Effect of Cytarabine and Decitabine in Combination in Human Leukemic Cell Lines

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Abstract

Purpose: 1-β-D-Arabinofuranosylcytosine (cytarabine; ara-C) is the most active agent in myeloid leukemia. 5-Aza-2′-deoxycytidine (DAC) is a cytosine analogue that inhibits DNA methylation and also has activity in myeloid leukemia. Therefore, we investigated combining these two drugs in human leukemia cell lines in vitro.

Experimental Design: We initially examined the effects of ara-C and DAC on human leukemia cell lines HL60, ML-1, RAji, and Jurkat. We measured IC50 of DAC and ara-C in these cell lines and calculated a combination index of these two drugs given either simultaneously or sequentially. In searching for mechanisms relative to epigenetic regulation for this effect, we examined DNA methylation of LINE and Alu repetitive elements as a surrogate for global genomic DNA methylation. In addition, we sorted AnnexinV positive and negative cells and measured differences in LINE methylation between them.

Results: The combination of DAC and ara-C showed additive induction of cell death in ML-1 and synergistic induction in HL60, RAji, and Jurkat. Sequentially, DAC followed by ara-C was a synergistic combination in all cell lines. Low-dose DAC induced more hypomethylation than high doses of the drug, whereas ara-C had no effects on methylation. The combination of ara-C with DAC either together or DAC followed by ara-C resulted in inhibition of LINE demethylation in HL60. The RIL gene, which is silenced by DNA hypermethylation, was activated by DAC, but the addition of ara-C to DAC reduced RIL gene activation. DAC treatment increased H3 Lys9 acetylation of Alu elements, whereas ara-C had no effect, and the addition of ara-C to DAC inhibited this effect. Finally, we showed that after DAC exposure, Annexin V positive cells were more hypomethylated than Annexin V negative cells.

Conclusion: The combination of DAC and ara-C showed additive or synergistic effects on cell death in four human leukemia cell lines in vitro, but antagonism in terms of epigenetic effects. One possible explanation for these paradoxical observations is that hypomethylated cells are sensitized to cell killing by ara-C. These data suggest that DAC used in combination with ara-C has clinical potential in the treatment of acute myeloid leukemia.

Epigenetics have acquired increased recognition as a driving force in human leukemia over the past few years, leading to the revival of interest in DNA methylation inhibitors as antineoplastic agents (1). 5-Aza-2′-deoxycytidine (DAC) is a potent hypomethylating agent that incorporates into DNA and traps DNA methyltransferase in the form of a covalent protein–DNA adduct. As a result, cellular DNA methyltransferase is rapidly depleted, and concomitantly genomic DNA is hypomethylated during continued DNA replication, resulting in replication-dependent DNA hypomethylation. Gene inactivation (“silencing”) of tumor-suppressor, growth-inhibitory, DNA repair, and several apoptosis genes can be mediated by DNA hypermethylation of gene promoters (2–5). DAC-induced hypomethylation is associated with reactivation of multiple genes, and it is thought that this effect on gene expression contributes to the mechanism of responding to the drug. DAC is active in myeloid leukemia (6) and was recently approved by the Food and Drug Administration for the treatment of MDS.

The deoxycytidine analogue 1-β-D-arabinofuranosylcytosine (cytarabine, ara-C) is the most commonly used drug in the treatment of acute myeloid leukemia (7). Ara-C exerts its cytotoxicity by inhibiting DNA polymerase or by terminating chain elongation upon incorporation into newly synthesized DNA (8, 9). DAC and ara-C have similar structures and metabolic pathways. Once inside the cell, both are phosphorylated

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Received 11/21/06; revised 4/4/07; accepted 5/9/07.

Grant support: NIH grants CA100632 and CA108631.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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doi:10.1158/1078-0432.CCR-06-2762
to the monophosphate form by the rate-limiting enzyme, deoxycytidine kinase. Subsequently, they are converted to diphosphate and triphosphate forms by other cellular kinases.

