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Pharmacokinetics of 1-β-D-Arabinofuranosylcytosine in Humans[1]

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SUMMARY

The pharmacokinetics of 1-β-D-arabinofuranosylcytosine (ara-C) in humans following rapid i.v. administration and slow i.v. infusion is reported, as is a high-pressure liquid chromatographic method for ara-C determination in biological fluids. Plasma concentrations of ara-C following a single i.v. dose of 100 mg had a biphasic decline with a mean terminal half-life of 2.62 hr. The half-life following a 4- to 5-day infusion was 2.47 hr. ara-C is rapidly deaminated to 1-β-D-arabinofuranosyluracil. The ratio of ara-C to 1-β-D-arabinofuranosyluracil excreted in urine in 48 hr was 1:4 after infusion and 1:6 after rapid injection. Most of the ara-C was excreted within 5 hr. Total drug and metabolite excretion was 70% in 48 hr. ara-C infusion was administered according to a Southwest Oncology Group treatment protocol in which the loading dose was one-fourth of the infusion dose over 4 hr. With this treatment schedule, minimum steady-state plasma concentrations were not achieved in most patients by 4 hr. In order to reach plasma concentrations rapidly, a schedule of a loading dose 3 times the hourly infusion rate is suggested.

INTRODUCTION

ara-C[3] is a pyrimidine analog with significant antitumor activity in animal models (6). Moreover, it is currently one of the most successful drugs in the treatment of adult acute granulocytic leukemia (2, 5, 10, 12). These effects are due to a profound disturbance in DNA metabolism apparently resulting from a selective inhibition of DNA polymerase (2, 9).

Prior pharmacokinetic studies have demonstrated that ara-C is rapidly deaminated to ara-U, has a short plasma half-life, and is poorly absorbed from the gastrointestinal tract (1, 3, 4, 7, 11, 14, 16, 19, 20). Recently, Ho and Frei (13) studied the pharmacology of ara-C in humans. They demonstrated, in contrast to previous reports, that the plasma disappearance of ara-C was biphasic, with a short half-life of 11 min and a longer plasma half-life of 111 min. In addition, they demonstrated dose-related pharmacokinetics which suggested that the deamination of ara-C was a saturable process.

Since there was some variance in the reported literature and since all of the studies to date followed the plasma decay for only a short time interval (3 to 8 hr), we have determined the pharmacokinetics of ara-C-^{3}H in patients with cancer, either after a single i.v. dose or during a constant infusion following a loading dose. This report summarizes our findings and compares results of the separation of ara-C and ara-U by both paper chromatography and high-pressure liquid chromatography.

MATERIALS AND METHODS

Patients. Fourteen patients, 21 to 74 years of age, were studied. Five had acute granulocytic leukemia and 9 had inoperable solid tumors. All of the patients had normal renal function (creatinine less than 1.5 mg/100 ml) and no evidence of myelosuppression at the time of the study. After obtaining informed consent, the patients were admitted to the Clinical Research Center for the studies. Whereas the patients with solid tumors received no other drugs, those with leukemia received a variety of medications including vincristine, prednisone, thioguanine, antibiotics, and other medications considered necessary for their adequate treatment.

Drug Administration. Chromatographically pure ara-C-^{3}H was purchased from New England Nuclear Corp. (Boston, Mass.) and mixed with commercial ara-C (Cytosar, Upjohn Co., Kalamazoo, Mich.) prior to administration. The single injection was 100 mg of ara-C containing 300 μCi ara-C-^{3}H/sq m. For the steady state infusions, the drug was administered according to an Oncology Group protocol. ara-C, 210 mg/24 hr was infused with a constant-rate infusion pump, except for 1 patient (R. M.) who received the drug by i.v. drip. Three patients (L. B., J. L., and J. T.) received ara-C as a continuous infusion for 5 days. During the final 4 hr, ara-C-^{3}H (300 μCi/sq m) was added to the infusion without changing the total dose of drug. Following a loading dose of 10 mg/sq m, 40 mg/sq m was administered during a 4-hr infusion on 5 consecutive days to Patients R. M., L. J., and E. B. On the 5th day, the ara-C administered contained ara-C-^{3}H (6 μCi/mg ara-C). Three subjects (V. D., A. L., and W. W.) without prior ara-C therapy received only one 4-hr infusion of ara-C which was equivalent to Day 5 for the previous group.

