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ORIGINAL ARTICLE

Lack of in vivo cross-resistance with 4′-thio-ara-C against drug-resistant murine P388 and L1210 leukemias

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Abstract
Purpose 4′-Thio-β-D-arabinofuranosylcytosine (4′-thio-ara-C), which has shown a broad spectrum of antitumor activity against human tumor systems in mice and is undergoing clinical trials, was evaluated for cross-resistance to seven clinical agents in order to identify potentially useful guides for patient selection for further clinical trials of 4′-thio-ara-C and possible noncross-resistant drug combinations with 4′-thio-ara-C.
Methods A drug resistance profile for 4′-thio-ara-C, which was administered intraperitoneally daily for nine consecutive days, was obtained using seven drug-resistant P388 and L1210 leukemias that were implanted intraperitoneally in mice.
Results Multidrug-resistant P388 leukemias (leukemias resistant to doxorubicin, etoposide, or paclitaxel) exhibited no cross-resistance to 4′-thio-ara-C. Leukemias resistant to camptothecin, cisplatin, and 5-fluorouracil were also not cross-resistant to 4′-thio-ara-C. Only the leukemia resistant to 1-β-D-arabinofuranosylcytosine was cross-resistant to 4′-thio-ara-C.
Conclusions The data suggest that (1) it may be important to exclude or to monitor with extra care patients who have previously been treated with 1-β-D-arabinofuranosylcytosine and (2) the lack of cross-resistance seen with 4′-thio-ara-C may contribute to therapeutic synergism when 4′-thio-ara-C is combined with other agents.

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Keywords 4′-thio-ara-C - in vivo cross-resistance · Drug-resistant murine leukemias

Abbreviations

ILS	Increase in life span
L1210/5-FU	5-Fluorouracil-resistant L1210 leukemia
L1210/0	Parental L1210 leukemia
P388/ADR	Doxorubicin-resistant P388 leukemia
P388/ARA-C	1-β-D-arabinofuranosylcytosine-resistant P388 leukemia
P388/CPT	Camptothecin-resistant P388 leukemia
P388/DDPt	Cisplatin-resistant P388 leukemia
P388/TAX	Paclitaxel-resistant P388 leukemia
P388/VP-16	Etoposide-resistant P388 leukemia
P388/0	Parental P388 leukemia

Introduction

4′-Thio-β-D-arabinofuranosylcytosine (4′-thio-ara-C) is a novel pyrimidine nucleoside antimetabolite, which differs from 1-β-D-arabinofuranosylcytosine (ara-C) by only one atom (O in the ribose ring replaced by S). The synthesis of the compound was reported by Whistler and co-workers over 30 years ago; however, because of the lengthy synthetic route used, only a small amount of the nucleoside was obtained [1, 2]. Cytotoxicity data were generated against only one tumor cell line (KB epidermoid) 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 [3]. Therefore, the compound was evaluated initially in vitro against a panel of human tumor cell lines, yielding IC_{50}

values typically in the range of 1 to 10 μM [3], and then evaluated in vivo against a spectrum of human tumor systems in mice [4]. Of the 15 tumors tested, eight were either cured or regressed following treatment with the compound. Of the remaining seven tumors, all but one exhibited sensitivity to the compound. The spectrum of activity was superior to that for ara-C, gemcitabine, fludarabine monophosphate, or clofarabine (approved in 2004 for treatment of pediatric acute lymphocytic leukemia) (unpublished data). Two phase I clinical trials have been conducted. As a result of these trials, additional clinical trials have been initiated for various leukemias/lymphomas. Drug resistance that may be either inherent or acquired seems likely to be encountered in these trials. Development of a drug resistance profile for 4′-thio-ara-C may identify potentially useful guides for patient selection for further clinical trials of 4′-thio-ara-C and possible noncross-resistant drug combinations with 4′-thio-ara-C. This information should aid in the design of strategies for the optimal use of the drug. In vivo models may be preferable for developing drug resistance profiles because of evidence that drug resistance may not be comparable between in vitro and in vivo models of the same cell line [5]. We report here the lack of in vivo cross-resistance seen for 4′-thio-ara-C against various drug-resistant murine leukemias.

Materials and methods

4′-Thio-ara-C was supplied by Gilead (Foster City, CA). The compound was dissolved in sterile saline containing 0.05% tween 80 (3.6 mg/ml), and appropriate dilutions were made with the vehicle. Dosing solutions of 4′-thio-ara-C were prepared fresh every 4–5 days and were stored at 4–8°C when not in use.