The fact that both DAC and ara-C are active in myeloid leukemia through different mechanisms of action has triggered interest in combining them. However, the effect of combining these two nucleoside analogues remains controversial. Combination of the two drugs showed antagonism in L1210 mouse leukemia, murine leukemic L5178 Y cell lines, and pediatric acute myeloid leukemia cells in vitro (10). The mechanism was presumed to be either that both of these drugs competed for deoxycytidine kinase (11–13) or that ara-C reduced DAC incorporation through inducing cell cycle arrest. A synergistic effect was found in a deoxycytidine kinase–deficient HL-60 cell line, and in pediatric patients with refractory acute lymphocytic leukemia by sequentially adding ara-C to DAC (14–16). The mechanism was proposed to be that ara-C induced deoxycytidine kinase silencing by hypermethylation, which could be reactivated by DAC. However, these studies used high doses of DAC, which may not be relevant to the clinic. There is little data on the epigenetic effects of combination of these drugs in human leukemia cell lines. This study investigated further the combination of ara-C and DAC in four human leukemic cell lines.

Materials and Methods

Cell culture and treatment protocols. The human leukemic cell lines HL60, ML-1, Raji, and Jurkat were obtained from American Type Collection. The cells were grown in RPMI 1640 plus 10% heat-inactivated FCS in plastic tissue culture plates in a humidified atmosphere containing 5% CO₂ at 37°C. For the growth inhibition assay, cells were plated at a density of 1 × 10⁶/mL in 5 mL of medium and split 24 h before treatment. Different concentrations of DAC and ara-C alone or in combination were added to the medium simultaneously. To measure IC₅₀, fresh DAC and ara-C was added every 24 h without changing medium. The doses that inhibited 50% proliferation (IC₅₀) were analyzed by the median-effect method (CalcuSyn software, Biosoft). In vitro cytotoxicity was assayed in triplicate by the following experimental conditions: DAC alone, ara-C alone, DAC + ara-C, and DAC followed by ara-C. The proportion of live cells in treated plates was measured by trypan blue exclusion. The effects of combinations were estimated using the CalcuSyn software, which was developed based on the median-effect method created by Chou and Talalay (17).

Pyrosequencing-based methylation analysis. Genomic DNA was prepared from cells and bisulfite conversion of genomic DNA was carried out. The Alu and LINE element PCR assay was used to measure global methylation and was modified for pyrosequencing-based methylation analysis (18). PCR cycling conditions were 96°C for 90 s, 43°C for 60 s, and 72°C for 120 s for 40 cycles. The PCR product was bound to Streptavidin Sepharose HP (Amersham Biosciences) and

![Fig. 1. Effects of DAC and ara-C on LINE methylation and cell viability. A, effects of DAC on cell growth in leukemia cell lines. Cells were plated in T-25 plates at a density of 1 × 10⁵/mL and treated with different doses of DAC from 30 nmol/L to 30 μmol/L. Cell viability was measured by trypan blue exclusion and IC₅₀ was calculated by the median-effect method. B, effects of ara-C on cell growth in leukemia cell lines. Cells were treated with different doses of ara-C from 10 nmol/L to 10 μmol/L. Cell viability was measured. C, low-dose DAC induced optimal hypomethylation in HL60. Cells were treated with different doses of DAC from 20 nmol/L to 50 μmol/L. Cell pellets were collected and DNA was extracted for bisulfite treatment. LINE methylation was measured by pyrosequencing-based analysis. D, recovery of LINE methylation. We treated HL60 with DAC 0.2 and 2 μmol/L, and then washed the medium away. The recovery of LINE methylation was measured at days 3, 5, and 20 after that.](www.aacrjournals.org)
the Sepharose beads containing the immobilized PCR product were purified, washed, denatured using a 0.2 mol/L NaOH solution, and washed again. Then, 0.3 μmol/L pyrosequencing primer was annealed to the purified single-stranded PCR product and pyrosequencing was done using the PSQ HS 96 Pyrosequencing System (Pyrosequencing, Inc.). Methylation quantification was done using the provided software. Primers were as follows: Alu forward primer (5′-GGGACGCGCAGCTAAGTTT-3′), reverse primer (5′-CCCCACAGCTACGTTTGA-3′), biotinylated universal primer (5′-GGGACGCGCAGCTAAGTTT-3′), and pyrosequencing primer (5′-AATACCTAAAATACCAAAC-3′); LINE forward primer (TTTTTTGATGTTTGGG), biotin-labeled reverse universal primer (GGGACGCGCAGCTAAGTTTCATCTGCTAATAATACCAAAC), and pyrosequencing primer (GGGTTGAGGAGG).