Sample Collection. Following administration of ara-C-^{3}H, blood, urine, and feces were collected at varying inter-

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[3] The abbreviations used are: ara-C, 1-β-D-arabinofuranosylcytosine; ara-U, 1-β-D-arabinofuranosyluracil.

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vals for 48 hr. Blood was collected in ice-cold tubes that contained 143 units sodium heparin and 10^{-4} M tetrahydrouridine, an inhibitor of blood deaminase (17). After mixing, the tubes were immediately placed in an ice bath and processed at 4° according to the method reported by Ho and Frei (13).

Sample Analysis. Total radioactivity of the samples was determined following oxidation to ^3H_2O. The concentration of ara-C was determined by both paper chromatography (13) and high-pressure liquid chromatography. The high-pressure liquid chromatography was performed on a Nester

Table 1

Comparison of paper chromatographic and high-pressure liquid chromatographic analyses of ara-C in urine

Time (hr)	Amount excreted (μM)	
	Paper chromatography	High-pressure liquid chromatography
4	19.70	19.56
6	9.16	11.48
8	3.75	3.10
10	1.76	2.12
12	1.01	1.12
24	4.20	3.43
36	1.02	0
48	0.42	0

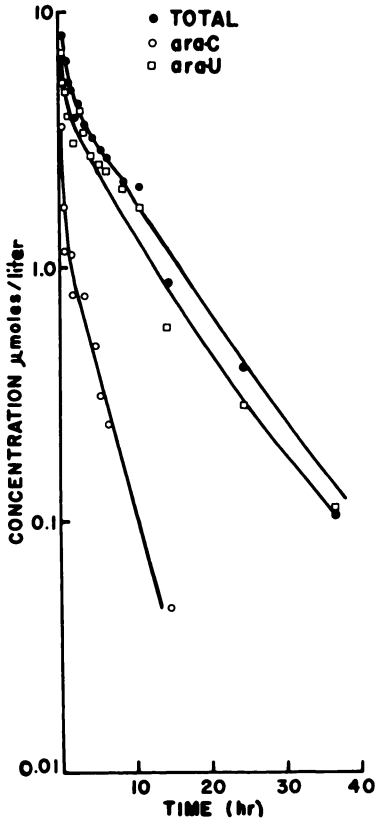

Chart 1. Plasma concentrations of ara-C, ara-U, and total drug following i.v. administration of 100 mg ara-C; Patient A. S.

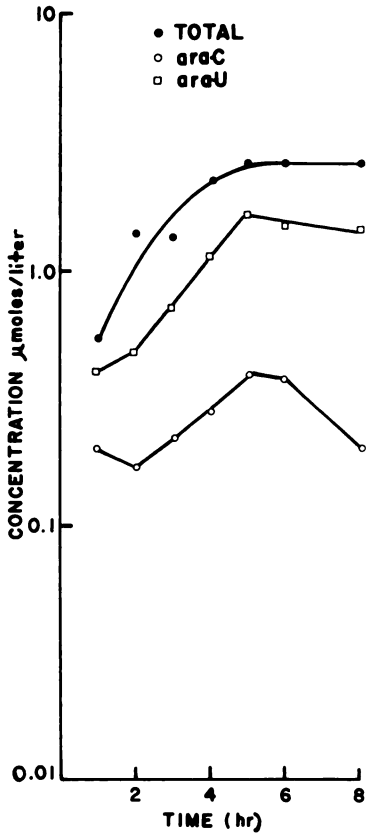

Chart 2. Plasma concentrations of ara-C, ara-U, and total drug during and after infusion of ara-C; Patient J. L.

Faust 1240, with a 254-nm UV detector. The 13-cm steel column was packed with Aminex A-7, a cation-exchange resin. The eluting buffer was 0.02 M potassium phosphate: potassium chloride, pH 4.3. The pressure was maintained at 1400 psi. Under these conditions, ara-C elutes from the column at 12 min and ara-U, at 4 min. However, ara-U was obscured by an endogenous compound with the same retention time.

