Cancer Chemother Pharmacol (2003) 51: 422–426
DOI 10.1007/s00280-003-0589-9

ORIGINAL ARTICLE

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Preclinical antitumor activity of 4′-thio-β-D-arabinofuranosylcytosine (4′-thio-ara-C)

Received: 24 January 2001 / Accepted: 15 January 2003 / Published online: 1 April 2003

Abstract *Purpose*: 4′-Thio-β-D-arabinofuranosylcytosine (4′-thio-ara-C), which has shown significant cytotoxicity against a panel of human tumor lines, was evaluated for antitumor activity against a spectrum of human tumor systems in mice. *Methods*: Antitumor activity was evaluated in 15 subcutaneously implanted human tumor xenografts. 4′-Thio-ara-C was administered intraperitoneally using either q1d×9 (daily treatment for nine consecutive days) or q4h×3/q1d×9 (three treatments each day separated by 4-h intervals for nine consecutive days). *Results*: 4′-Thio-ara-C exhibited an excellent spectrum of activity. Treatment with the compound was curative against HCT-116 colon, SW-620 colon, NCI-H23 NSCL, and CAKI-1 renal tumors and resulted in partial/complete regressions in the DLD-1 colon, NCI-H522 NSCL, DU-145 prostate, and PANC-1 pancreatic tumor models. Tumor stasis was noted for HT29 colon and NCI-H460 NSCL tumors. Tumor inhibition was observed for A549 NSCL, PC-3 prostate, LNCAP prostate, and MDA-MB-435 breast tumors. Of the 15 tumors examined, only CFPAC-1 pancreatic was unresponsive to the compound. In contrast, 1-β-D-arabinofuranosylcytosine was minimally active at best against CAKI-1 renal, HCT-116 colon, NCI-H460 NSCL, and SW-620 colon tumors. Schedule- and route-dependency studies were conducted using the NCI-H460 NSCL tumor. The activity of 4′-thio-ara-C was independent of schedule when comparing q2d×5 (every other day for five treatments), q1d×9, and q4h×3/q1d×9 treatment schedules. 4′-Thio-ara-C was equally effective by the intravenous and intraperitoneal routes of administration, with the oral route being less efficacious. *Conclusions*: On the basis of these results, 4′-thio-ara-C appears to have a profile distinct from other nucleoside antitumor agents and is being advanced to clinical trials.

Keywords Ara-C · 4′-Thio-ara-C · Anticancer drugs · Human tumor xenografts

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Introduction

In recent years three nucleoside analogs have received FDA approval for the treatment of cancer. These agents are fludarabine phosphate (1991), cladribine (1993), and gemcitabine (1996) [3]. Whereas fludarabine phosphate and cladribine are used in the treatment of hematopoietic cancers (chronic lymphocytic leukemia and hairy cell leukemia, respectively), as are almost all of the clinically approved nucleoside drugs [e.g., cytarabine (ara-C)], gemcitabine has been approved for the treatment of some solid tumors [i.e., non-small-cell lung (NSCL) and pancreatic cancers). The Southern Research Institute has had a program designed to look for nucleosides with improved properties, agents that might have either an increased activity/selectivity or a broader application to different types of tumors. As a part of that program, a number of classes of 4′-thionucleosides have been synthesized. One of these compounds, 4′-thio-β-D-arabinofuranosylcytosine (4′-thio-ara-C, OSI-7836), has shown significant cytotoxicity against a panel of human tumor lines (CAKI-1 renal, NCI-H23 NSCL, DLD-1 colon, SK-MEL-28 and LOX IMVI melanomas, SNB-7 CNS, PC-3 prostate, and ZR-75-1 breast), with IC_{50} values ranging from 1 to 10 μ*M* [9]. We present here data resulting from the evaluation of 4′-thio-ara-C against a spectrum of human tumor systems in mice, which shows a broad spectrum of in vivo antitumor activity [10]. In contrast ara-C has shown greater cytotoxicity against human tumor lines than 4′-thio-ara-C

[9]; however, ara-C has exhibited little to no in vivo antitumor activity against human tumor xenografts [10].