Annexin V staining and sorting. For this experiment, cells were treated with fresh drugs and medium daily for 4 days. Cells were collected, washed, and stained with 100 μL Annexin V detection kit (TACS kits, TREVIGEN). Apoptosis was analyzed by the BD FACS Calibur assay and Annexin V positive and negative cells were sorted with the BD FACS Vantage Hi-Flow Speed Flow Cytometer. Annexin V FITC and propidium iodide emission was collected with 530/30 and 635/65 nm filters, respectively. DNA was extracted from both Annexin V positive and negative cells for bisulfite treatment. LINE methylation was analyzed by pyrosequencing-based analysis.

Quantitative reverse transcription-PCR. Real-time quantitative reverse transcription-PCR was done with the ABI 7700 Sequence Detector (Applied Biosystems). We used commercially available primers sets with minor groove binder probe for PDLIM4 (RIL) and β-actin as an internal control (Applied Biosystems). Total cellular RNA was extracted by TRIzol reagent (Life Technologies). RNA was eluted with RNase-free water, quantified at an absorbance at 260/280 nm, and used for first-strand cDNA synthesis. Reactions for quantitative reverse transcription-PCR were done with the TaqMan universal PCR Master Mix kit (Applied Biosystems) in 96-well plates. Each sample was measured in triplicate. Assembled plates were then covered and run using the following conditions: an initial denaturation step of 95 °C for 10 min followed by 45 cycles at 95 °C for 15 s and 60 °C for 1 min. The resulting data were analyzed with ABI Prism 7000 SDS software (Applied Biosystems). The threshold cycles (Ct) were determined and the differences in the Ct values for β-actin and RIL were calculated.

Chromatin immunoprecipitation assays. The protocols for chromatin immunoprecipitation have been described previously (19). Briefly, cells were treated with formaldehyde to cross-link histones to DNA. After washing, the cells were sonicated. The lysate was then divided into three fractions; the first and second ones were diluted in lysis buffer, and the third one was used for input control. The first lysate was incubated with 10 μL of anti-Lys 9 acetylated histone H3 antisera (Upstate Biotechnology) at 4 °C overnight. To collect the immunoprecipitated complexes, protein A-Sepharose beads (Pharmacia Biotech) were added and incubated for 1 h at 4 °C. After washing, the beads were treated with RNase for 30 min at 37 °C and then proteinase K overnight. The cross-links were then reversed by heating the sample at 65 °C for 6 h. DNA was extracted, and PCR amplification of DNA was carried out on diluted DNA aliquots, using Alu forward primer (GCACCGCTGATCGTTTATTACCAAAC) and Alu reverse primer (CGTGGTTCGTCGCTTGAGTTTGC). The PCR products were visualized by agarose gel electrophoresis and quantitated by capillary electrophoresis using the Agilent 2100 bioanalyzer (Agilent Technologies).

