phase I trial was conducted to identify a safe dose of F-ara-AMP administered by continuous IV infusion for 5 days once every 4 weeks in adults with advanced solid tumors.

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Patients and methods

All patients had pathological proof of cancer and had tumors resistant to conventional modalities of treatment. A Karnofsky performance status of at least 50%, a white blood count of 4000/mm^3 or greater, and a platelet count of 150 000/mm^3 or greater were required, as were a normal serum bilirubin (< 1.4 mg/dl) and a creatinine clearance of at least 60 ml/min.

F-Ara-AMP was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, Md) in sterile vials containing 200 mg prepared as a white lyophilized powder with sodium hydroxide to adjust the pH. The drug was reconstituted by adding 2 ml sterile water for injection (USP) to the vial. The appropriate dose for each patient was prepared fresh daily and added to 1000 ml 5% dextrose in water (USP) and given as a continuous 24 h IV infusion daily for 5 days. The initial dose of F-ara-AMP in this study was 35 mg/m^2/day, approximately 1/30th of the murine LD_{10}.

For each patient automated blood count, serum creatinine, and 12-channel biochemical profile were obtained at least once weekly. As the study progressed, automated blood counts were obtained more frequently. Qualitative toxicities were graded on a scale of 0 to 4+. Standard criteria for therapeutic response were employed when applicable. A course was considered evaluable if the 5 day treatment was completed and if the patient was observed for 21 days. In addition, if dose-limiting toxicity was encountered but the patient died with progressive disease within 21 days, the course was also considered evaluable.

Table 1. Characteristics of 27 evaluable patients treated with F-Ara-AMP

Age (years)			
	Median		58
	Range		41–74
Performance status (Karnofsky)			
	Median		70
	Range		50–90
Sex			
	Male		19
	Female		8
Prior therapy			
	None		2
	Chemotherapy only		8
	Radiotherapy only		2
	Chemotherapy + radiotherapy		15
Diagnosis:			
	Lung cancer		12
	Adenocarcinoma	4	
	Epidermoid carcinoma	5	
	Small cell carcinoma	3	
	Sarcoma		4
	Colon cancer		3
	Melanoma		3
	Breast cancer		2
	Unknown primary adenocarcinoma		1
	Head/neck epidermoid carcinoma		1
	Lymphocytic lymphoma		1

Results

Thirty-one patients entered this study. Table 1 shows the characteristics of the 27 patients who received evaluable courses of F-ara-AMP.

Although the initial daily dose was 35 mg/m^2, only one patient was entered at this level. This patient, like several patients treated with the initial dose level at other institutions, exhibited moderate leukopenia [5, 7]. The next dose evaluated, therefore, was 20 mg/m^2; three additional dose levels were also studied (Table 2). In no patient was the dose escalated to a level greater than the first dose received; the dose was reduced in the first patient because of the myelosuppression encountered.

The only major toxicity was hematologic (Table 2). With two exceptions, all patients had some degree of myelotoxicity within 3 weeks of their first dose of F-ara-AMP, and myelosuppression was frequently limiting at doses of 27.5 mg/m^2/day and higher. In general, marrow toxicity was manifested as leukopenia, with only occasional thrombocytopenia. The WBC nadir was seen at approximately 2 weeks after the first dose of F-ara-AMP. When it occurred, thrombocytopenia was seen at approximately 3 weeks from the day of the first dose of F-ara-AMP. Eleven patients received more than one 5-day course of F-ara-AMP at the same dose. Six had more pronounced leukopenia with their second course of treatment than with their first. Of the six, two had persistent thrombocytopenia which prevented further cytotoxic therapy. Both had received prior chemotherapy, and one had extensive prior chemotherapy and radiotherapy. Thus, there appeared to be cumulative marrow toxicity, especially in previously treated patients. Drug-related anemia was not observed.

Of the 27 evaluable patients, 4 had transient vomiting during the 5-day infusion, and an additional 5 had nausea without vomiting. Six patients reported mild diarrhea at some point during therapy. One patient developed a creatinine level of 3.4 mg/dl 2 weeks after F-ara-AMP treatment; this occurred in the setting of progressive disease and intravascular volume depletion and was probably not related to the drug.