RESULTS

The ara-C concentration in urine measured by the paper chromatographic method and by liquid chromatography is shown in Table 1. There is good correlation between the values obtained by the 2 different methods. However, the liquid chromatographic method is considerably faster and does not require that radioactivity be given the patient. The measurement of ara-U in plasma by the liquid chromatographic method was interfered with by the endogenous compounds present. However, if future separation of these peaks is accomplished, it would be possible to measure ara-U concentrations as low as 0.1 μg/ml.

The plasma half-lives of ara-C and ara-U after i.v. injection and infusion were comparable to those obtained by Ho and Frei (13). The plasma disappearance of ara-C, ara-U, and total radioactivity for a representative patient (A. S.) is given in Chart 1. The plasma disappearance of

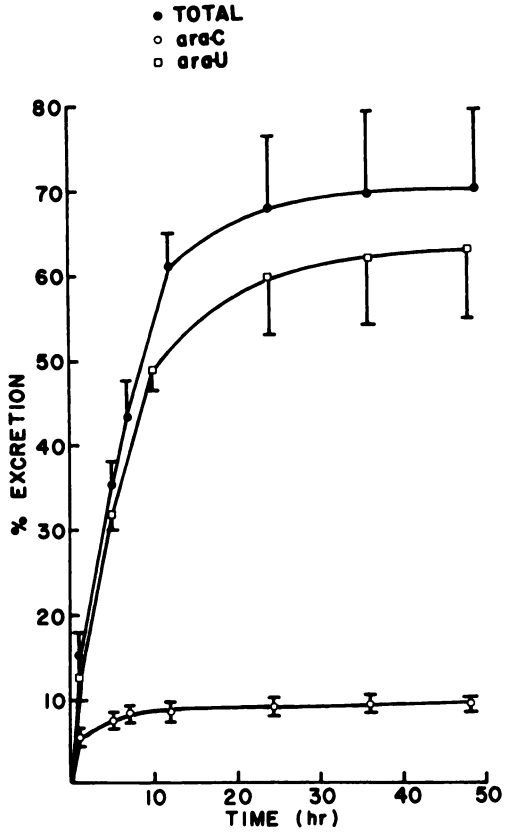

Chart 3. Cumulative excretion of ara-C, ara-U, and total drug following i.v. administration of ara-C; mean ± S.E.

ara-C was biphasic with a short half-life of 0.12 hr and a long half-life of 2.62 hr. Half-life values were obtained by fitting data to a 2-compartment open model, by means of the BMDX85 program. In Chart 2, the plasma concentrations of ara-C, ara-U, and total radioactivity are presented for a typical patient during the 4-hr infusion. In this patient, steady state levels were not obtained until 3 to 4 hr after initiation of infusion. This was true for all patients studied who received treatment by the infusion route. This could be due to the inadequate nature of the loading dose in the Southwest Oncology Group protocol.

Charts 3 and 4 give the cumulative urinary excretion following a single i.v. injection in 5 patients and a 4-hr infusion in 6 patients, respectively. Both ara-C and ara-U are rapidly excreted in the urine, with 50% of the dose excreted in 8 to 10 hr and 70% excreted in 36 hr. The ratio of ara-C to ara-U in urine was 1:4 after infusion and 1:6 after i.v. administration. Most of the ara-C was excreted within 5 hr. Radioactivity was not detectable in feces.

The plasma half-lives and excretion of ara-C and ara-U in urine after i.v. administration are given in Table 2. In 3 patients, the rapid half-life could not be determined accurately due to our inability to obtain frequent enough samples. The 48-hr excretion of ara-C and ara-U was 70%, with 10.4% of it as ara-C and 63.9% as ara-U. In Table 3, the same parameters are presented for patients who received ara-C by infusion. As demonstrated, the long half-life and excretion in urine are similar to those values given for the i.v. injection.

Apparent volumes of distribution were high. The initial volume of distribution, or volume of the central pool, ranged from 33 to 63 liters. Apparent volume of distribution at steady state was 171 to 186 liters. The relatively large initial volume of distribution may be an artifact caused by high blood deaminase activity. Metabolic and renal clearances following an i.v. dose are shown in Table 2. The values were obtained by dividing the dose and cumulative ara-C excretion, respectively, by the area under the plasma concentration-time curve. Renal clearance was much lower compared to elimination by metabolism, which had a mean of 845 ml/min.

