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Pharmacokinetic study of fludarabine phosphate (NSC 312887)*

Marla R. Hersh[1, 2], John G. Kuhn[1, 2], Jerry L. Phillips[1, 3], Gary Clark[1], Thomas M. Ludden[1, 2], and Daniel D. Von Hoff[1]

[1] Department of Medicine and [2] School of Pharmacy, University of Texas Health Science Center at San Antonio,
[3] Audie L. Murphy Memorial Veterans Hospital and the Cancer Therapy and Research Center, San Antonio, TX 78284, USA

Summary. Characterization of the pharmacokinetics of 2-FLAA has been completed in seven patients receiving 18 or 25 mg/m^2 daily $\times 5$ of 2-FLAMP over 30 min. Assuming 2-FLAMP was instantaneously converted to 2-FLAA, the plasma levels of 2-FLAA declined in a biexponential fashion. Computer fitting of the plasma concentration-time curves yielded an average distribution half-life ($t_{1/2}\alpha$) of 0.60 h and a terminal half-life ($t_{1/2}\beta$) of 9.3 h. The estimated plasma clearance was 9.07 ± 3.77 l/h per m^2 and the steady state volume of distribution, 96.2 ± 26.0 l/m^2. There was a significant inverse correlation between the area under the curve (AUC) and absolute granulocyte count ($r = -0.94$, $P < 0.02$). A relationship between creatinine clearance and total body clearance was noted, but was not statistically significant ($r = 0.828$; $P < 0.1$). Approximately $24\% \pm 3\%$ of 2-FLAA was excreted renally over the 5-day course of drug administration.

Fludarabine phosphate (9-β-arabinofuranosyl-2-fluoroadenine-5'-mono-phosphate), 2-FLAMP, is a novel nucleotide analogue of adenine mononucleotide and adenine arabinoside. It was designed to be more soluble and resistant to deamination than adenine arabinoside, because of the fluorine atom substituted at the 2-position on the purine ring [3]. 2-FLAMP is believed to be dephosphorylated by serum phosphatases to 2-fluoro-ara-A (2-FLAA) [9]. The 2-FLAA is probably phosphorylated by deoxycytidine kinase and then converted to the active triphosphate derivative [4].

A pharmacokinetic study was conducted in conjunction with our phase I clinical trial to characterize the disposition of 2-FLAMP in humans [5].

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Offprint requests to: J. G. Kühn, Department of Medicine, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284, USA

Abbreviations used: 2-FLAA-9-β-D-arabinofuranosyl-2-fluoroadenine; 2-FLAMP, the 5'-monophosphate of 2-FLAA, also known as fludarabine phosphate; AUC, area under the curve; AGC, absolute granulocyte count; TPC, total plasma clearance; Vd_{ss}, volume of distribution at steady state; Vd, volume of distribution; Creat Cl, creatinine clearance; SGOT, serum glutamic-oxaloacetic transaminase; WBC, peripheral white blood cell count

Materials and methods

Patient selection and characteristics. Seven adult patients, six men and one woman, with a median age of 57, completed the study. All patients had histologically documented tumors refractory to all conventional modalities; most had adequate blood counts (white blood counts $>4000/\mu l$, platelets $>100000/\mu l$) and adequate liver and renal function (bilirubin <2.0 mg/dl, SGOT <1.5 times normal, serum creatinine <2.0 mg/dl, and creatinine clearance >35 ml/min). Two patients whose laboratory values were not within these specified ranges were entered. Patient #4 had an elevated alkaline phosphatase (180 nl 30–115 units/l) due to bone metastases documented by CT scan. Patient #5 had an elevated alkaline phosphatase due to liver metastases (266) documented by liver-spleen scan. Written informed consent was obtained from all patients according to institutional and federal policies.

Treatment schedules. 2-FLAMP was obtained from the National Cancer Institute (NCI) as a sterile lyophilized powder, 200 mg/vial, with no preservatives. The drug was reconstituted with 2 ml sterile water USP. The prescribed dose of 2-FLAMP was removed and further diluted in 100 ml 0.9% sodium chloride. The drug was administered i. v. over 30 min through a free-flowing i. v. line daily for 5 days every 28 days. Pharmacokinetic studies were performed on days 1 through 5 during the first course of treatment in all patients receiving either 18 or 25 mg/m^2 per day.