Table 2. Hematologic toxicity of the first course of F-ara-AMP in 27 evaluable patients

Dose (mg/m^2)	Number of patients		WBC nadir ($\times 10^3$/mm^3)			Platelet nadir ($\times 10^3$/mm^3)		
	Entered	Evaluable	Median	Range	Day	Medium	Range	Day
20	5	5	3.2	2.4–3.5	13	165	92–245	15
25	8	7	3.3	1.3–7.8	13	200	106–428	20
27.5	11	8	1.8	0.5–4.3	11	262	99–420	11
30	6	6	1.8	0.4–6.7	14	198	39–476	23
35	1	1	1.0		14	132		14

Four patients complained of fatigue, which generally coincided with the period of leukopenia. One man complained of visual hallucinations suggestive of delerium tremens during therapy. He had a history of ethanol abuse in the remote past, but denied recent ethanol use or withdrawal. At the time of the hallucinations he was receiving narcotic analgesics and was dyspneic. The symptoms did not recur following the 5-day treatment. No other acute neurologic symptoms were reported by patients in this study.

Most patients did not have measurable lesions, and therefore, therapeutic responses could generally not be quantitated. However, one patient with an epidermoid carcinoma of the base of the tongue experienced $<50\%$ reduction in a metastatic chest wall lesion; this response lasted 1 month. Two patients with advanced colon cancer had transient reduction in serum CEA, but no clinical benefit could be documented.

Discussion

This phase I clinical study demonstrates that F-ara-AMP can be safely administered to patients with advanced solid tumors by continuous IV infusion at a dose of 25 mg/m^2/day daily for 5 days. The acute dose-limiting toxicity was leukopenia. Considerable variation between patients was noted in the degree of myelosuppression, and some patients can certainly tolerate higher doses. This difference did not appear to be related solely to extent of prior therapy, and may be related to differences in drug metabolism between patients. Since pharmacokinetic studies were not performed as part of this clinical trial, this cannot be definitely stated.

Six patients treated with more than one course of F-ara-AMP exhibited greater myelosuppression following their second course than following the initial 5-day treatment. Furthermore, although thrombocytopenia was uncommon following the first treatment, profound and persistent thrombocytopenia was observed in two patients following their second courses of therapy. Both of these patients had received other myelosuppressive chemotherapy prior to treatment with F-ara-AMP. This pattern of myelosuppression may reflect exhaustion of the marrow stem cell component, and therefore has important implications for further testing and extended use of this drug.

Nonhematologic toxicities were generally mild, and not clearly dose dependent. Overall the drug was extremely well tolerated in terms of patients symptoms.

Similar clinical toxicity has been reported with similar total doses of this drug but different schedules of administration [5, 7]. Regardless of the schedule, however, the myelosuppressive potency of this drug is far greater than that predicted by preclinical toxicology studies in mice and dogs. Laboratory animals tolerated far higher doses of F-ara-AMP; perhaps this explains the absence of the anticipated clinical toxicites, such as renal injury. The difference in toxicity between species may be explained by the pharmacokinetic behavior of the drug. In vivo, F-ara-AMP is rapidly dephosphorylated [5]. Thereafter, F-ara-A is extensively deaminated in the dog, but not in man [5]. In man, F-ara-A has a long terminal half-life of 9–10 h [5, 7] compared to a terminal half-life of 1.6 h in dogs [5], and the total body plasma clearance of F-ara-A in the dog is nearly twice that observed in man.

F-ara-AMP is not simply a biologic or biochemical equivalent of Ara-C plus deoxycoformycin (dCF). In cells, F-ara-A is phosphorylated by deoxycytidine kinase, unlike ara-A which is phosphorylated by both deoxycytidine and adenosine kinases. F-ara-A is inactive against L1210 resistant to ara-C, whereas ara-A + dCF is active in that system which lacks deoxycytidine kinase [2]. Leukemia P388 resistant to ara-C + dCF is sensitive to F-ara-A, however [10]. The mechanism of resistance in this system is not understood. In addition, in the sensitive L1210 leukemia, F-ara-A is most active on a daily schedule, whereas the combination of ara-A plus dCF shows minimal activity when used daily [10].

These clinical and laboratory observations suggest that further clinical evaluation of F-ara-AMP is indicated. Based on available human pharmacokinetic data, a continuous infusion of the drug is not necessary to achieve continuous plasma concentrations of F-ara-A [5]. Phase II studies can be initiated at a dose of 25 mg/m^2/day daily for 5 days in patients with good performance status, adequate blood counts, and good renal function, but dose modification may be necessary to avoid cumulative myelosuppression, especially in pretreated patients. Phase I studies in a patients with acute leukemia can be initiated at the same dose.

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