DISCUSSION

Our results confirm the observations of Ho and Frei (13) on the biphasic plasma disappearance of ara-C. The rapid deamination and short half-life of ara-C is evidence that the drug must be administered as an infusion rather than as single intermittent doses. ara-C is a cycle-dependent drug and its efficacy depends upon dose scheduling (18) as well as duration of exposure to the drug. By administering ara-C over a longer period of time by infusion to maintain continuous plasma levels between 0.05 and 0.1 μg/ml, the

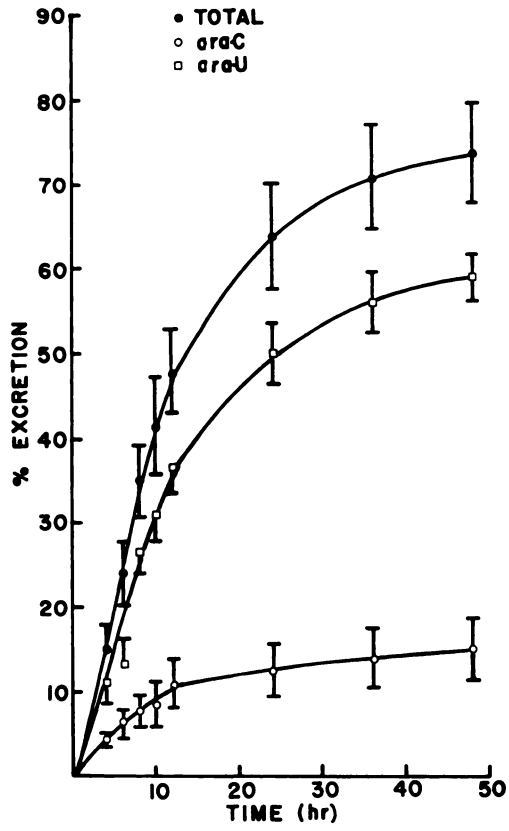

Chart 4. Cumulative excretion of ara-C, ara-U, and total drug following infusion of ara-C; mean ± S.E.

antitumor activity should be enhanced. Therapeutic responses in patients support this dose schedule (unpublished data presented by L. B. Mellett at the First Annual Experimental Pharmacology Workshop, Michigan, May 1973).

Although deoxycytidylic deaminase activity is increased by ara-C in mammalian cultures (15), it appears that prior therapy with ara-C has no influence on its pharmacokinetics, since the half-lives and *in vivo* metabolism were similar following both the single dose and the i.v. infusion studies. This is in keeping with clinical observations (8) which indicate that the toxicity to ara-C is not affected by prior ara-C treatment.

Moreover, the narrow range of half-lives suggests that

Table 2

Plasma half-lives of ara-C and excretion of ara-C and ara-U in urine following i.v. administration

Patient	1st $t_{\frac{1}{2}}$ (hr)	2nd $t_{\frac{1}{2}}$ (hr)	Metabolic clearance (ml/min)	Renal clearance (ml/min)	% dose excreted	% ara-C	% ara-U
D. S.		0.9	953	119	94.7	9.5	76.7
R. E.		3.5	1089	100	63.0	6.5	53.8
A. S.	0.15	4.5	807	66	91.6	13.7	83.4
A. D.	0.10	2.1	891	55	80.2	13.3	65.5
D. J.		2.1	486	48	45.7	9.9	40.0
Mean	0.12	2.62	845	78	70.4	10.6	63.9
S. E.		0.63	101	14	9.6	7.8	7.8

Table 3

Plasma half-lives of ara-C and excretion of ara-C and ara-U in urine following 4-hr infusion

Patient	$t_{\frac{1}{2}}$	% dose excreted	% ara-C	% ara-U	Comment
L. B.	3.7	74.2	10.8	57.2	
J. L.	2.7	33.8	3.7	28.6	Incomplete urine collection
R. M.		103.2	38.7	69.3	Infusion by i.v. drip
V. M.	3.0	79.3	13.6	70.8	
A. L.		59.2	8.8	57.8	3-hr infusion
W. W.		56.3	8.2	51.9	Concentration variable
J. J.	2.4	86.5	20.0	57.6	
J. T.	0.7	53.3	5.7	52.3	
E. B.	2.3	75.8	13.8	55.3	
Mean	2.47	73.47	14.94	59.03	
S. E.	0.41	5.98	3.73	2.54	
n	6	8	8	8	

