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Pharmacology of 5′-Esters of 1-β-D-Arabinofuranosylcytosine[1]

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SUMMARY

Pharmacological studies of 5′-esters of 1-β-D-arabinofuranosylcytosine (ara-C) were performed in three species (mouse, pig, and man). In mice, after a single i.p. injection of a suspension of tritiated 1-β-D-arabinofuranosylcytosine 5′-palmitate (PalmO-ara-C) at a therapeutic dose of 150 mg/kg, 30% of the administered radioactivity was recovered in the urine in 24 hr and 56% was recovered after 7 days. Excretion was less rapid after s.c. administration. ara-C and 1-β-D-arabinofuranosyluracil each accounted for about 50% of the excreted radioactivity, and no PalmO-ara-C was found. Plasma ara-C concentrations of greater than 0.1 μg/ml were detected 24 hr after i.p. administration of PalmO-ara-C (150 mg/kg). Single doses of PalmO-ara-C were effective against L1210 leukemic mice when administered 5 to 7 days before tumor inoculation. In a pig, after i.m. injection of tritiated PalmO-ara-C (60 mg/kg, two sites), only 7% of the administered radioactivity was recovered in the urine over a 1-week period. Similar low rates of excretion were also observed in patients treated i.m. with PalmO-ara-C or 1-β-D-arabinofuranosylcytosine 5′-benzoate. No ara-C was detected in the plasma, which is consistent with the absence of clinical toxicity or myelosuppression in Phase 1 trials of PalmO-ara-C at doses up to 1500 mg/sq m every 3 weeks for as many as eight courses.

INTRODUCTION

ara-C[2] is an effective antiviral, immunosuppressive, and antitumor agent in experimental systems and in humans (1, 2, 6, 12–14). In experimental animal tumor systems, continuous or frequent administration of ara-C produces a substantially greater therapeutic index than daily single injections (17). The pharmacological and cytokinetic bases for this dose schedule dependency have been demonstrated (4, 20). In patients with acute myelogenous leukemia, the therapeutic activity of ara-C by continuous i.v. injections is superior to that by daily injections (1, 3). Clinical pharmacological studies of ara-C have indicated that the continuous i.v. infusion regimen yields low, but sustained and effective, plasma ara-C levels (10). A single injection of certain ara-C esters in experimental animals is as effective as ara-C therapy on an optimum schedule of multiple, closely spaced doses at appropriate intervals, suggesting that these esters are depot forms of ara-C (15, 16, 19). Pharmacological studies with one of these agents [1-β-D-arabinofuranosylcytosine 5′-adamantoate (NSC 117614)] in mice and rats have been reported (5, 15). This paper describes the pharmacological studies of PalmO-ara-C and BzO-ara-C in mice, a pig, and man.

MATERIALS AND METHODS

Female C57BL/6 × DBA/2 F_1 (hereafter called $BD2F_1$) mice weighing 20 ± 2 g were purchased from The Jackson Laboratory, Bar Harbor, Maine. A female Yorkshire pig weighing 13 kg was obtained from H. Gray, Vicksburg, Mich. Six patients were included in the pharmacological studies: 3 with melanoma and 1 each with oat cell carcinoma of the lung, adenocarcinoma of the breast, and adenocarcinoma of the right kidney. All chemotherapeutic agents, including [^{3}H]PalmO-ara-C, tritiated in positions 5 and 6 of the pyrimidine base (specific activity, 1.79 mCi/mg), [^{14}C]PalmO-ara-C (specific activity, 19.3 μCi/mg) and [^{14}C]BzO-ara-C (specific activity, 25 μCi/mg), were supplied by The Upjohn Company, Kalamazoo, Mich. The latter 2 agents were synthesized by Dr. R. S. P. Hsi, The Upjohn Company. The radiochemical purities of the [^{3}H]PalmO-ara-C, [^{14}C]PalmO-ara-C, and [^{14}C]BzO-ara-C, as determined by radiochemical and thin-layer and paper chromatographic techniques, were 95, 98, and 98%, respectively.