The in vivo sensitivity of P388/0 or L1210/0 and seven drug-resistant P388 or L1210 leukemias (all maintained as in vivo lines) to 4′-thio-ara-C was determined as previously described for other antitumor agents [6]. $CD2F_1$ mice were implanted i.p. with 10^6 cells of either P388/0 or P388/drug-resistant leukemia on day 0 (or with 10^5 cells of either L1210/0 or L1210/5-FU leukemia). 4′-Thio-ara-C was evaluated at three i.p. dose levels. There were six mice in each dose group; tumor-bearing control mice (12/experiment) were untreated. Mice were observed for life span and were euthanized when they were unable to get to food or water or were moribund. In each experiment, additional groups were treated with two doses of an appropriate drug to confirm the resistance of a P388/drug-resistant (or L1210/5-FU) leukemia. In each experiment, two P388/drug-resistant leukemias were compared directly with the parent or wild-type P388/0 leukemia, and the three parallel groups of mice were treated identically with a single-drug preparation. All studies were repeated for confirmation. Procedures were approved by Southern Research Institute's Institutional Animal Care and Use Committee, which conformed 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 was assessed on the basis of the percentage of median ILS and the net $\log_{10}$ cell kill. Calculations of the net $\log_{10}$ cell kill were made from the tumor-doubling time that was determined from an internal tumor titration consisting of implants from serial 10-fold dilutions of P388 (or L1210) cells [7]. Long-term (60-day) survivors were excluded from calculations of ILS and tumor cell kill. For the assessment of tumor cell kill at the end of treatment, the survival difference between treated and control groups was adjusted to account for regrowth of tumor cell populations that may occur between individual treatments [8]. The net $\log_{10}$ cell kill was calculated as follows:

$$\text{Net } \log_{10} \text{ cell kill} = \frac{(T - C) - (\text{duration of treatment in days})}{3.32 \times T_d}$$

where $(T - C)$ is the difference in the median day of death between the treated (T) and the control (C) groups, 3.32 is the number of doublings required for a population to increase 1 $\log_{10}$ unit, and T_d is the mean tumor-doubling time (days) calculated from a log-linear least-squares fit of the implant sizes and the median days of death of the titration groups.

Cross-resistance was defined as a decrease in the sensitivity (>2 $\log_{10}$ units of cell kill) of a drug-resistant leukemia to 4′-thio-ara-C when compared with that concurrently observed for the parental leukemia.

Results and discussion

The in vivo cross-resistance profile for 4′-thio-ara-C is shown in Table 1. Multidrug-resistant P388 leukemias (P388/ADR, P388/VP-16, and P388/TAX) exhibited no cross-resistance to the drug. The P388/ADR and P388/TAX sublines were more sensitive to the drug than the parental P388 line based on % ILS values, whereas the P388/VP-16 subline and the parental P388 line exhibited comparable % ILS values. P388/CPT, P388/DDPt, and L1210/5-FU sublines were also not cross-resistant to the drug with the P388/CPT and P388/DDPt sublines exhibiting comparable % ILS values in comparison with the parental P388 line. Unexpectedly, a 5-FU dosage of 90 mg/kg/dose was toxic to mice bearing the L1210/5-FU subline but was not toxic to mice

Table 1 Activity of 4′-thio-ara-C against drug-resistant P388 and L1210 leukemias in vivo (ADR doxorubicin, VP-16 etoposide, CPT camptothecin, TAX paclitaxel, DDPt cisplatin, ARA-C 1-β-D-arabinofuranosylcytosine, 5-FU 5-fluorouracil)

Expt. No.	Resistant leukemia	Optimal i.p. dosage[a] ($\leq LD_{10}$, mg/kg/dose)	Therapeutic response						
			Sensitive Leukemia			Resistant Leukemia[c]			
			Median % ILS	Net $\log_{10}$ cell kill[b]	60-Day survivors/ total	Median % ILS	Net $\log_{10}$ cell kill[b]	60-Day survivors/ total	Cross-resistance?
3	P388/ADR	90	+133	+5.7	3/6	+227	+6.6	1/6	No
7		90	+167	+6.8	0/6	+235	+6.9	0/6	
2	P388/VP-16	90	+150	+6.5	1/6	+150	+6.8	2/6	No
5		90	+143	+6.8	0/6	+107	+5.2	0/6	
3	P388/CPT	90	+133	+5.7	3/6	+164	+6.7	1/6	No
7		90	+167	+6.8	0/6	+150	+7.0	0/6	
1	P388/TAX	90	+195	>+6.6	0/6	+250	>+6.7	1/6	No
4		90	+142	+6.7	0/6	+188	+6.7	2/6	
2	P388/DDPt	90	+150	+6.5	1/6	+155	>+6.8	0/6	No
5		90	+143	+6.8	0/6	+142	+7.2	0/6	
1	P388/ARA-C	90	+195	>+6.6	0/6	+125	+0.9	0/6	Yes
4		90	+142	+6.7	0/6	+55	−1.0	0/6	
6	L1210/5-FU	90	+200	+4.6	5/6	+25	Toxic	0/6	No
		45	+186	+3.8	1/6	–	>+5.8	6/6	
8	L1210/5-FU	90	–	>+5.8	6/6	+11	Toxic	0/6	
		45	+250	+5.8	1/6	–	+6.2	6/6	

[a] 4′-Thio-ara-C was administered on days 1–9 using an injection volume of 0.5 ml/20 g animal body weight

[b] Net $\log_{10}$ reduction in the tumor cell population between the beginning and the end of therapy, based on the median day of death of mice that died