Table 4

Patient characteristics and route of administration of ara-C

Patient	Age	Sex	Wt (kg)	Route	Creatinine (mg/100 ml)	Creatinine clearance (ml/min)	BUN[a] (mg/100 ml)	Alkaline phosphatase: Bessey-Lowry units/l	Total bilirubin (mg/100 ml)	SGOT (units/ml)
R. E.	62	M	61	i.v.	1.0	72	16	6.1	0.4	12
A. D.	51	M	66	i.v.	0.6	N.D.	11	34.8	1.1	66
D. S.	54	F	37	i.v.	0.6	N.D.	14	7.5	0.4	25
A. S.	74	M	80	i.v.	0.9	67	14	3.3	N.D.	18
D. J.	40	M	65	i.v.	0.7	N.D.	10	3.2	0.3	14
I. J.	45	F	45	Infusion	0.6	65	34	4.9	N.D.	30
E. B.	61	F	56	Infusion	0.7	N.D.	13	9.7	0.5	16
V. C.	67	F	50	Infusion	0.6	N.D.	9	54.0	0.2	32
A. L.	61	M	67	Infusion	1.2	N.D.	16	8.7	0.1	25
W. W.	64	M	65	Infusion	1.0	N.D.	13	31.8	1.0	79
J. T.	46	M	56	Infusion	0.7	96	5	3.5	0.6	14
R. M.	21	M	80	Infusion	1.0	N.D.	17	2.8	0.6	18
J. L.	70	M	66	Infusion	1.0	74	14	23.0	0.4	23
L. B.	60	M	77	Infusion	0.7	90	9	3.9	0.6	16

[a] BUN, blood urea nitrogen; SGOT, serum glutamic oxalopyruvic transaminase; N.D., not done.

Table 5

Diagnosis and previous therapy in patients receiving ara-C

Patient	Previous therapy	Diagnosis
R. E.	X-ray	Cancer of the esophagus
A. D.	None	Metastatic undifferentiated cancer
D. S.	X-ray	Squamous cell cancer of the tongue
A. S.	None	Lymphosarcoma
D. J.	X-ray, Mtx[a]	Metastatic adenocarcinoma
I. J.	Cyclo, ara-C, Thio	Acute myelomonocytic leukemia
E. B.	ara-C, Vin, Thio	Acute leukemia
V. C.	Cyclo, Mtx, 5-FU, Vin, Pred	Metastatic breast cancer
A. L.	X-ray	Cancer of the lung, bronchogenic
W. W.	None	Bronchogenic cancer
J. T.	DOAPx3, Thio, ara-C	Acute myeloblastic leukemia
R. M.	Vin, Thio	Acute myelocytic leukemia
J. L.	ara-C, Cyclo, Vin, ara-C, Pred	Acute myelocytic leukemia
L. B.	ara-C, Vin, pred	Acute myeloblastic leukemia

[a] Mtx, methotrexate; Cyclo, cyclophosphamide; Thio, thioguanine; Vin, vincristine; 5-FU, 5-fluorouracil; Pred, prednisone; DOAP, daunorubicin, vincristine, ara-C, prednisone.

other drugs (*e.g.*, prednisone, vincristine, and thioguanine) did not markedly influence the pharmacokinetics of ara-C. Although the ara-C deaminase activity in erythrocytes is higher in patients with solid tumors (13), our results failed to demonstrate differences in ara-C pharmacokinetics between the solid tumor patients and those with acute leukemia. The diversity in type of neoplastic disease, previous therapy, and some pertinent patient characteristics are provided in Tables 4 and 5.

The failure to achieve steady state rapidly following the loading dose in the infusion studies is explained by the failure to administer a sufficiently large loading dose. Since we were evaluating patients who were being treated according to a current Southwest Oncology Group treatment protocol, the loading dose was only the equivalent of the hourly rate of infusion over 4 hr. This is considerably less than the calculated loading dose and the loading dose administered by Ho and Frei (13) and explains why steady state concentrations were achieved rapidly in their studies. We suggest that the loading dose be 3 times the hourly rate of infusion in order to achieve rapid steady state concentrations.

Preliminary experience with high-pressure liquid chromatography suggests that this method is quite useful for measuring ara-C in patients, obviating the need for administering radioactive drug to patients in future studies.

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