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9-β-D-Arabinofuranosyladenine 5'-Phosphate Metabolism and Excretion in Humans[1]

G. A. LePage, S. R. Naik, S. B. Katakkar, and Abdul Khaliq

The McEachern Laboratory, University of Alberta [G. A. L., S. R. N.] and the Dr. W. W. Cross Institute [S. B. K., A. K.] Edmonton, Alberta, Canada T6G 2E1

SUMMARY

9-β-D-Arabinofuranosyladenine (ara-A) was converted chemically to the 9-β-D-arabinofuranosyladenine 5'-phosphate (ara-A-5'-P) and administered i.v. to four cancer patients in seven experiments. Urinary excretion and plasma levels of radioactivity were monitored for 24 hr in each case. Radioactivity present as unchanged ara-A-5'-P, ara-A, and the deamination product of ara-A, 9-β-D-arabinofuranosylhypoxanthine, was determined. Excretion was, as in earlier studies with ara-A, given i.v., largely as 9-β-D-arabinofuranosylhypoxanthine. However, in contrast to the 88 to 97% excretion of ara-A and products in 24 hr when ara-A was given by i.v. push, excretion was 41.47 to 79.1% in 24 hr when ara-A-5'-P was given. With the exception of one experiment at a low dose, where plasma ara-A levels were significant for 6 hr, the plasma levels of ara-A were sustained at significant levels for 24 hr after a single dose of ara-A-5'-P. The doses of ara-A-5'-P given were well tolerated by the four patients. Indications are that this derivative provides important advantages (solubility and sustained blood levels) over ara-A.

INTRODUCTION

The nucleoside analog, ara-A,[2] has had preliminary trials both as an antiviral agent for DNA viruses (2) and as an anticancer agent in hematological cancers (1), with indications of efficacy in both. Due to the distributions of the deaminases and kinases involved in catabolism and activation, respectively, ara-A would be expected to have a different antitumor spectrum from that of ara-C, and, unlike ara-C, it is not immunosuppressive (3). However, ara-A, like ara-C, has a relatively short half-life *in vivo* (4) and has had to be administered in large volumes of fluid because of low solubility. A remedy was suggested whereby the agent would be supplied as the ara-A-5'-P (5). This derivative is very water soluble, and it was assumed that it would, on i.v. injection, be largely confined to the plasma space. Gradual conversion to ara-A would hopefully occur because of phosphomonoesterase activity. In the limited study carried out earlier (5), this principle appeared to operate. Efficacy of ara-A-5'-P as an antiviral treatment for experimental DNA virus infections has been demonstrated (6). A large number of animal models were tested in our laboratory, and all cleaved ara-A-5'-P to ara-A at rapid rates. Thus an animal model resembling the human was not available, although ara-A-5'-P was in all cases metabolized to ara-A and excreted as the deaminated derivative ara-H. Consequently, to extend the study we obtained permission from the hospital ethics committee and the Canadian Food and Drug Administration for a further limited pharmacology experiment in which ara-A-5'-P, labeled with tritium on position 2 of the base, would be administered to 4 terminal cancer patients who had normal liver and kidney function. The metabolism and excretion of the drug were studied in these 4 patients. To minimize risk and yet reach doses equimolar with those of ara-A-producing antitumor effects, we began by administering a very low dose to the first patient. When it had been ascertained that no deleterious effects were observed, successive patients received first the higher dose used in the preceding patient and later a doubled dose. The doses for Patients 3 and 4 were then in the "therapeutic" range.

MATERIALS AND METHODS

[^{3}H]ara-A was purchased from New England Nuclear, Boston, Mass. ara-A was supplied by Drug Research and Development Branch, National Cancer Institute, Bethesda, Md. [^{3}H]ara-A-5'-P was synthesized by an adaptation of the procedure of Yoshikawa *et al.* (7). In a typical batch, 4.8 g of ara-A in suspension in 50 ml of ethanol:water (3:7) were mixed with 1 ml of [^{3}H]ara-A solution (1 mCi), and the mixture was evaporated in a vacuum to dryness. The dry solid was cooled to 0°, and an ice-cold solution of $POCl_3$ (5.0 ml) in trimethyl phosphate (54 ml) was added slowly with stirring. The flask was sealed with a drying tube and magnetically stirred for 2 hr at 0°. Thin-layer chromatography on silica gel of a small aliquot, hydrolyzed 2 to 3 min in water, was carried out with CH_3CN:0.1 M NH_4Cl (7:3) and revealed that about 70 to 75% conversion had occurred. Additional $POCl_3$ (1.5 ml) was added slowly during the next 2 hr. Chromatography now indicated a 95% conversion. The clear reaction mixture was poured into ice water (600 ml) and kept at 0–4° for 16 hr. The solution was diluted with 200 ml water, neutralized to pH 5.0 with concentrated NH_4OH, and extracted twice with

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[2] The abbreviations used are: ara-A, 9-β-D-arabinofuranosyladenine; ara-C, 1-β-D-arabinofuranosylcytosine; ara-A-5'-P, 9-β-D-arabinofuranosyladenine 5'-phosphate; ara-H, 9-β-D-arabinofuranosylhypoxanthine.