Blood and urine sampling. Blood samples 8 ml in volume were obtained through a heparin lock at ten time intervals (time 0, 5, 10, 20, 40, and 60 min and 2, 4, 8, and 12 h) after the infusion on days 1 and 5. On days 2, 3, and 4 only a pre-dose sample was obtained. A total of 22 blood samples were collected from each patient with the exception of patient #6, whose day-5 samples were inadvertently omitted. Blood was collected in heparinized tubes and immediately centrifuged by cold centrifugation at 4 °C at 2500 *g*. Plasma samples were deproteinized in Amicon CF conical filters by centrifugation at 1000 *g* for 20 min, and the ultrafiltrates were flash frozen. The 24-h urines were collected daily for 6 days. Urines were processed using a common precipitate method as follows. Samples of 250 µl urine were each placed in a centrifuge tube and 500 µl 0.3 *N* $Ba(OH)_2$ was added, after which the tube was vortexed.

Then 500 µl 5% $ZnSO_4 \cdot 7H_2O$ was added and the sample revortexed. The mixture was then centrifuged at 1700 *g* for 15 min, and the supernatant was collected for analysis. All samples were filtered through 0.45-µm filters before HPLC analysis. Plasma and urine samples were maintained at −20 °C until analysis.

HPLC analysis. An HPLC assay for 2-FLAA was developed using a modification of the assays reported by Plunkett et al. [10]. The assay for 2-FLAA was developed using a Beckman model 314 chromatograph at a wavelength of 254 nm. Analytical grade 2-FLAA was obtained from the Drug Synthesis and Chemistry Branch of the NCI. 2-FLAA was separated from other nucleosides on a 25 cm × 4.6 mm ID Altex Ultrasphere C18 reverse-phase column (5 µm) with 10 m*M* $NH_4H_2PO_4$ (pH 4.15) containing 6% methanol. At a flow rate of 1–1.2 ml/min, 2-FLAA eluted at approximately 55 min.

The lower limit of detection for 2-FLAA was approximately 12.5 pmol. The assay for 2-FLAA clearly resolved 2-FLAMP from 2-FLAA. Drug recovery from plasma samples was 80%±2%, while recoveries from urine samples was ≥98%.

Pharmacokinetic analysis. Analysis was performed by nonlinear least-squares regression analysis (NON-LIN) with a weighting of 1/y [8]. The plasma concentration-time data were fitted to two- and three-compartment models using a zero-order infusion input and first-order elimination for all samples obtained on days 1 through 5. The two-compartment model was parameterized in terms of central and peripheral volume of distribution and tissue and plasma clearances. The volume of distribution at steady state (Vd_{ss}) was taken as the sum of the central and peripheral volumes of distribution. Tissue clearance describes intercompartmental clearance and represents the movement of drugs into and out of the peripheral compartment [6]. The *F*-test [2] and Akaike information criterion [12] were applied to determine the best presentation for the time-course data.

Results

In vitro studies indicate that 2-FLAMP is rapidly dephosphorylated to 2-FLAA [9]. This also appears to occur in man, because none of the parent compound was detected in any of the patients' plasma 5 min after discontinuation of the infusion. The following results represent data from six of the seven patients studied. The pharmacokinetic data from one patient (≠2) are omitted from the mean and standard deviation, because his calculated pharmacokinetic parameters were greater than 2 standard deviations from the mean (Table 1). Although this patient's terminal half-life was greater than 30 days, his peak concentration on day 5 was only 0.377. There is no clear explanation of this phenomenon. It is of note that patient ≠2 had a normal creatinine clearance and no laboratory evidence of liver dysfunction.

As noted in Table 1, peak plasma levels of 2-FLAA ranged from 0.199 µg/ml to 0.88 µg/ml and appeared to be related to the dose and rate of infusion. Mean peak plasma levels of 2-FLAA on days 1 and 5 in patients receiving 18 mg/m² were 0.39 and 0.51 µg/ml, respectively, and in those receiving 25 mg/m², 0.57 and 0.54 µg/ml. These concentrations may be useful for in vitro drug sensitivity studies [11]. There was no accumulation of the drug during the 5-day treatment schedule.