Materials and methods

Drugs

4′-Thio-ara-C was synthesized at the Southern Research Institute following the procedure of Tiwari et al. [9]. PalmO-ara-C was synthesized using a previously described procedure [4]. 1-β-D-Arabinofuranosylcytosine (ara-C) was provided by the Drug Synthesis and Chemistry Branch, National Cancer Institute (NCI) (Bethesda, Md.). For the in vivo studies 4′-thio-ara-C was prepared fresh every 5 days in saline containing 0.05% Tween 80 and kept at 2–8°C between injections.

Experimental chemotherapy

Mice, obtained from various commercial suppliers, were housed in microisolator cages and were allowed commercial mouse food and water ad libitum. The various human tumors were obtained from the Developmental Therapeutics Program Tumor Repository (Frederick, Md.) and were maintained in in vivo passage. Only tumor lines that tested negative for antibodies to selected viruses were used. For the in vivo evaluation of the sensitivity of human tumors to the compounds, NCr-*nu* athymic mice (scid mice for LNCAP prostate and CFPAC-1 pancreatic) were implanted subcutaneously (s.c.) with 30–40 mg of tumor fragments (10^7 cultured cells in 0.2 ml Matrigel for LNCAP). The tumor implantation day was designated day 0. In each experiment, 4′-thio-ara-C was tested at two or three dosage levels. The procedures were approved by the Southern Research Institute's Institutional Animal Care and Use Committee, which conforms to the current Public Health Service "Policy on Humane Care and Use of Laboratory Animals" and the "Guide for the Care and Use of Laboratory Animals".

Antitumor activity assessment

Antitumor activity was assessed on the basis of delay in tumor growth (T–C). The delay in tumor growth is the difference in the median of times poststaging for tumors of the treated and control groups to double in mass two, three, or four times. Drug-related deaths, tumor-free survivors, and any other animal whose tumor failed to attain the evaluation size were excluded. Tumors were measured in two dimensions (length and width) twice weekly, and the tumor weight was calculated using the formula (length×width^2)/2 assuming unit density. The mice were also weighed twice weekly.

Statistical analysis

Statistical analysis was performed using SYSTAT version 7.0 (life table analysis using the time to n doublings as the survival endpoint, i.e., Mantel statistic).

Results

The therapeutic effectiveness of 4′-thio-ara-C against 15 human tumor xenografts (four colon, four NSCL, three prostate, two pancreatic, one renal, and one breast) implanted s.c. in immunodeficient mice is shown in Table 1. The compound was administered intraperitoneally (i.p.) using one of two treatment schedules: q1d×9 (daily treatment for nine consecutive days) or q4h×3/q1d×9 (three treatments each day separated by 4-h intervals for nine consecutive days). 4′-Thio-ara-C exhibited an excellent spectrum of activity. Treatment with the compound was curative against HCT-116 colon, SW-620 colon, NCI-H23 NSCL, and CAKI-1 renal tumors and resulted in partial/complete regressions in the DLD-1 colon, NCI-H522 NSCL, DU-145 prostate, and PANC-1 pancreatic tumor models. Tumor stasis was noted for HT29 colon and NCI-H460 NSCL tumors. Figures 1 and 2 illustrate the growth-inhibitory activity of 4′-thio-ara-C against CAKI-1 and NCI-H460 tumors, respectively. Tumor inhibition was observed for A549 NSCL, PC-3 prostate, LNCAP prostate, and MDA-MB-435 breast tumors. Of the 15 tumors examined, only CFPAC-1 pancreatic was unresponsive to the compound.