Mice were given i.p. injections of PalmO-ara-C (suspended in 0.9% NaCl solution containing about 0.1% Tween 80) at 19 to 150 mg/kg (57 to 450 mg/sq m), and heparinized blood was obtained from the vena cava at intervals; 6 animals were used for each time period. Blood was pooled, plasma was obtained by centrifugation, and the plasma was assayed for ara-C (or its cytotoxic equivalent) by means of a previously described microbiological assay (8, 15). For urinary excretion, [^{3}H]PalmO-ara-C, 150 mg/kg (52 μCi/mouse) was administered i.p. (8 mice/group) or s.c. (4 mice/group). Each mouse received 0.2 ml of a suspension in 0.25% aqueous methylcellulose (Upjohn Vehicle 122). The mice were housed in metabolism cages (Acme Metal Products, Chicago, Ill.), and urine and feces were collected at various intervals over a period of 8 days. Radioactivity of aliquots of urine was determined directly by liquid scintillation techniques. Feces were extracted with water, and radioactivity was determined. Results were confirmed by

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[2] The abbreviations used are: ara-C, 1-β-D-arabinofuranosylcytosine (NSC 63878, U-19,920, cytarabine, Cytosar); PalmO-ara-C, 1-β-D-arabinofuranosylcytosine 5′-palmitate (ara-C 5′-palmitate, NSC 135962); BzO-ara-C, 1-β-D-arabinofuranosylcytosine 5′-benzoate (ara-C 5′-benzoate, NSC 165022); ara-U, 1-β-D-arabinofuranosyluracil; ILS, increased life-span.

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combustion techniques. Fecal radioactivity was low (<10% of that observed in urine) and probably represented contamination by urine. Tritium exchange (indicated by the presence of ^3H_2O) was determined by lyophilizing the urine samples (i.p. experiment only), collecting the water, and determining the radioactivity in both the water and the residue. In no case did the amount of ^3H_2O exceed 3% of the total radioactivity in the sample. Urine samples (i.p. experiment only) were chromatographed with silica gel thin-layer plates (Brinkmann Instruments, Inc., Westbury, N. Y.). Chromatograms were developed in 2 different solvent systems: Solvent 1 (methyl ethyl ketone:acetone:water, 7:2:1) and Solvent 2 (isopropyl alcohol:concentrated NH_4OH: water, 7:1:2). R_F values for ara-C, ara-U, and PalmO-ara-C were (*a*) Solvent 1: 0.15, 0.45, 0.45; and (*b*) Solvent 2: 0.50, 0.65, 0.75.

Therapy experiments in L1210 leukemic mice were carried out as described previously (16). Briefly, PalmO-ara-C was administered in a single i.p. or s.c. dose as a 0.2-ml suspension in Vehicle 122 to groups of 8 $BD2F_1$ mice. The agent was administered at various times relative to inoculation of the animals with 1×10^5 L1210 cells per mouse on Day 0. Where the agent was administered on Day 0 (see Chart 3), administration was 3 hr after tumor inoculation. The dose used was 150 mg/kg. Mean survivals (with S.D.) were calculated, and percentage of ILS was calculated from mean survivals of treated and untreated (control) groups. Animals surviving for 45 days were considered cured and were omitted from calculation of the mean survival times.

A 15-ml suspension of [^{3}H]PalmO-ara-C (2800 μCi) in Vehicle 122 was administered i.m. (2 sites) at 60 mg/kg to a 13-kg pig. Urine samples were collected daily for 9 days, and radioactivity was determined as described above. The maximum exchange observed (determined as above) was 10% (Day 9 sample).