A representative plasma decay curve for 2-FLAA after a dose of 25 mg/m² per day for 4 days is illustrated in Fig. 1. Assuming instantaneous conversion of 2-FLAMP to 2-FLAA, the two-compartment model best described the pharmacokinetic profile of 2-FLAA. The kinetic parameters for the patients are shown in Table 1. The central volume of distribution was 37.8 ± 15.4 l/m², with a Vd_{ss} of 96.2 ± 26.0 l/m². The average tissue clearance was 20.1 ± 10.9 l/h per m², and the plasma clearance was 9.07 ± 3.77 l/h per m². Plasma levels declined in a biexponential fashion with a harmonic mean distribution half-life ($t_{1/2}\alpha$) of 0.6 h and an elimination half-life ($t_{1/2}\beta$) of 9.2 h. Approximately 24% of 2-FLAMP was excreted in the urine as 2-FLAA during the 5-day treatment schedule (Table 2).

Table 1. 2-FLAA kinetic parameters

Patient	BSA (m²)	Dose		Duration of infusion (min)		Peak conc. µg/ml		Clearance rates (l/h per m²)		Volumes of distribution (l/m²)		$t_{1/2}$ (h)	
		mg/m²	mg	Day 1	Day 5	Day 1	Day 5	Plasma	Tissue	VD_{ss}	$VD_{central}$	α	β
1	1.57	18	27	32	30	0.285	0.285	13.43	28.3	115.4	48.6	0.59	7.0
2[a]	1.74	18	31	25	30	0.199	0.377	1.51	28.1	1629.9	75.3	1.69	787.5
3	1.62	18	29	38	30	0.693	0.856	4.35	19.8	59.8	16.1	0.37	10.7
4	1.90	25	48	30	30	0.876	0.611[b]	10.38	23.8	91.9	22.9	0.39	7.8
5	1.94	25	48	35	30	0.509	0.550	8.30	5.1	86.4	46.8	1.99	10.6
6	1.74	25	43	33	30	0.550	–[c]	5.28	9.9	88.6	37.0	1.26	13.9
7	2.06	25	51	30	30	0.336	0.458[b]	12.71	33.8	135.2	55.2	0.59	8.44
Mean								9.1	20.1	96.2	37.8	0.89[d]	9.22[d]
SD								3.8	10.9	26.0	15.4	–	–

[a] Patient omitted from calculation of mean and SD
[b] Day 5 levels drawn on day 4
[c] Day 5 levels not studied
[d] Harmonic mean half-life $\left(\frac{n}{\Sigma \frac{1}{x}}\right)$

Table 2. Urinary excretion of 2-FLAA

Patient	% Dose in urine						Creatinine clearance ml/min
	Day 1	Day 2	Day 3	Day 4	Day 5	5-Day average	
1	14	25	31	7	53	26	76
2	72	16	19	14	9	25	73
3	28	29	29	24	7	24	37
4	25	12	20	38	–	24	77
5	20	20	14	20	13	17	59
6	14	23	27	18	35	23	50
7	17	25	35	45	8	26	73
Mean	27	21	25	24	21	24	63
±SD	21	6	7	13	19	3	15

The urines of all patients were analyzed for the presence of 2-fluoro-adenine [1]. The metabolite was only detected in two of the seven patients' urine ($\neq 2$ and $\neq 4$) (personal communication from Dr Vassilios Avramis).

In an attempt to apply these pharmacological parameters to future trials, a variety of clinical parameters were examined to determine their correlation to the area under the curve (AUC) and the pharmacokinetic parameter, total plasma clearance (TPC). Since the dose-limiting toxicity of 2-FLAMP is granulocytopenia, the clinical parameters were again examined to determine their relationship to absolute granulocyte count. The clinical parameters studied included creatinine clearance, serum creatinine, SGOT, alkaline phosphatase, total bilirubin, hemoglobin, and hematocrit. A correlation was noted between the decreasing absolute granulocyte count and the AUC. As noted in Table 3, which is arranged according to granulocyte nadir, the severity of granulocytopenia increased as the AUC increased. The Spearman rank correlation coefficient between absolute granulocyte count and AUC was -0.94 ($P<0.02$). Since absolute granulocyte count correlated to AUC and AUC is influenced by the dose of the drug and the TPC of the drug, the absolute granulocyte count would be expected to be related to the TPC. The Spearman rank correlation coefficient between absolute granulocyte count and TPC was 0.94, which is significant at the 0.02 level. The TPC of 2-FLAA would be expected to be influenced by both renal and hepatic elimination. The correlation coefficient between creatinine clearance and TPC was 0.828 ($0.05 < P < 0.01$). As more patients accrue, these data may become statistically significant. No correlation was noted between TPC and any of the clinical measurements of liver function.