Table 1 Response of s.c.-implanted human tumor xenografts to 4′-thio-ara-C

Tumor	Schedule	Optimal i.p.dosage (mg/kg/dose)	Median tumor size range (mm^3)	T–C (days)	Tumor-free survivors
HCT-116 colon	q4h×3, days 6–14	20[a]	100–144	>43.1[b]	6/6
DLD-1 colon	Days 9–17	90	94–126	32.7[b]	0/6
HT-29 colon	Days 12–20	90	113–144	>37.1[b]	0/6
SW-620 colon	Days 7–15	90	126–135	>49.2[c]	3/6
A549 NSCL	Days 14–22	90	126–162	>30.9[b]	0/6
NCI-H23 NSCL	Days 14–22	90	144–172	>33.8[c]	4/6
NCI-H460 NSCL	Days 7–15	90	135–144	22.1[c]	0/6
NCI-H522 NSCL	Days 8–16	90	104–135	31.1[b]	2/6
DU-145 prostate	q4h×3, days 11–19	30	76–111	31.8[b]	1/6
PC-3 prostate	Days 13–21	90	132–162	>31.2[b]	0/6
LNCAP prostate	Days 9–17	40	162–189	27.9[b]	0/6
CAKI-1 renal	Days 14–22	90	245–336	>66.5[b]	4/6
	q4h×3, days 14–22	30		>66.5[b]	2/6
MDA-MB-435 breast	Days 13–21	90	135–144	19.3[b]	0/6
CFPAC-1 pancreatic	Days 8–16	60	94–126	9.7[d]	0/6
PANC-1 pancreatic	Days 14–22	90	144–153	43.3[c]	0/6

[a]Highest dosage tested
[b]The difference in the median of times poststaging for tumors of the treated (T) and control (C) groups to double in mass twice
[c]The difference in the median of times poststaging for tumors of the treated (T) and control (C) groups to double in mass three times
[d]The difference in the median of times poststaging for tumors of the treated (T) and control (C) groups to double in mass four times

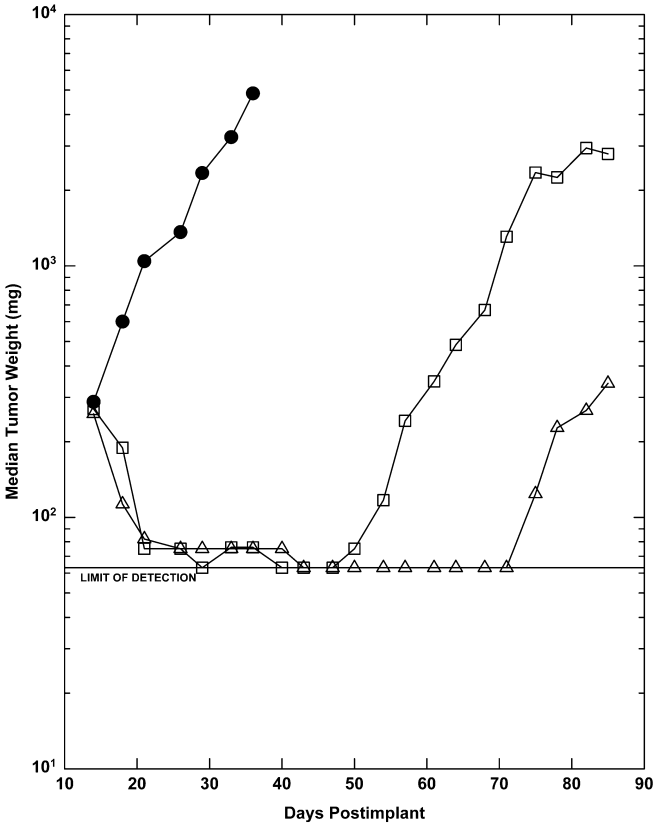

Fig. 1 Response of s.c.-implanted CAKI-1 renal tumor to optimal treatment with 4′-thio-ara-C. 4′-Thio-ara-C was administered i.p. either daily on days 14–22 (△ 90 mg/kg/dose) or three times a day (q4h×3) on days 14–22 (□ 30 mg/kg/dose, ● 0 mg/kg/dose). The growth curves are for only the mice bearing tumors (i.e., △, □, and ● had 4/6, 2/6, and 0/12 tumor-free survivors, respectively)