[^{3}H]PalmO-ara-C (250 μCi) was administered i.m. at 200 mg/sq m to 2 patients as a homogenized suspension in 5 ml "steroid-suspending vehicle." Each ml of the vehicle contained 9 mg sodium chloride, 5 mg sodium carboxymethylcellulose-7LP, 4 μl polysorbate 80, and 9 μl benzyl alcohol. Heparinized blood was collected in tubes containing tetrahydrouridine (1×10^{-4} M). All blood and urine samples were processed at 4° and analyzed as previously described (10). The samples were applied to Whatman No. 1 papers which were developed (descending) for 6 to 8 hr in a solvent consisting of isopropyl alcohol:H_2O:ethylacetate, 22.5:12.5:65. The R_F values of ara-C, ara-U, and PalmO-ara-C are 0.1, 0.32, and 0.92, respectively. The possible presence of tritiated H_2O in the samples was determined as described above. In contrast to the animal experiments, 35 to 90% of the tritium in human plasma samples from 4 to 192 hr was present as tritiated H_2O. This invalidated the results, and ^{14}C-labeled drug was used in later studies. [^{14}C]PalmO-ara-C (100 μCi) at 300 mg/sq m and [^{14}C]BzO-ara-C (100 μCi), 250 mg/sq m, were given i.m. to patients. In addition, a patient who had been fasted overnight received a p.o. dose of [^{14}C]PalmO-ara-C (300 mg/sq m) in 40 ml orange juice.

RESULTS

Plasma concentrations of ara-C in mice at various times after receiving different doses of PalmO-ara-C i.p. are shown in Chart 1. Maximum levels were achieved about 1 hr after administration. The plasma ara-C level still exceeded 0.1 μg/ml 24 hr after a dose of 150 mg/kg.

The results of the urinary excretion studies in mice given [^{3}H]PalmO-ara-c (150 mg/kg) i.p. or s.c. are presented in Chart 2. At 24 hr after i.p. administration, 30% of the radioactivity had been excreted; about 15% was excreted after s.c. administration. The rate of excretion slowed thereafter such that after 1 week, only 58% (i.p.) or 43% (s.c.) of the dose was recovered. However, the rate of excretion at that time, although low, was still significant (1 to 2% of administered dose per day). Residual precipitated drug could be observed in the peritoneal cavity of mice upon dissection 8 days after i.p. administration. No intact PalmO-ara-C was

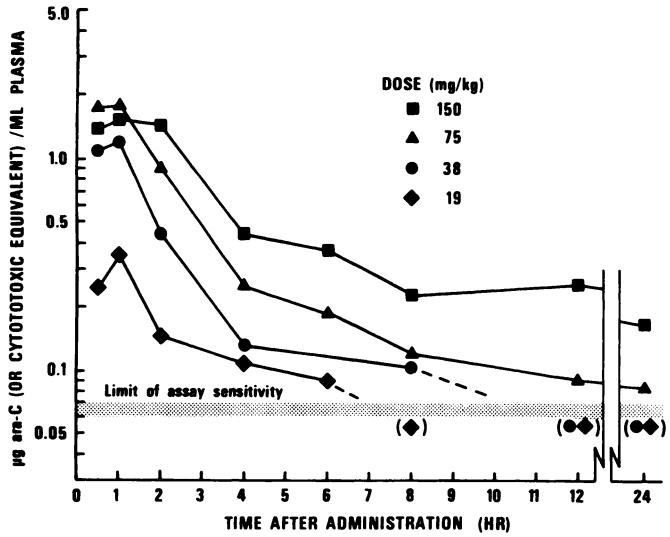

Chart 1. ara-C concentrations in mouse plasma after a single i.p. injection of PalmO-ara-C as a suspension in 0.9% NaCl solution containing *ca.* 0.1% Tween 80. Blood from 6 mice/time was pooled for preparation of plasma. ara-C levels were determined by microbiological assay. The limit of assay sensitivity (*stippled area*) was 0.06 to 0.07 μg/ml. *Symbols* in *parentheses* indicate that levels were not detected.

