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Evaluation of Cytarabine Against Ewing Sarcoma Xenografts by the Pediatric Preclinical Testing Program

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Abstract

Treatment with the nucleoside analog cytarabine has been shown to mimic changes in gene expression associated with down-regulation of the EWS-FLI1 oncogene in Ewing sarcoma cell lines, selectively inhibit their growth in vitro, and cause tumor regression in athymic nude mice. For this report cytarabine was studied in vitro against a panel of 23 pediatric cancer cell lines and in vivo against 6 Ewing sarcoma xenografts. Acute lymphoblastic leukemia cell lines were the most sensitive to cytarabine in vitro (median IC₅₀ 9 nM), while Ewing sarcoma cell lines showed intermediate sensitivity (median IC₅₀ 232 nM). Cytarabine at a dose of 150 mg/kg administered daily 5× failed to significantly inhibit growth of five xenograft models, but reduced growth rate of the A673 xenograft by 50%. Cytarabine shows no differential in vitro activity against Ewing sarcoma cell lines and is ineffective in vivo against Ewing sarcoma xenografts at the dose and schedule studied.

Keywords

Ewings/PNET; oncogenes; pediatric hematology/oncology

INTRODUCTION

Cytarabine is a nucleoside analog cytotoxic to a wide variety of proliferating mammalian cells in culture. It exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S phase) and under certain conditions blocks the progression of cells from the G1 phase to the S phase. Cytarabine is an established drug for remission induction in acute myeloid leukemia (AML) of adults and pediatric patients and is also used in the treatment of acute lymphoblastic leukemia (ALL). Recently, Stegmaier et al. [1] made the observation

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that cytarabine selectively and specifically induced similar changes in gene expression to those observed when EWS-FLI1 was turned “off” using RNA interference (RNAi). EWS-FLI1 expression is essential for Ewing sarcoma cells, as targeting of EWS-FLI1 with antisense oligonucleotides or RNAi inhibits proliferation, survival, and oncogenic transformation. Ewing sarcoma cell lines were more sensitive to cytarabine than cells not expressing EWS-FLI1, suggesting cell type selectivity [1]. Further, cytarabine treatment reduced the levels of EWS-FLI1 protein in these cells, and caused regression of a Ewing sarcoma line grown in athymic nude mice. The authors concluded that, based on these novel observations, clinical trials testing cytarabine in poor prognosis patients with Ewing sarcoma were warranted. In this study we have expanded in vitro testing of cytarabine to a panel of 23 cell lines and in vivo testing to an additional 5 Ewing sarcoma xenograft lines as well as A673 used in the original report [1]. Cytarabine showed potent in vitro activity, particularly against ALL cell lines, but demonstrated low activity in vivo against Ewing sarcoma xenografts, consistent with recently published clinical data for intermediate dose cytarabine in patients with refractory Ewing sarcoma [2].

MATERIALS AND METHODS

In Vitro Testing

In vitro testing was performed using DIMSCAN, a semi-automatic fluorescence-based digital image microscopy system that quantifies viable cell numbers in tissue culture multiwell plates [3]. Cells were incubated in the presence of cytarabine for 96 hr at concentrations from 0.1 nM to 1.0 μ M and analyzed as previously described [4].

In Vivo Tumor Growth Inhibition Studies

CB17SC-M *scid*^{-/-} female mice (Taconic Farms, Germantown, NY), were used to propagate subcutaneously implanted Ewing, sarcoma xenografts, as previously described [5]. Characteristics of these tumors can be obtained at (pftp.stjude.org/documents), and all are positive for the EWS-FLI1 translocation. All mice were maintained under barrier conditions and experiments were conducted using protocols and conditions approved by the institutional animal care and use committee of this institution. Tumor volumes (cm³) were determined as previously described [5]. Responses were determined using three activity measures as previously described [5]. An in-depth description of the analysis methods is included in the Supplemental Response Definitions Section.

Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SAS®, was used to compare event-free survival distributions between treatment and control groups. *P* values were two-sided and were not adjusted for multiple comparisons given the exploratory nature of the studies. The Mann–Whitney test was used to test the difference of medians of IC₅₀ values between the groups of lines with similar tumor types to the remaining lines of the panel.

Drugs and Formulation

Cytarabine was provided to the Pediatric Preclinical Testing Program by the Division of Cancer Treatment and Diagnosis (DCTD), NCI. Cytarabine was dissolved in D5W USP and administered intraperitoneally, daily 5× at a dose of 150 mg/kg.

RESULTS

The median IC₅₀ for cytarabine against the 23 cell lines of the PPTP in vitro panel was 242 nM, but there was wide variation across panels. For the ALL panel, the median IC₅₀ was 9 nM, which was significantly lower than that for the remaining PPTP cell lines ($P = 0.003$). Conversely, for the rhabdomyosarcoma panel, the median IC₅₀ was >1,000 nM. The median IC₅₀ values for the Ewing sarcoma and neuroblastoma cell lines were intermediate between these extremes (232 and 386 nM, respectively).

Cytarabine administered at 150 mg/kg for 5 days resulted in 4 of 60 deaths (6.7%) whereas no mice died in the control groups (n = 60; Supplemental Table I). Cytarabine extended the EFS T/C to 1.5 and 1.6 in CHLA258 and A673 tumors, respectively, but did not significantly alter EFS in the other tumor lines. Results for each tumor response criteria (T/C, EFS T/C, Objective Response) are summarized in Table II. This analysis shows that cytarabine has low activity using any of the standard criteria utilized by the PPTP to assess agent activity.