Results

Low-dose DAC induces optimal hypomethylation. We initially examined effects of DAC and ara-C on global genomic methylation and cell viability. We treated with DAC and ara-C at different doses daily for 4 days. DAC and ara-C induced cell death in a dose-dependent manner as measured by trypan blue exclusion. At high doses, ara-C resulted in virtually no viable cells, whereas a fraction of cells was still alive at the highest dose of DAC (Fig. 1A and B). Global methylation using the DNA repetitive element LINE as a marker was measured by pyrosequencing-based analysis. LINE was heavily methylated in HL60. We treated HL60 cells with DAC at 0.02, 0.2, 1, 2, 5, 20, and 50 μmol/L daily for 4 days. Low-dose DAC induced hypomethylation and this effect was lost at high doses (Fig. 1C). The methylation level was almost normal after 50 μmol/L DAC treatment. Ara-C treatment at different doses had no effect on global methylation (data not shown). Hypomethylation of LINE was not stable, as the methylation level quickly recovered in drug-free medium back to normal levels. Interestingly, we observed that low-dose DAC (0.2 μmol/L) treated cells had a slower recovery of methylation level than those treated at a higher dose (DAC 2 μmol/L; P < 0.01 on day 3 and P < 0.05 on day 5; Fig. 1D).

Synergism of combining DAC and ara-C in leukemia cell lines. We first measured IC50 of DAC and ara-C in different cell lines. IC50 for DAC varied from 54 nmol/L in Raji to 1,200 nmol/L in Jurkat, whereas IC50 for ara-C varied from 16 nmol/L in Raji and 72 nmol/L in Jurkat (Table 1). To evaluate the effects of combining ara-C and DAC on cell viability, we used a fixed ratio of IC50 for DAC and ara-C in all the groups, which is equivalent to 1/8, 2/8, 4/8, 6/8, and 8/8 IC50 of DAC and ara-C, respectively. The combination of DAC and ara-C simultaneously or sequentially was synergistic in most cell lines, indicated by combination index (CI) values of <0.8 (Fig. 2). In ML-1 only, combining DAC and ara-C was additive rather than synergistic. For example, The CI value in Raji cells ranged from 0.35 to 0.87 with DAC and ara-C simultaneously, and from 0.19 to 0.46 with ara-C after DAC. No antagonism (CI > 1.2) was observed in any cell line. To determine whether apoptotic cell death is responsible for ara-C- and DAC-induced decrease in cell viability, we did flow cytometry analysis (fluorescence-activated cell sorting) and Annexin V staining. Cells were treated with ara-C 50 nmol/L in combination with DAC 0.2 and 2 μmol/L. Fresh medium and drugs were added every 24 h. The synergistic effect of DAC plus ara-C on apoptosis was observed. (Fig. 3A).

Ara-C inhibits DAC-induced epigenetic effects. In search for mechanisms of synergistic or additive effects of combining DAC and ara-C, we examined the effects of ara-C and DAC on DAC-induced epigenetic modulation. We used LINE and Alu as markers of global methylation (20). First, we studied LINE methylation after ara-C alone and found that ara-C 50 or