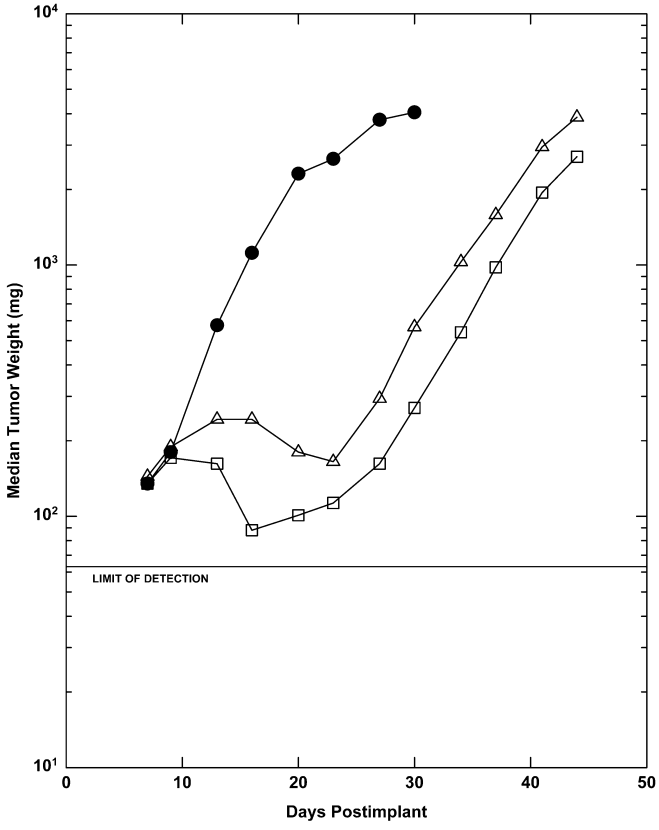

Fig. 2 Response of s.c.-implanted NCI-H460 NSCL tumor to treatment with 4′-thio-ara-C. 4′-Thio-ara-C was administered i.p. daily on days 7–15 at dosages of 90 (□), 60 (△), and 0 (●) mg/kg/dose. There were no tumor-free survivors in this study

In contrast, ara-C (or its depot form, palmO-ara-C) exhibited in general little or no activity against human tumor xenografts. Table 2 summarizes some historical NCI data for these two compounds against six human tumors (three colon, two NSCL, and one renal). Of the tumors cured by 4′-thio-ara-C, SW-620 was unresponsive to both ara-C and palmO-ara-C; HCT-116 was unresponsive to ara-C and minimally responsive to palmO-ara-C; and CAKI-1 was minimally responsive to ara-C and extremely sensitive to palmO-ara-C. For the NCI-H460 and NCI-H522 NSCL tumors, neither ara-C nor palmO-ara-C had any activity, whereas 4′-thio-ara-C resulted in either tumor stasis or regression.

Table 2 Response of s.c.-implanted human tumor xenografts to ara-C and palmO-ara-C

Tumor	Ara-C				PalmO-ara-C			
	Schedule	Optimal i.p. dosage (mg/kg/dose)	T−C (days)	Tumor-free survivors	Schedule	Optimal i.p. dosage (mg/kg/dose)	T−C (days)	Tumor-free survivors
HCT-116 colon	q4h×3, days 6–14	13.3	3.3[a]	0/6	Days 6–14	22.5	15.1[a]	0/6
SW-620 colon	q4h×6, days 9,13, 17	25	0.7[b]	0/6	Days 9, 13, 17	75	1.6[b]	0/6
COLO 205 colon	q4h×6, days 15,19, 23	25	7.1[a]	0/6	Days 15, 19, 23	75	9.0[a]	0/6
NCI-H460 NSCL	q4h×6, days 8,12, 16	25	5.0[b]	0/6	Days 8–16	23	5.9[b]	0/6
NCI-H522 NSCL	q4h×6, days 20,24, 28	16.8	2.3[c]	0/6	Days 20–28	15	4.1[c]	0/6
CAKI-1 renal	q4h×3, days 7–15	13.3	11.0[c]	0/6	Days 7–15	22.5 (LD_{17})	26.5[c]	0/6
	q4h×6, days 16,20, 24	25	7.2[a]	0/6	Days 16–20	45[d]	> 12.3[a]	2/6

