Optimization of cytarabine (ARA-C) therapy for acute myeloid leukemia

Richard L Momparler

Abstract

Cytarabine (cytosine arabinoside) is one of the most effective drugs for the treatment of acute myeloid leukemia. The standard dose of cytarabine used to treat this leukemia is 100 mg per square meter. In an attempt to improve the effectiveness of cytarabine against acute myeloid leukemia, a high-dose treatment (3,000 mg per square meter) was introduced into therapy. The side effects of high-dose cytarabine was a major concern, especially its neurological toxicity. A review of recent clinical trials indicates that this high-dose cytarabine can be replaced by the intermediate-dose of 1,000 mg per square meter without loss of efficacy and with less toxicity. This is an important step to improve the efficacy of cytarabine for the treatment of acute myeloid leukemia. Despite the improvements in the therapy for this leukemia, the current overall survival rate for adult patients is less than 30%. To optimize the cytarabine therapy, it is important to determine how some leukemic stem cells survive treatment. Preclinical data suggest that survival of the leukemic stem cells could be due to the long 12 hour interval between infusions of cytarabine, which permits some leukemic cells to escape its S phase specific action. Among the other factors that can lead to leukemic cell survival are the high levels in the liver and spleen of cytidine deaminase, the enzyme that inactivates cytarabine and drug resistance due to deficiency in deoxycytidine kinase, the enzyme that activates the prodrug, cytarabine. Several approaches are proposed in this commentary to overcome these impediments with the goal of increasing the effectiveness of cytarabine for the treatment of acute myeloid leukemia.

Keywords: Cytarabine, Cytosine arabinoside, Acute myeloid leukemia, Pharmacology, Drug resistance

Introduction

Cytarabine (cytosine arabinoside, ARA-C) has been used for the treatment of acute myeloid leukemia (AML) for approximately 40 years [1]. One of the first standard dose-schedules (SD) of ARA-C was 100 mg per square meter per day administered as a continuous i.v. infusion for 7 days [1].

The rationale for the use of HD-ARA-C for the treatment of AML was first proposed in 1974 [2]. High-dose (HD) ARA-C was introduced into clinical therapy for AML in 1979 and the early 1980s [3].

Several investigators performed pilot studies in patients with AML using intensive doses of ARA-C and reported several positive results [4-6]. The most comprehensive clinical study was published in 1983 by Herzig and colleagues. They determined that the maximal tolerated dose (MTD) of ARA-C was 3,000 mg per square meter administered as a one-hour infusion every 12 hours for 6 days [6]. The MTD of a drug is not necessarily its most effective therapeutic dose. One of the major concerns of this HD-ARA-C was its toxicity. In addition to myelosuppression, severe neurologic toxicity was also observed in some patients treated with HD-ARA-C [7]. This CNS toxicity was dose-related because it was more severe at the higher ARA-C dose of 4,500 mg per square meter [6]. The pathogenesis of this CNS toxicity remains to be clarified and may be due to the formation of minor metabolites of ARA-C, such as ARA-C-diphosphocholine. Normal cells treated with ARA-C can synthesize significant amounts of ARA-C-diphosphocholine [8], which can interfere with the function of the normal metabolite cytidine diphosphocholine. In support of this hypothesis is the interesting data on the use cytidine diphosphocholine (citicoline) for its “brain-repair” action in the treatment of stroke [9,10].
Due to the concern of the severe side effects of HD-ARA-C observed in some patients with AML, clinical investigators initiated studies using an intermediate dose (ID) of ARA-C in the range of 1,000 to 2,000 mg per square meter [11]. In a recent commentary by Löwenberg entitled: “Sense and nonsense of high-dose cytarabine for acute myeloid leukemia,” he states that the data from several clinical trials indicate that it does not make sense to use HD-ARA-C and that it can be replaced by ID-ARA-C with similar clinical efficacy but less toxicity [12]. Investigations of the metabolism of ARA-C to its active inhibitor, ARA-CTP, by kinases in myeloid leukemic cells from treated patients support this conclusion [13]. This latter study showed that at the 1,000 mg per square meter dose of ARA-C, maximal formation of ARA-CTP is achieved and not increased by the use of the 3,000 mg per square meter dose. During the infusion of HD-ARA-C the steady state plasma level of ARA-C is >100 μM [14]. The enzymes involved in the activation of ARA-C are completely saturated at the lower dose. This is an interesting example of how in vitro studies on leukemic cells from patients can provide useful guidelines to optimize the dose of ARA-C for the treatment of AML.

The replacement of HD-ARA-C with ID-ARA-C to improve its therapeutic index is an important advancement for the treatment of AML. The next key question to consider is whether there are other ways to further improve the effectiveness of ARA-C therapy for AML. To make further progress it is important to determine why patients with AML relapse after treatment with ARA-C. In Table 1 is a list of different mechanisms by which leukemic stem cells can possibly ‘escape’ ARA-C treatment and suggestions to overcome these potential impediments to improve the effectiveness of chemotherapy.