### Table 1. Measurement of IC50 of DAC and ara-C in human leukemia cell lines

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>IC50 (DAC nmol/L)</th>
<th>IC50 (ara-C nmol/L)</th>
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<tbody>
<tr>
<td>HL60</td>
<td>200 ± 54</td>
<td>37 ± 6.1</td>
</tr>
<tr>
<td>ML-1</td>
<td>98 ± 27</td>
<td>17 ± 1.9</td>
</tr>
<tr>
<td>Raji</td>
<td>54 ± 21</td>
<td>16 ± 3.2</td>
</tr>
<tr>
<td>Jurkat</td>
<td>1,200 ± 180</td>
<td>72 ± 9.1</td>
</tr>
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NOTE: Values are presented as mean ± SE of three independent experiments.
Fig. 2. CI plots of DAC–ara-C combinations in human leukemia cell lines. A. CI plots treated with DAC and ara-C simultaneously. The effect of the combinations was assessed by trypan blue exclusion 4 d after cells were incubated with drugs: DAC alone, ara-C alone, and DAC + ara-C. B. CI plots treated with ara-C after DAC. Cells were divided into three groups: DAC alone, ara-C alone, and DAC followed by ara-C. Cells were treated with DAC for 4 d, maintained in fresh medium for 1 d, and then treated with ara-C for 4 d. The combinations were in a fixed molar ratios based on the IC50 values of each drug. The effects of combinations were estimated using the CalcuSyn software, which was developed based on the median-effect method. CI < 0.8 indicates synergy; CI = 0.8 to 1.2 is additive; and CI > 1.2 means antagonism.
Fig. 3. Ara-C inhibits DAC-induced epigenetic effects. A, the combination of DAC and ara-C simultaneously was synergistic on apoptosis. HL60 cells were treated with DAC 0.2 and 2 μmol/L and ara-C 50 nmol/L alone or simultaneously for 4 d. Cells were harvested, fixed with cold 70% ethanol, stained with propidium iodide, and analyzed by fluorescence-activated cell sorting. Apoptosis was assayed by the appearance of a sub-G1 population. B, combination of ara-C with DAC reduced LINE demethylation. After HL60 cells were treated with DAC and ara-C, LINE methylation was measured. C, combination of ara-C and DAC reduced Alu demethylation. D, RIL gene methylation in HL60. Aberrant methylation was detected by bisulfite-PCR followed by restriction enzyme digestion. Unmethylated bands (top) and methylated bands (bottom, arrow) were quantitated by densitometry. E, RIL gene expression. RIL gene expression was assayed by RT-PCR using S-14 internal control or real-time PCR using β-actin as internal control (data not shown). F, Alu H3 Lys9 acetylation. Chromatin immunoprecipitation assay was conducted using antibodies against H3 Lys9. PCR of Alu repetitive elements was done. The intensity of the PCR bands was quantitated by Agilent 2100 bioanalyzer. Columns, ratios of precipitated DNA over input calculated as a relative precipitated fold.
Fig. 4. Ara-C preferentially kills hypomethylated cells. A, ara-C after DAC treatment was synergistic on apoptosis. HL60 cells were treated with DAC 0.2 μmol/L for 4 d; cells were centrifuged and maintained in drug-free medium for 1 d before the addition of ara-C for 4 d. The sub-G1 fraction of cells were determined by fluorescence-activated cell sorting analysis. B, ara-C after DAC treatment reduced LINE demethylation. After treatment, LINE methylation was measured. C, Annexin V staining for apoptosis assay in HL60 cells. Apoptosis was assayed by Annexin V staining and fluorescence-activated cell sorting analysis. The percentage of apoptotic cells (Annexin V positive) was indicated in the top right gradient. D, LINE methylation in Annexin V positive and negative HL60 cells. Cells were treated and centrifuged everyday to remove the debris during treatment. At the end of the treatment, cells were stained with Annexin V. Annexin V positive and negative cells were sorted using a BD FACSVantage Hi-Speed Flow Cytometer. LINE methylation was measured. Columns, mean of three separate experiments undertaken in triplicate plates. Student's t test was used to compare the significance between two groups (*, P < 0.05). E, a model to explain how ara-C reduces LINE demethylation. DAC-induced hypomethylation in HL60 is not homogeneous. Cells with less methylation are prone to death or to be Annexin V positive, whereas cells with higher methylation are Annexin V negative. The addition of ara-C to DAC killed more hypomethylated cells, leading to elevation of LINE methylation in both Annexin V positive and negative cells.
500 nmol/L had no effects on methylation. After DAC 0.2 and 2 μmol/L alone, the methylation decreased from 64% to 18% and 22%, respectively (P < 0.01 compared with control). When ara-C 50 nmol/L was added, LINE methylation decreased to 31% and 42%, respectively (P < 0.01 compared with DAC alone; Fig. 3B). High-dose ara-C (500 nmol/L) added to DAC 0.2 and 2 μmol/L was more efficient at inhibiting hypomethylation (42% and 57%, respectively) than low-dose ara-C. Similarly, ara-C treatment inhibited Alu hypomethylation by DAC (Fig. 3C). The ara-C effects were observed with DAC at 0.2 and 2 μmol/L, which induced optimal hypomethylation. To investigate whether ara-C affects DAC-induced gene reactivation, we studied RIL, a gene previously found methylated in leukemia and silenced by DNA hypermethylation in HL60. DAC treatment at 0.2, 2, and 5 μmol/L induced hypomethylation (Fig. 3D) and gene activation (Fig. 3E). Ara-C alone had no effect on gene expression. However, the addition of ara-C to DAC inhibited RIL gene expression by reverse transcription-PCR (Fig. 3E) and real-time PCR (P < 0.01, data not shown).