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$CHCl_3$ (500 ml). The $CHCl_3$ was discarded and the aqueous layer was passed through a column of Barneby-Cheney AU4 activated charcoal (2.4 × 60 cm) that had been washed with 1 N HCl (granular charcoal obtained from Barneby-Cheney Ltd., St. John's, Quebec, Canada). The column was washed with water until salt free ($AgNO_3$ test for chloride). The column was eluted with 3.5 liters of ethanol:H_2O:concentrated NH_4OH (70:24:6). Solvent was removed in a vacuum, and the residue was taken up in water (10 ml), 50 g of silica gel were added (60 to 200 mesh silica gel, J. T. Baker Chemical Co., Phillipsburg, N. J.), and the mixture was dried in a vacuum. The dry mixture was applied to the top of a dry-packed column of the same material (5.6 × 47 cm). The column was eluted with CH_3CN:1 N NH_4OH (85:15), and 10 ml fractions were collected. The first 2000 ml of eluate contained the unreacted ara-A, as determined by paper and thin-layer chromatography. The elution was continued with a total of 6 liters of acetonitrile:1 M NH_4OH (4:1). This was evaporated to dryness in a vacuum and 4.5 g of ara-A-5'-P were obtained as the ammonium salt. This material gave 1 spot on paper chromatograms developed with isobutyric acid:H_2O:NH_4OH (66:33:1), R_F 0.38, and 1 spot on thin-layer chromatography with CH_3CN:1 N NH_4Cl (7:3). R_F's for ara-A-5'-P and ara-A were 0.22 and 0.73, respectively. The product had an absorption maximum at 259 nm. It was also established by the chromatography that >99% of the radioactivity was present as [^{3}H]ara-A-5'-P. A small amount of ara-A-3'- (or 2'-P) was obtained separately from the column purification. This was not used. However, tests in mice indicated that this material was not cleaved to ara-A and was excreted in the urine unchanged. Analyses by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y., indicated that the product had 1 molecule of water of hydration.

Informed consent was obtained from the 4 patients participating in this study. They were patients with metastatic cancers that had not responded to sufficient trials of conventional chemotherapy. They had normal liver and kidney function. The dosages of [^{3}H]ara-A-5'-P were made up as neutral solutions, 30 mg/ml (isotonic), and tritium content was 80 to 150 μCi/dose. The dose was made up just before use and administered in each case by i.v. push through a Swinnex-25 filter with 0.22-μm pores (Millipore Corp., Bedford, Mass.). Blood samples were taken in heparinized vacuum tubes and immediately chilled. It was demonstrated by incubations with normal blood that this refrigeration prevented dephosphorylation and deamination of ara-A-5'-P and ara-A, respectively, in such samples. Urine collections were immediately refrigerated. Blood samples were counted in a Nuclear-Chicago Unilux II scintillation system with a polar scintillation solution at 60% efficiency. Other aliquots were chromatographed on Whatman No. 3MM paper sheets with ethyl acetate:isopropyl alcohol:water (64:22.5:12.5). Markers were used for ara-A-5'-P, ara-A, and ara-H (R_F's 0.01, 0.3, and 0.35, respectively). Solvent was run off the end of the sheets in descending chromatography in order to obtain good separation of ara-A and ara-H. Separations were distinct and did not involve contamination of one component by the other. Aliquots of urine samples were used directly, counted, and chromatographed similarly to those from blood plasmas. The appropriate areas of the chromatograms (determined by examination in UV) were cut out and eluted in minimal volumes of 0.1 M HCl, and aliquots of the eluates were counted. These were related quantitatively to the total