DISCUSSION

The development of RNAi-mediated gene silencing has opened the way to identifying the biological significance of downregulating oncogenes. Stegmaier et al. [1], screened a small library of biologically active compounds and found that cytarabine selectively and specifically recapitulated changes in gene expression found when EWS-FLI was silenced using RNAi. Interestingly, doxorubicin, a drug with known utility in treatment of Ewing sarcoma had similar effects, as did rapamycin. However, in the PPTP study, rapamycin did not exert significant activity against Ewing sarcoma models in vivo [6]. Based on the novel findings in the Stegmaier report, COG has recently conducted a clinical trial of cytarabine in relapsed or refractory patients with Ewing sarcoma [2]. Despite significant grade 4 hematologic toxicity in all 10 patients, there was only transient stabilization of disease progression in a single patient on study. The conclusion was that at the dose and schedule of cytarabine administered (500 mg/m² b.i.d. daily 5×), this agent showed minimal therapeutic activity.

In vitro testing of cytarabine against a panel of pediatric cancer cell lines failed to identify a pattern of distinctive sensitivity for Ewing sarcoma cell lines. Consistent with prior reports, we observed that many leukemia cell lines have IC₅₀ values in the low nanomolar range, whereas IC₅₀ values for solid tumor cell lines are higher [7–10]. The variation in in vitro activity is consistent with the clinical activity observed for cytarabine (i.e., leukemias much more responsive than solid tumors) and highlights the need for surveying a range of cancer cell lines before concluding that an agent shows selectivity for a particular histology.

In this study we evaluated the in vivo antitumor activity of cytarabine in a panel of Ewing sarcomas, including the A673 line reported to regress on cytarabine treatment [1]. Our study used a more intensive schedule (3.1-fold) of cytarabine administration compared to that reported by Stegmaier et al. (150 mg/kg daily 5× vs. 60 mg/kg daily 4×). All tumors progressed on treatment including A673, although the growth rate of this line was inhibited ~50%. While the increase in time to event was statistically significant for CHLA258 and A673, this did not meet criteria for intermediate activity (EFS (T/C) 2), thus had “low” activity by all criteria (Table II). Another difference between the studies, is that whereas in the PPTP tumors are established (>200 mm³) at start of treatment, Stegmaier et al. used a luciferase reporter to estimate tumor response, hence it is difficult to assess actual tumor volume at initiation of treatment. However, the results reported here seem to closely parallel the lack of clinical response to this therapy reported by DuBois et al. [2]. Panels of pediatric tumor xenografts have been valuable in accurately identifying novel agents [11–13], combinations [14–16], and schedules that subsequently showed robust clinical activity. Further, the xenograft models used by the PPTP identify cytotoxic agents with known clinical utility against pediatric solid tumors and ALL [5,17]. Thus, while the approach to identifying novel therapeutic approaches by identifying agents that modulate oncogenes is intrinsically appealing, the results presented here suggest that more comprehensive preclinical results should be used to validate the approach before initiating clinical trials in children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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TABLE I

Activity of Cytarabine Against the PPTP In Vitro Cell Line Panel

Cell line	Histology	IC ₅₀ (nM)
RD	Rhabdomyosarcoma	>1,000
Rh41	Rhabdomyosarcoma	>1,000
Rh18	Rhabdomyosarcoma	>1,000
Rh30	Rhabdomyosarcoma	317
BT-12	Rhabdoid	>1,000
CHLA-266	Rhabdoid	>1,000
TC-71	Ewing sarcoma	242
CHLA-9	Ewing sarcoma	105
CHLA-10	Ewing sarcoma	496
CHLA-258	Ewing sarcoma	222
GBM2	Glioblastoma	69
NB-1643	Neuroblastoma	212
NB-EBc1	Neuroblastoma	493
CHLA-90	Neuroblastoma	278
CHLA-136	Neuroblastoma	620
NALM-6	ALL	39
COG-LL-317	ALL	9
RS4;11	ALL	181
MOLT-4	ALL	10
CCRF-CEM	ALL	9
Kasumi-1	AML	51
Karpas-299	ALCL	302
Ramos-RA1	NHL	46
Median		242
Minimum		9
Maximum		1,000

TABLE II

Summary of Cytarabine Antitumor Activity in Ewing Sarcoma Xenograft Models

Xenograft line	KM estimate of median time to event	P-value	EFS/TC	Median final RTV	Tumor volume T/C	P-value	Overall group response	T/C activity	EFS activity	Response activity
SK-NEP-1	8.6	0.07	1.1	>4	0.79	0.247	PD1	Low	Low	Low
EW5	12.1	0.993	1.1	>4	0.75	0.579	PD1	Low	Low	Low
EW8	11.5	0.012	1.1	>4	0.9	0.460	PD1	Low	Low	Low
TC-71	8.8	0.52	1.2	>4	0.87	0.360	PD1	Low	Low	Low
CHLA258	16.5	0.002	1.5	>4	0.55	0.027	PD1	Low	Low	Low
A673	10.2	0.023	1.6	>4	0.50	0.001	PD2	Low	Low	Low