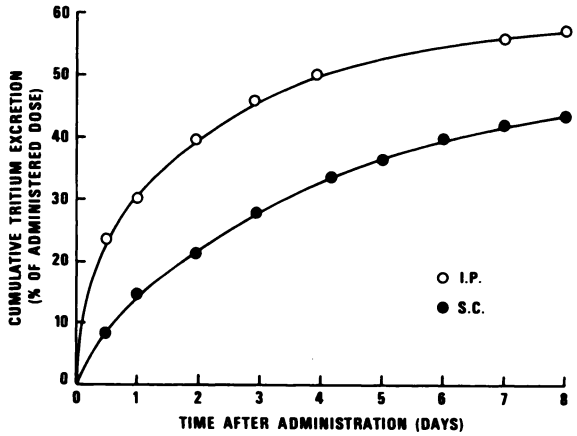

Chart 2. Cumulative excretion of total radioactivity (as a percentage of total dose administered) from mice after a single injection of [^{3}H]PalmO-ara-C (150 mg/kg, 2.66 mCi/kg) as a suspension in 0.25% aqueous methylcellulose (○, 8 mice; ●, 4 mice). Less than 10% of excreted radioactivity was found in feces.

found in urine at any time. ara-C and ara-U each accounted for about 50% of the radioactivity excreted.

The results of an experiment in which PalmO-ara-C (150 mg/kg) was administered i.p. or s.c. to $BD2F_1$ mice at various times before or after L1210 tumor inoculation are shown in Chart 3. L1210 cells were inoculated i.p. on Day 0. The maximum therapeutic effect (169% ILS, i.p.; and 91% ILS, s.c.) were observed when treatment was on Day 0, 3 hr after tumor inoculation. However, significant therapeutic activity (>25% ILS) was observed when the agent was administered up to 6 to 7 days before tumor inoculation.

After an i.m. injection (in 2 sites) of [^{3}H]PalmO-ara-C (60 mg/kg; 215 μCi/kg) into a pig, the rate of excretion of radioactivity was very low. After 3 and 9 days, only 5 and 9% of the administered radioactivity was recovered in the urine. Chromatographic identification of the radioactive components was not attempted.

The results of studies of urinary excretion of patients after receiving [^{14}C]PalmO-ara-C are shown in Chart 4 (300 mg/sq m, i.m., in Chart 4*A*; 300 mg/sq m, p.o., in Chart 4*B*). Excretion was very slow and incomplete. After i.m. administration, recoveries of radioactivity in the urine at 24 hr and 3 days were 5 and 21%, respectively; after p.o. administration, they were 15 and 28%, respectively. The urinary excretion of total radioactivity after i.m. administration of [^{14}C]BzO-ara-C (250 mg/sq m) (Chart 4*C*) was also slow (1% at 24 hr; 10% at 3 days). By chromatography, less than 1% of the radioactivity present in the urine was ara-C and the remaining 99% was ara-U. As in the mouse studies, no intact drug was detected.

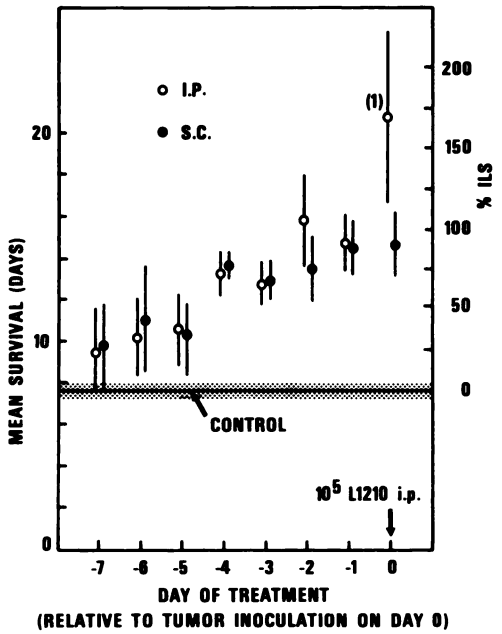

Chart 3. Depot effect of PalmO-ara-C in the treatment of L1210 leukemic mice. $BD2F_1$ mice were treated with single injections of PalmO-ara-C (150 mg/kg) at various times (Day −1 to Day −7) before i.p inoculation of 10^5 L1210 cells per mouse on Day 0. Mice treated on Day 0 were given injections 3 hr after inoculation. Survival times (mean ± S.D.) and percentage ILS are shown as a function of day of treatment. Control survival (mean ± S.D.) is represented by the *stippled area*. *Numbers* in *parentheses*, number of cures.