Table 1

Patients involved in pharmacological study of ara-A-5'-P

Patient	Description	Treatment
P. E.	65-yr-old male with malignant melanoma, including pulmonary involvement BSA:[a] 2.0 sq m Hb:[b] initial 14.2 → 15.6 WBC: initial 6300 → 10,100 Platelets: initial 224,000 → 301,000	Day 1: ara-A-5'-P, 162 mg/sq m, by i.v. push Day 8: ara-A-5'-P, 324 mg/sq m, by i.v. push
S. K.	53-yr-old male with adenocarcinoma of the cecum BSA: 1.9 sq m Hb: initial 13.1 → 13.9 WBC: initial 10,400 → 8,900 Platelets: initial 169,000 → 203,000	Day 1: ara-A-5'-P, 324 mg/sq m, by i.v. push Day 30: ara-A-5'-P, 648 mg/sq m, by i.v. push
S. V.	74-yr-old female with carcinoma of the stomach with local extension BSA: 1.5 sq m Hb: initial 7.4 → 9.3 WBC: initial 4600 → 5000 Platelets: initial 273,000 → 204,000	Day 1: ara-A-5'-P, 648 mg/sq m, by i.v. push Day 30: ara-A-5'-P, 1296 mg/sq m, by i.v. push
P. H.	64-yr-old male with carcinoma of the colon with hepatic metastases BSA: 2.0 sq m Hb: 14.6 WBC: 7200 Platelets: 133,000	Day 1: ara-A-5'-P, 1296 mg/sq m, by i.v. push Day 30: Returned but was not given a 2nd dose

[a] BSA, body surface area.
[b] Hb, hemoglobin, g/100 ml.

counts in the samples to determine proportions present as the unchanged [^{3}H]ara-A-5′-P and as [^{3}H]ara-A or [^{3}H]ara-H.

RESULTS

Data concerning the 4 patients involved in the study are shown in Table 1. No immediate or delayed side effects were experienced by the patients except for a short episode of nausea and vomiting in Patient 4 (P. H.), who was already experiencing intermittent nausea and vomiting prior to this treatment, probably as a result of his disease.

The findings concerning urinary excretions after i.v. injections with [^{3}H]ara-A-5′-P are shown in Table 2. Separate aliquots of each urine sample were counted directly and, after evaporation to dryness in a vacuum and reconstitution to the original volume in distilled water, as a test for volatile tritium that might have arisen by oxidation at position 2 on the purine ring of ara-H, releasing the tritium. Evaporated and reconstituted samples gave counts that were 98 to 104% of those from direct counts. Thus, there was no indication, as far as these analyses indicated, that such oxidation had occurred. Urinary excretion was composed mostly of ara-H at the earlier times and the components ara-H, ara-A, and ara-A-5′-P constituted almost all the radioactivity excreted. In the 6- to 24-hr period there appeared to be some deficiency in this total, possibly

Table 2

Urinary excretions after i.v. injection of [^{3}H]ara-A-5′-P

Doses given patients contained 80 to 150 μCi, with higher specific radioactivity used for lower doses, to allow for similar accuracy of analyses. Counting was such as to limit error to ±2%.

Patient	Dose (mg)	Time (hr)	ara-A-5′-P equivalent excreted (mg)	% of dose	% present as		
					ara-H	ara-A	ara-A-5′-P
P. E.	324	0–2	61.3	18.9	67.5	6.0	2.8
		2–4	36.3	11.2	82.5	4.4	0.6
		4 6	18.1	5.6	97.5	0.2	0.2
		6–24	34.7	10.7	72.4	1.8	1.1
Totals			150.4	46.4	76.0	3.4	1.7
P. E.	600	0–2	125	20.8	94.1	0.4	0.3
		2–4	52.1	8.7	95.5	1.8	1.8
		4–6	3.4	5.9	82.8	1.9	4.4
		6–10	20.4	3.4	88.0	2.0	4.4
		10–24	33.6	5.6	45.3	4.5	4.6
Toals			266.5	44.4	86.2	1.5	2.0
K. S.	615	0–2	89.9	14.6	81.5	1.3	<1
		2–10	125.5	20.4	81.9	<1	<1
		10–24	271	44.1	46.0	<1	<1
Totals			486.4	79.1	53.6	<1	<1
K. S.	1230	0 2	232	18.9	78.0	20.0	1.7
		2–4	108	8.8	95.0	3.5	1.6
		4–6	88.0	7.15	88.0	7.5	0.5
		6–10	66.5	5.4	83.0	9.0	0.8
		10–24	33.2	2.7	73.0	1.2	1.4
Totals			527.7	42.95	83.5	12.0	1.3
S. V.	972	0–2	217	22.3	77.0	19.0	3.7
		2–4	97.0	9.9	83.0	13.0	4.0
		4–6	33.5	3.45	95.0	3.2	2.1
		6–10	22.0	2.27	96.0	1.9	1.2
		10–24	34.5	3.55	62.0	19.0	2.0
Totals			404.0	41.47	80.0	16.2	3.3
S. V.	1944	0–2	406	20.9	74.2	20.3	4.0
		2–4	278	14.3	90.0	6.5	2.0
		4–6	257	13.2	90.0	6.0	2.9
		6–10	109	5.6	84.8	10.1	3.9
		10–24	21	1.1	53.5	27.3	1.1
Totals			1071	55.1	82.6	12.4	3.2
P. H.	2592	0–2	254	9.8	82.4	14.1	2.4
		2–4	269.5	10.4	89.5	9.0	1.4
		4–10	529	20.4	93.0	5.0	0.8
		10–24	277.5	10.7	93.0	4.6	0.1
Totals			1330	51.3	90.5	7.4	1.1

indicating another product. This pattern of excretion matches that reported by patients given ara-A instead of ara-A-5′-P by i.v. push (4) except that excretion was delayed when ara-A-5′-P was given. Excretion of radioactivity was almost complete (88 to 97%) in 24 hr when ara-A was given but was only 41.47 to 55.1% in 6 of these experiments and 79.1% in 1 experiment at a relatively low dose of ara-A-5′-P.