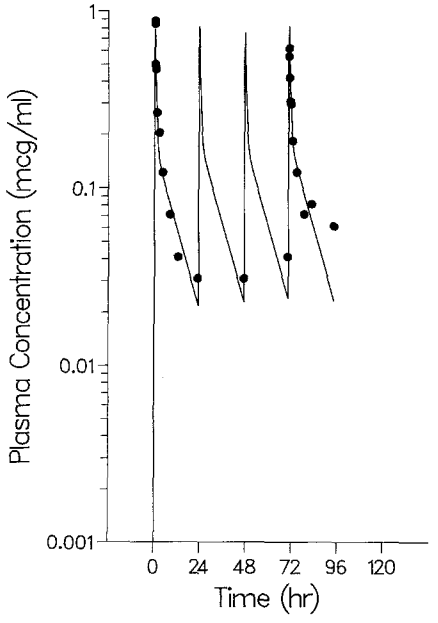

Fig. 1. Plasma concentration-time profile of 2-FLAA in plasma. The patient ($\neq 5$) received 30-min infusions of 2-FLAMP (25 mg/m^2 per day) i. v., ending at times 0.5, 23.75, 47.8, and 70.75 h. The *solid line* is the computer-generated line of best fit and the *filled circles* indicate observed concentrations

Discussion

We have found that 2-FLAMP is rapidly dephosphorylated to 2-FLAA. Assuming instantaneous conversion of 2-FLAMP to 2-FLAA, the plasma levels of 2-FLAA decline in a biexponential fashion. Computer fitting of the plasma concentration-time curves yielded an average $t_{1/2}\alpha$ of 0.60 h and a $t_{1/2}\beta$ of 9.2 h. The estimated TPC was 9.07 l/h per m^2 and the Vd_{ss} was 96.2 l/m^2. There was a significant correlation between the AUC and absolute granulocyte count.

Several interesting pharmacokinetic parameters were noted. The mean Vd_{ss} was 96 l/m^2, which is about twice that of body weight, suggesting that tissue binding of the drug occurs. 2-FLAA is cleared from plasma with a termi-

Table 3. Comparison of AUC with absolute granulocyte nadir and creatinine clearance

Patient	Dose mg/m^2 per day × 5	AUC[a] mg-h per l	AGC	Creatinine clearance ml/min
1	18	6.4	3999	76
7	25	9.73	1916	73
4	25	12.2	624	77
5	25	14.9	608	59
6	25	23.4	299	50
3	18	20.5	176	37

[a] Days 0–5

nal half-life of about 9 h. Although only 24% of the drug was renally excreted during the 5-day treatment schedule, patients with decreased renal function appeared to experience greater toxicity from the drug, as noted in Table 3. This is based on the association between creatinine clearance and TPC and the relationship between TPC and AUC. If the creatinine clearance was ≤ 50 ml/min the absolute granulocyte count seemed particularly low (≤ 208).

Malspies et al. [7] described the plasma elimination of 2-FLAA after a single i. v. bolus dose of 2-FLAMP as triphasic with a terminal half-life of 10 h a Vd_{ss} of 44 l/m^2, and a total body clearance of 69.0 ml/min per m^2 (4.1 l/h per m^2). The discrepancies between the two- and three-compartment models described for 2-FLAA are probably due to differences in the sampling schedules. Our first sample was drawn 5 min after the end of the infusion. The $t_{1/2\alpha}$ reported by Malspies was 5.42 min, so we may have missed the initial rapid distribution phase of 2-FLAA. Since both plasma clearance and Vd_{ss} were only about one-half of that observed in our patients, the terminal half-lives of the drug were very similar, at 9 h vs 10 h.

Our data on 2-FLAMP kinetics revealed a positive correlation between granulocytopenia, the dose-limiting toxicity of 2-FLAMP, and the TPC of 2-FLAA. Although the correlation between creatinine clearance and toxicity was not statistically significant, a positive relationship was noted. Unfortunately, we cannot make a definitive dosage recommendation for patients receiving 2-FLAMP with poor renal function, because only two patients studied had creatinine clearances ≤ 50 ml/min. However, patients receiving 2-FLAMP whose creatinine clearances are less than 50 ml/min should be closely monitored. Further kinetic studies are necessary for more accurate definition of the relationship among a patient's clinical parameters (such as creatinine clearance) and pharmacokinetic parameters, and granulocytopenia, the major toxicity of 2-FLAMP.

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