[c] In these studies, the average degree of resistance of a drug-resistant subline in comparison with the parental line was as follows: ADR, 8 $\log_{10}$ units; VP-16, 8 $\log_{10}$ units; CPT, 8 $\log_{10}$ units; TAX, 4 $\log_{10}$ units; DDPt, 8 $\log_{10}$ units; ARA-C, 7 $\log_{10}$ units; and 5-FU, 5 $\log_{10}$ units

bearing the parental L1210 line. The L1210/5-FU subline was more sensitive to the drug than the parental L1210 line at a lower dosage of 45 mg/kg/dose (6/6 vs. 1/6 cures); however, the higher dosage of 90 mg/kg/dose was also curative against the parental line. Only the P388/ARA-C subline exhibited cross-resistance to the drug as evidenced by lower % ILS values in comparison with the parental P388 line and differences in net cell kill of approximately 7 $\log_{10}$ units.

Resistance to doxorubicin in P388/ADR leukemia (selected for resistance in vivo) is multifactorial (i.e., decreased drug accumulation, decreased formation of DNA single- and double-strand breaks, increased GSH transferase activity, earlier onset of DNA repair, reduced DNA topoisomerase II activity and protein levels, and elevated P-glycoprotein) [9, 10]. Concerning the mechanisms of resistance of P388/VP-16 leukemia, pyrimidine triphosphate pools [11] as well as the specific phorbol diester receptor and protein kinase C [12] were decreased in comparison with the parental P388/0 line. Studies in our laboratories have shown that P388/VP-16 leukemia (selected for resistance in vivo) does not overexpress the *mdr*-1 gene, which is probably a reflection of the fact that the line was selected for resistance in vivo. The line does exhibit decreased DNA topoisomerase II activity in comparison with the parental line [13]. Even though the mechanisms of paclitaxel resistance operative in P388/PTX leukemia have not been investigated, studies conducted with other tumor cell lines have revealed the resistance to be multifactorial—overexpression of the *mdr*-1 gene, molecular changes in the target molecule (β-tubulin), changes in apoptotic regulatory and mitosis checkpoint proteins, and changes in lipid composition and potentially the overexpression of interleukin 6 [14].

Resistance to camptothecin in P388/CPT leukemia (selected for resistance in vivo) has been attributed to a decrease in DNA topoisomerase I activity due to a rearrangement of the topoisomerase I gene [15]. Concomitant with this change has been an increase in DNA topoisomerase II activity.

Resistance to cisplatin in P388/DDPt leukemia (selected for resistance in vivo prior to chronic in vitro exposure to cisplatin) is multifactorial [16, 17]. P388/DDPt cells exhibit decreased drug accumulation, elevated GSH, and increased DNA polymerase β activity. We have found that our in vivo P388/DDPt subline contains elevated DNA

topoisomerase II activity in comparison with P388/0 (unpublished results).

Resistance to 5-fluorouracil in L1210/5-FU leukemia has been attributed to increased N^5,N^{10}-methylenetetrahydrofolate and thymidylate synthetase [18]. Resistance to 5-fluorouracil in P388/5-FU leukemia has been attributed to reduced initial uptake of drug, decreased levels of uridine kinase and uracil phosphoribosyltransferase, and reduced levels of 5-fluorouracil nucleotides [19, 20].

For P388/ARA-C leukemia, there appears to have been no published data concerning the mechanisms(s) of resistance. Studies conducted with human and other murine (L1210, L5178Y, and P815) leukemia cells resistant to ara-C have revealed several mechanisms of resistance: decreased membrane nucleoside-binding sites, decreased deoxycytidine kinase activity, increased cytidine deaminase activity, and increased intracellular CTP and dCTP pools [21, 22]. Clinical studies with AML indicate that resistance correlates best with increases in DNA polymerase α and 5′-nucleotidase activities and decreases in hENT transporter. Other studies show that resistance correlates with decreased deoxycytidine kinase and increased deoxycytidine deaminase activities [23].

As new agents enter phase II and III clinical trials, the selection of patients, most of whom have been treated previously with one or more drugs, may be critical to the success of the trials [24]. Information on the patterns of cross-resistance among various antitumor agents may be helpful in the selection of patients for treatment with 4′-thio-ara-C. For these trials, it may be important to exclude or to monitor with extra care patients who have previously been treated with ara-C.

The observation of a lack of cross-resistance of P388/ADR, P388/VP-16, P388/TAX, P388/CPT, P388/DDPt, and L1210/5-FU leukemias to 4′-thio-ara-C suggests that a combination of doxorubicin, etoposide, paclitaxel, camptothecin [or other topoisomerase I inhibitors (e.g., irinotecan)], cisplatin, or 5-FU with 4′-thio-ara-C might exhibit therapeutic synergism. As always, none of the above approaches may be applied clinically without caution and concern for the recognized gap between preclinical prediction and clinical validation.

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Conflict of interest 4′-Thio-ara-C was discovered at Southern Research Institute, which receives licensing fees and royalty payments from the commercial development and use of this agent. Southern Research Institute does distribute some of this money to Drs. Secrist and Waud.

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