Analyses of plasma samples from the 7 experiments are given in Table 3. At the lowest dose (162 mg/sq m) in the 1st patient, giving ara-A-5′-P maintained an appreciable level

Table 3

Plasma levels of ara-A-5′-P and metabolites after i.v. injection of ara-A-5′-P

Counting was such that errors would be limited to ±5%. Samples where radioactivity was less than 1.5 times background are indicated (*e.g.*, <0.1).

Patient	Dose (mg)	Time (hr)	ara-H	ara-A (μmoles/liter)	ara-A-5′-P
P. E.	324	0.25	14.9	0.69	6.2
(BSA[a] 2.0 sq m)		0.5	13.4	1.1	3.2
		1	12.9	1.2	1.5
		2	10.0	0.53	0.41
		4	6.6	0.42	<0.1
		6	4.4	0.22	<0.1
		24	2.3	<0.1	<0.1
P. E.	600	0.25	8.15	1.69	32.2
(BSA 1.85 sq m)		0.5	15.2	2.2	22.6
		1	14.7	4.95	9.05
		2	12.5	6.4	3.48
		4	5.95	8.05	1.68
		6	3.05	4.4	0.78
		24	1.89	2.05	<0.01
S. K.	615	0.25	13.1	0.99	14.3
(BSA 1.9 sq m)		0.5	16.1	0.18	5.45
		1	12.6	0.28	2.18
		2	12.1	0.40	0.54
		4.4	7.4	0.52	<0.01
		6.1	5.1	0.57	<0.01
		24	2.32	<0.01	<0.01
S. K.	1230	0.25	39.7	17.0	23.5
(BSA 1.9 sq m)		0.5	25.2	8.4	15.4
		1	18.5	12.6	7.9
		2	13.4	10.7	9.1
		4	14.9	7.0	8.2
		6	6.15	3.16	7.3
		24	2.15	2.25	4.7
S. V.	972	0.25	39.7	11.5	12.2
(BSA 1.5 sq m)		0.5	33.2	5.0	12.1
		1	23.6	9.4	10.7
		2	15.8	6.4	10.7
		4	9.5	4.75	9.0
		6	5.9	3.3	6.25
		24	1.57	1.95	3.75
S. V.	1944	0.25	54.9	38.2	30.0
(BSA 1.5 sq m)		0.5	51.5	30.5	21.3
		1	44.0	29.2	28.5
		2	32.7	18.1	23.2
		4	22.4	13.7	17.3
		6	15.6	13.8	13.8
		24	9.05	9.9	8.9
P. H.	2592	0.25	123	12.0	33.6
(BSA 2.0 sq m)		0.5	86.7	14.5	35.6
		0.75	93.5	11.6	19.0
		1.25	72.6	9.8	14.7
		2.25	56.0	5.2	10.2
		4.25	30.6	3.25	14.8
		6.25	27.8	2.8	6.5
		24	6.2	4.1	3.05

[a] BSA, body surface area.

of ara-A in plasma for 6 hr. In the subsequent experiment in the same patient at a higher dose (324 mg/sq m) and in the subsequent tests in the other patients at higher doses, a significant level of ara-A was maintained in the plasmas throughout the 24-hr period.

DISCUSSION

Since ara-A-5′-P is very soluble (20% solutions can be made), it can apparently provide a suitably soluble formulation that is well tolerated and maintains a sustained blood plasma level of ara-A without the need for the continuous infusion used in earlier tests (1).

The levels of deaminase for ara-A in human and mouse tumors vary widely. It seems probable that variations would also occur in the deaminating capacity of organs from one patient to another. It appears that Patient K. S., for whom analyses in Table 2 indicated a more rapid conversion of ara-A-5′-P to ara-A and then to ara-H, has higher phosphomonoesterase capacity and/or deaminase capacity. A higher dose appeared to suffice to saturate these capacities and give sustained blood levels of ara-A.

In certain instances, the analyses for ara-A-5′-P, ara-A, and ara-H do not account for all of the radioactivity. This is most evident in later urine samples and indicates an additional metabolite that has not been identified.

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