[a]The difference in the median of times poststaging for tumors of the treated (T) and control (C) groups to double in mass twice
[b]The difference in the median of times poststaging for tumors of the treated (T) and control (C) groups to double in mass four times
[c]The difference in the median of times poststaging for tumors of the treated (T) and control (C) groups to double in mass three times
[d]Highest dosage tested

Table 3 Schedule dependency study of 4′-thio-ara-C administered i.p. using s.c.-implanted human NCI-H460 NSCL tumor

Schedule	I.p. dosage (mg/kg/dose)	Loss in body weight on day 15 (%)	T−C (days)[a]	Tumor-free survivors
Days 7, 9, 11, 13, 15	360	33	Toxic	0/6
	240	14	17.4	0/6
	160	17	18.5	0/6
	107	13	16.4	0/6
Days 7–15	135	25	Toxic	0/6
	90	9	16.2	0/6
	60	14	16.2	0/6
	40	9	16.2	0/6
q4h×3, days 7–15	30	23	Toxic	1/6
	20	22	17.7	0/6
	13.3	14	17.4	0/6
	8.9	17	16.0	0/6

[a]The difference in the median of times poststaging for tumors of the treated (T) and control (C) groups to double in mass three times

Table 4 Route dependency study of 4′-thio-ara-C using s.c.-implanted human NCI-H460 NSCL tumor

Route	Dosage[a] (mg/kg/dose)	Loss in body weight on day 20 (%)	T−C (days)[b]	Tumor-free survivors
I.v.	135	24	21.4	0/6
	90	20	21.3[c,d]	0/6
	60	15	19.2	0/6
	40	16	19.3	0/6
I.p.	90	20	21.4[c,e]	0/6
	60	15	16.8	0/6
	40	20	18.6	0/6
	27	11	11.3	0/6
Oral	200	15	8.9[d,e]	0/6
	135	16	10.7	0/6
	90	11	6.2	0/6
	60	5	0.6	0/6

[a]Treatment was on days 9–17
[b]The difference in the median of times poststaging for tumors of the treated (T) and control (C) groups to double in mass four times
[c]Statistically insignificant difference ($P = 0.32092$), using a life table analysis with the time to four doublings as the survival endpoint
[d]Statistically significant difference ($P = 0.00051$)
[e]Statistically significant difference ($P = 0.00051$)

Schedule- and route-dependency studies were conducted using the NCI-H460 NSCL tumor. Three i.p. treatment schedules were evaluated: q2d×5 (every other day for five treatments), q1d×9, and q4h×3/q1d×9. Toxicity was reached for each treatment schedule. The total tolerated dose decreased as the treatment became more chronic (i.e., 1200 mg/kg for q2d×5, 810 mg/kg for q1d×9, and 540 mg/kg for q4h×3/q1d×9). The activity of 4′-thio-ara-C was schedule-independent, as evidenced by similar T−C values for the three treatment schedules. No dose response was observed. Two other studies using the NCI-H460 tumor showed a modest dose response (see Table 4; for Table 1, a dosage of 60 mg/kg/dose produced a T−C of 18.7 days in comparison to 22.1 days for a dosage of 90 mg/kg/dose). The data are summarized in Table 3.

For the route-dependency study, three routes were investigated: intravenous (i.v.), i.p., and oral (by gavage). Toxicity was approached for the i.v. and i.p. routes. The i.v. route permitted as much compound to be administered (if not more) as the i.p. route. An i.p. dosage of 135 mg/kg/dose given q1d×9 is always lethal to nude mice. At least twice as much compound can be administered by the oral route in comparison to the i.v. and i.p. routes. 4′-Thio-ara-C was equally effective by the i.v. and i.p. routes, with the oral route being less efficacious. The data are summarized in Table 4.

Discussion

The synthesis of 4′-thio-ara-C was reported by Whistler and co-workers 30 years ago; however, because of the lengthy synthetic route used, only a small amount of the nucleoside was obtained [6, 11]. Cytotoxicity data were generated against only one tumor cell line (KB epidermoid, EC_{50} 0.42 μM) and no further biological results were reported. Tiwari et al. at our institute developed a facile synthetic procedure that can be used to generate gram quantities of the compound [9]. Therefore, the compound was evaluated in vitro against a panel of human tumor cell lines, yielding IC_{50} values typically in the range 1 to 10 μM. For comparison, ara-C exhibited lower IC_{50} values typically in the range 0.2 to 2 μM [9].