DNA methylation targets histone modifications, which also play critical roles in epigenetic silencing (21, 22). Therefore, we examined the effects of inhibition of DNA methylation on Alu H3-Lys9 acetylation (a mark of active chromatin). To this end, we did chromatin immunoprecipitation on HL60 cells treated with DAC 2 μmol/L daily for 4 days. As shown in Fig. 3F, Alu elements showed an increasing level of H3-Lys9 acetylation after DAC. Ara-C alone had no effects on acetylation, but the addition of ara-C to DAC reduced H3-Lys9 acetylation.

**Ara-C preferentially kills hypomethylated cells.** The data discussed above show that ara-C inhibited DAC-induced epigenetic activation (hypomethylation, gene expression, histone acetylation). This could be due to direct inhibition of DAC activity (e.g., reduced incorporation into DNA), to the fact that both drugs work on the same subset of cells (dividing cells), or to preferential killing of hypomethylated/gene-activated cells by ara-C. To test these possibilities, we first examined the effects of ara-C administered after DAC treatment, reasoning that ara-C given after DAC should not interfere with incorporation or direct activity of DAC. HL60 cells were treated with DAC (0.2 μmol/L) daily for 4 days, followed by 1 day of rest. After that, cells were exposed to control medium or to ara-C at 50 and 500 nmol/L. As shown in Fig. 4A, ara-C 50 nmol/L after DAC 0.2 μmol/L was synergistic on apoptosis. DAC induced hypomethylation at day 4 (from 59% to 20%) and cells exposed to control medium showed recovery of methylation (from 20% to 42%; Fig. 4C). When cells were treated with ara-C 50 and 500 nmol/L, methylation seemed to increase significantly (47% with ara-C 50 nmol/L; 52% with ara-C 500 nmol/L, P < 0.01 and P < 0.05 compared with DAC alone, respectively; Fig. 4C). Because ara-C alone has no effects on methylation, we interpret this result as suggesting that ara-C is killing preferentially the most hypomethylated cells.

To directly test the hypothesis that global genomic hypomethylation enhances sensitivity to ara-C, we sorted Annexin V positive and negative cells treated with DAC 0.2 and 2 μmol/L, and ara-C 50 nmol/L, alone or simultaneously for 4 days using a BD FACSVantage Hi-Speed Flow Cytometer, and investigated the LINE methylation levels of each fraction. LINE methylation levels in Annexin V positive (dying) cells were lower compared with Annexin V negative cells after DAC 0.2 μmol/L treatment (24.1% versus 26.4%, P = NS), and DAC 2 μmol/L treatment (26.4% versus 30%, data P < 0.05). When ara-C 50 nmol/L was added to DAC 0.2 μmol/L, LINE methylation of Annexin V positive and negative cells was decreased to 32% and 35%, respectively (P < 0.01 and P < 0.01 compared with DAC alone, respectively; Fig. 4D). These are minimal estimates of the differences because dead cells would not be sorted in this experiment. Our data are consistent with a model whereby hypomethylation sensitizes cells to ara-C, leading to cell death at a higher LINE methylation threshold (Fig. 4E).