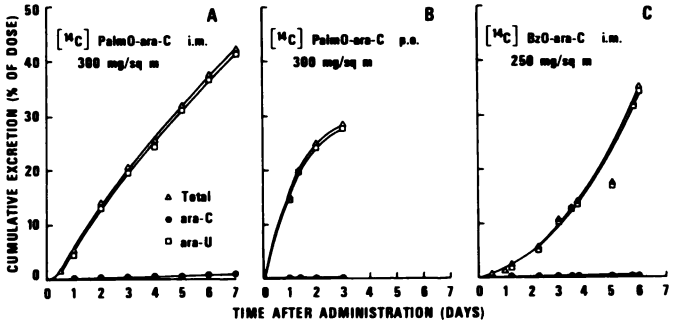

Chart 4. Cumulative excretion of total radioactivity, ara-C, and ara-U in 3 patients after a single administration of an ara-C ester.

DISCUSSION

The antitumor effects of ara-C in the treatment of mouse L1210 leukemia are exquisitely schedule sensitive. Optimum results are achieved when the agent is administered to mimic constant infusion such that sustained tissue and body fluid levels are maintained (17). Several 5′-esters of ara-C have been shown to act as depot or sustained action forms of ara-C in mice (7, 15, 16, 19). Single i.p. or s.c. injections of PalmO-ara-C or BzO-ara-C are markedly effective in the treatment of L1210 leukemic mice (Refs. 7, 17; Chart 3). For a given total dose, therapeutic results are relatively insensitive to the schedule used. For example, single-dose administration of PalmO-ara-C was approximately as effective as daily administration (5 days) of the same total dose of this agent and was approximately as effective as ara-C administered on its optimum schedule of injections every 3 hr over a 24-hr period, repeated at suitable intervals to allow for host recovery.

Chart 1 shows that plasma concentrations of ara-C persisted (at levels greater than 0.1 μg/ml) for at least 24 hr after administration of a single dose of PalmO-ara-C (150 mg/kg) to mice. These results were similar to those observed previously with another ester, the adamantoate (15). In contrast, after an equimolar dose of ara-C, levels above 0.1 μg/ml could not be maintained for more than 4 hr (15). An ara-C concentration of 0.1 μg/ml approximates the minimum growth-inhibitory concentration against mouse leukemia cells in culture (4, 10, 20). The significant antitumor activity of PalmO-ara-C administered up to 5 to 7 days before tumor inoculation provides additional evidence for the persistence of ara-C. This receives further support from the mouse urinary excretion experiments (Chart 2). From studies with a large number of 5′-acylates of ara-C (8, 15, 16, 19), we have concluded that the most important characteristics of the depot-active materials are, 1st, solubility of the ester in water and, 2nd, the rate of hydrolysis by the mixed esterase systems in body fluids (in particular, plasma). In experimental animals, low-water solubility is a necessary but not sufficient requirement for a high degree of single-dose (depot) activity. Rapid hydrolysis to ara-C is also required to liberate the parent agent. Primarily on the basis of these considerations, PalmO-ara-C was chosen for initial pharmacology studies in large animals and humans.[3]

The therapeutic dose of ara-C by continuous i.v. infusion is of the order of 150 to 200 mg/sq m over a 5-day period. For a single-dose depot form to mimic this infusion effec-

[3] The aqueous solubility of PalmO-ara-C is 1.5 to 2 μg/ml and hydrolysis to ara-C is rapid in mammalian plasma (19).