4′-Thio-ara-C was then evaluated against a spectrum of human tumor systems in mice. In contrast to previous results for ara-C, 4′-thio-ara-C showed outstanding

selectivity in the in vivo tumor studies. Of the 15 tumors tested, 8 were either cured or regressed following treatment with the compound. Of the remaining 7 tumors, all but one (CFPAC-1 pancreatic) exhibited sensitivity to the compound. This broad spectrum of in vivo antitumor activity suggests that 4′-thio-ara-C should be considered seriously for advancement to clinical trials. In contrast, ara-C has exhibited little to no activity against human tumor xenografts and is used primarily against hematopoietic cancers in the clinic [1].

Little if any information has been published concerning the sensitivity of the CFPAC-1 pancreatic tumor line (derived from a metastatic lesion in the liver of a cystic fibrosis patient) to standard chemotherapeutic agents. Our limited use of the line in vivo (just nucleosides) has shown gemcitabine to be the most efficacious agent with 5-FU being minimally active at best (similar to 4′-thio-ara-C).

Interestingly, treatment with 4′-thio-ara-C was independent of schedule (at least for the three treatment schedules investigated). Comparable activity was observed whether the compound was given as 5 or 27 injections over a 9-day period. This result is in contrast to the behavior of ara-C, which is highly schedule-dependent. Skipper et al. have shown that ara-C is curative against murine L1210 leukemia when given eight times each day separated by 3-h intervals (q3h×8) and then repeated four times at 4-day intervals (q4d×4) but only resulted in a 63% increase in life-span and no long-term survivors when given once a day on a q4d×4 schedule [8].

Parker et al. have studied the metabolism of 4′-thio-ara-C in human CEM leukemia cells to determine how the biochemical pharmacology of this compound differs from that of ara-C [7]. Although there are many quantitative differences in the metabolism of the two compounds, the basic mechanism of action of 4′-thio-ara-C is similar to that of ara-C—namely, phosphorylation to the triphosphate (i.e., 4′-thio-ara-CTP and ara-CTP, respectively) and inhibition of DNA synthesis. The major differences between the two compounds are the following: (1) 4′-thio-ara-C is phosphorylated to active metabolites at 1% of the rate for ara-C; (2) 4′-thio-ara-CTP is 10- to 20-fold more potent as an inhibitor of DNA synthesis than ara-CTP; (3) the half-life of 4′-thio-ara-CTP is twice that of ara-CTP; (4) the catalytic efficiency of 4′-thio-ara-C with cytidine deaminase is 10% that for ara-C; and (5) the 5′-monophosphate of ara-C is a better substrate for deoxycytidine 5′-monophosphate deaminase than the 5′-monophosphate of 4′-thio-ara-C. Of these differences in metabolism between the two compounds, the prolonged retention of 4′-thio-ara-CTP is considered to be the major factor contributing to the activity of 4′-thio-ara-C against human solid tumors.

Gemcitabine, another cytidine nucleoside analog, also exhibits a broad spectrum of in vivo antitumor activity against human tumor xenografts, being most active against colon carcinoma (HT29, HC1, GC3,and VRC5), head and neck squamous cell carcinoma (HNX-14C and HNX-22B), soft tissue sarcoma [S.La(C)], ovarian carcinoma (OVCAR-3 and A2780), and breast carcinoma (MX-1) [2, 5].

On the basis of the results presented here, 4′-thio-ara-C appears to have a profile distinct from other nucleoside antitumor agents (including gemcitabine) and is being advanced to clinical trials by OSI Pharmaceuticals.

Acknowledgements This work was supported by NCI grant PO1-CA34200. The authors gratefully acknowledge the technical assistance of the staff of the Cancer Therapeutics and Immunology Department. J. Tubbs and G. Jones assisted with data management, and K. Cornelius prepared the manuscript.

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