**Discussion**

DAC and cytarabine are two of the drugs that have the highest single-agent activity in myeloid neoplasms, making a combination of the two an obviously attractive clinical proposition. However, both are cytosine analogues that use deoxycytidine kinase as a first step toward DNA incorporation (23, 24). Consequently, there has been serious theoretical and experimental concern for antagonism between the two drugs (11). However, we show clearly that, at low but clinically relevant doses of DAC, no antagonism is observed; rather, synergistic effects are seen in most experimental conditions with additivity seen in one. Interpretation of these data and extrapolation to the clinic are complicated by the limitations of the assays used. Although cytarabine and induction of cell death are appropriate end points for cytarabine, which works primarily by interrupting DNA synthesis, the clinical effects of DAC are thought to reflect its epigenetic mechanism of action (reactivation of genes), which may not be well represented in cytarabine assays. Indeed, clinical responses to this agent in vivo take a long time, often requiring multiple exposures, and have been hypothesized to include differentiation and immune activation components (1), all effects that are difficult to model *in vitro*. Nevertheless, our data show that DAC does not lessen cytarabine cytotoxicity and indeed may potentiate it in most conditions tested. It is reassuring enough that a clinical trial of a combination of the two agents can now be seriously envisaged.

Interestingly, although the combination of cytarabine and DAC showed greater apoptosis induction than either agent alone, we also observed antagonism in terms of epigenetic effects (hypomethylation, gene reactivation). Given that cytarabine has no epigenetic effects alone, and the antagonism is seen when giving the agents together or when cytarabine follows DAC (after a period of drug wash out), it is likely that the antagonism is apparent but not real and reflects killing of the most hypomethylated cells. In other words, if cytarabine were truly antagonistic, the antagonism should not have been observed when it was given after DAC. Rather, our data are most consistent with chemosensitization by hypomethylation. Thus, if the most hypomethylated cells are killed most readily by cytarabine, the apparent molecular effect (which is determined on surviving cells) is of reducing the amount of measured hypomethylation. Indeed, we show that cells undergoing apoptosis (based on Annexin V staining) have measurably lower levels of methylation. Although the difference is low, it is an underestimate of the true difference given that cells that already died cannot be sorted by Annexin V and therefore do not contribute to the measured results.

It is interesting to consider possible mechanisms for chemosensitization to cytarabine by DAC. One possibility relates to...
the additive effects of DNA adducts induced by both cytarabine and DAC. However, the sequential studies suggest that hypomethylation sensitizes cells to cytarabine in the absence of active drug (DAC) in the medium. A simple hypothesis to explain this effect relates to activation of proapoptotic genes by the epigenetic effects of DAC. Indeed, multiple genes such as TMS1, HRK, and others that directly affect the process of apoptosis are silenced in cancer and their activation by DAC could be one of the key determinants of chemosensitization by the drug (25–27).

The finding of a U-shaped dose-response curve for hypomethylation induced by DAC is of considerable clinical interest. It recapitulates previous data on differentiation induced by the drug in vitro (28), and it stresses the dual nature of the mechanism of action of this drug—epigenetic at low doses and cytotoxic at high doses (via DNA adduct formation). Clinically, the data stress the importance of using low doses of DAC to maximize its epigenetic effects, a strategy that has had some success in myeloid malignancies (29–31). Following up on this, the current data suggest that low-dose DAC may potentiate the activity of cytarabine in myelogenous leukemia, a concept that deserves testing in the clinic.

Acknowledgments

We thank Dr. Randall Evans for the Flow Cytometry analysis and Annexin V sorting and Drs. William Plunkett and Rong Chen for general advice and help in using the CalcuSyn software.

References

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