tively, it would have to be absorbed[4] at a rate such that approximately 150 to 200 mg of ara-C per sq m would be released into the general circulation daily. For the ideal (and probably unachievable) case, in which 20% of the dose was absorbed each day, a single dose of about 1500 to 2000 mg of PalmO-ara-C per sq m would be required. Lower absorption rates would, of course, require higher doses. The mouse data suggested that effective absorption rates might be achieved. For example, with s.c. doses of PalmO-ara-C of 150 mg/kg (450 mg/sq m), initial excretion rates (over the 1st 24 hr) were about 15%/day. However, i.m. administration in a pig (60 mg/kg; *ca.* 1600 mg/sq m) yielded initial excretion rates of only 1 to 2%/day. The initial excretion rate observed in the human patient (8 mg/kg; 300 mg/sq m) was only about 5 to 6%/day. This result suggested that doses of PalmO-ara-C considerably in excess of 5000 mg/sq m[5] would be required to give adequate levels of ara-C. Such doses (suspensions containing greater than 8 g of drug for an average adult) are clearly impractical.

Clinical Phase 1 trials of PalmO-ara-C were carried out concomitantly with studies of its pharmacology at the M. D. Anderson Hospital (E. J Freireich, personal communication). Eleven patients with metastasized solid tumors were treated with single i.m. injections at an initial dose of 225 mg/sq m. Doses were increased by 20% increments every 21 days, up to a maximum dose of 1500 mg/sq m. Among the 11 patients, 2 each received 1, 2, 3, 4, or 6 courses and 1 had 8 courses. No patient showed any toxic effect or myelosuppression. In most patients, persistent masses at the site of injection were observed. In 1 case, autopsy material was obtained from the area of the injection site from a patient 4 days after a single i.m. injection of [^{14}C]PalmO-ara-C. The tissue was homogenized with 2 volumes of distilled H_2O in a Waring Blendor. After further dilution with 4 volumes of glacial acetic acid, radioactivity in the homogenate was determined. The total amount recovered from this injection site was approximately 92% of the unrecovered radioactivity. This direct evidence of poor drug absorption is consistent with the lack of biological effect.

The rate of absorption of a depot form of ara-C would be expected to be influenced by the aqueous solubility of the agent. Thus, a more soluble derivative, BzO-ara-C, was chosen for further study. BzO-ara-C is about 40 times more soluble (75 to 90 μg/ml water) than PalmO-ara-C and is even more rapidly hydrolyzed in mammalian plasma (19). It has good single-dose activity in leukemic mice (7, 19). Unfortunately, the rate of absorption (estimated by excretion rate) in the 1 patient studied was not superior to that observed with PalmO-ara-C (Chart 4, *A* and *C*), and clinical trials were not attempted.

It appears that the solubilities of the 2 derivatives selected for clinical pharmacological investigation were not adequate to allow the desired rates of absorption, especially from poorly perfused muscle tissue. Other depot forms, including more water-soluble esters (19), may hold some promise. In addition, concomitant administration of the deaminase inhibitor, tetrahydrouridine, to prevent deamination of ara-C to ara-U may allow the reduction of doses of these depot forms to practical levels. Daily administration of cyclocytidine, a very soluble agent that is slowly hydrolyzed to ara-C and is excreted at a slower rate, may represent an even more attractive alternative (9, 11, 18). This study also emphasizes the importance of clinical pharmacology studies before the clinical trial of new antineoplastic agents.

[4] Because of the expected rapid hydrolysis of PalmO-ara-C to ara-C (confirmed by our findings that only ara-C and its metabolite, ara-U, were observed in the urine) and the rapid elimination (by metabolism and excretion) of ara-C, rates of recovery of radioactivity in the urine can be considered to reflect the absorption rate.

[5] Because of the differences in molecular weight (ara-C, 243; PalmO-ara-C, 481), 1.98 mg of PalmO-ara-C would be required to release 1.0 mg ara-C.

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