Duration of interval between ARA-C infusions

The long 12-hour interval between the one-hour infusions of HD-ARA-C provides a window for leukemic stem cells to escape its therapeutic action. Because ARA-C has a short plasma half-life [7], 3 hours after the end of the infusion its concentration falls below the therapeutic range. Cell kinetic analysis shows that the S phase of AML cells from some patients can be as short as 8 h [15]. Because ARA-C is an S phase specific agent, some leukemic cells can progress through this phase during the 12-hour interval with minimal or no exposure to cytotoxic concentrations. The original model for intense dose ARA-C for the treatment of AML used a 6-hour interval between drug infusions to reduce the probability of leukemic cells passing through the S phase without exposure to cytotoxic concentrations of this analogue [2]. Laboratory studies using a colony assay show that reduction of the interval between ARA-C exposures to less than 12 hours increases its cytotoxic action against human myeloid leukemic cells [16].

Drug resistance to ARA-C

Drug resistance to ARA-C can occur by several different mechanisms [17]. ARA-C is a prodrug that is activated by phosphorylation by deoxycytidine (CdR) kinase. Because the human CdR kinase gene is located on chromosome 4, there are two copies of the gene in the cell [18]. Complete drug resistance due to a deficiency in CdR kinase is rare because it requires the gene inactivation of both alleles. It is important to determine if relapse after ARA-C therapy is due to a deficiency in CdR kinase. A simple in vitro drug sensitivity test can be performed on blood leukemic blasts. The leukemic cells are incubated with radioactive thymidine and the inhibition of its incorporation into the DNA by ARA-C is determined [19]. Leukemic cells that show some deficiency in CdR kinase show less inhibition of DNA synthesis by ARA-C. We used this in vitro test to detect CdR kinase deficiency in leukemic cells from patients treated with the related CdR analogue, 5-aza-2’-deoxycytidine (decitabine) [19,20]. Resistance to ARA-C due to deficiency of CdR kinase in AML cells has also been reported by other investigators [18].

One interesting approach to overcome the problem of ARA-C drug resistance due to a deficiency in CdR kinase is to use 3-deazauridine (3-DU) [21]. 3-DU is a competitive inhibitor of CTP synthetase that reduces the intracellular levels of CTP and dCTP. Wild type leukemic cells can escape the cytotoxic action of 3-DU by using CdR kinase, while the incorporation of ARA-CTP into DNA [22]. The drug-resistant leukemic cells are more sensitive to the chemotherapeutic action of 3-DU than the wild type leukemic cells [20,21]. 3-DU also enhances the cytotoxic action of ARA-C on AML cells by increasing its incorporation into DNA [22].

Another mechanism of drug resistance to ARA-C is due to an increase in the intracellular level of dCTP in leukemic cells [23]. dCTP has a dual action to antagonize the action of ARA-C. First, it acts as a natural competitive inhibitor of the incorporation of ARA-CTP into DNA. Second, dCTP is a feedback inhibitor of CdR kinase that reduces the phosphorylation of ARA-C. As discussed above, 3-DU can reverse both these effects.

The deamination of ARA-C to uracil arabinoside by cytidine (CR) deaminase results in a complete loss of antineoplastic activity. Cells that overexpress CR deaminase show signs of drug resistance to ARA-C [24,25]. We detected an increased expression of CR deaminase in leukemic blasts after treatment with decitabine [19]. Tetrahydrouridine, a potent inhibitor of CR deaminase, can be used to overcome this type of drug resistance [26,27].
Inactivation of ARA-C by CR deaminase in the liver and spleen
The liver and spleen contain very high levels of CR deaminase [28] that provide a biochemical sanctuary in these organs for leukemic cells due to the deamination of ARA-C to very low concentrations with minimal or no antineoplastic activity [27]. This event probably occurs frequently when SD-ARA-C is used because the plasma level of ARA-C is low. Tetrahydrouridine can be used to enhance the antineoplastic action of CdR analogues on tumor cells present in the liver and spleen [27].

ARA-C can block progression of some leukemic cells from G1 to S phase
The regulation of the progression of cells from G1 to S phase is a complex event that involves a series of activations of different cyclins by phosphorylation [29]. The inhibition of DNA replication by ARA-C in leukemic cells at this G1/S phase check point can lead to a block in the progression of some leukemic cells into S phase. Evidence for this rare event comes from studies that compare the antineoplastic activity of ARA-C and decitabine in leukemic cells [30]. Both these agents are deoxycytidine analogues and S phase specific agents because their antileukemic action is due to their incorporation into DNA. However, their mechanisms of action are different; ARA-C inhibits DNA synthesis whereas decitabine inhibits DNA methylation. Because both of these agents target the same cohort of leukemic cells in the S phase, they should produce an identical loss of survival at equal exposure times and therapeutic concentrations. However, colony assays show that more myeloid leukemic cells survive after ARA-C treatment than after decitabine [30]. Additionally, in a mouse model of L1210 leukemia using the same duration of infusion, decitabine cured the mice, but ARA-C at its MTD did not [31]. The partial G1/S phase block by ARA-C can permit a small fraction of the leukemic cells to escape its chemotherapeutic action. There are two possible approaches than could be used to overcome this problem. First, it is possible that some leukemic cells may recover from this block when an intermittent ARA-C treatment is used rather than a continuous exposure. Second, it is possible to replace ARA-C with decitabine, whose epigenetic action does not block progression of the G1 cell into S phase [32]. It would be interesting to use a colony assay to compare the antineoplastic activity of ARA-C and decitabine on leukemic cells to identify the patients that would benefit from decitabine therapy.

High frequency of leukemic stem cells
Research shows that upon analysis of leukemic cells from patients with AML, only a small fraction of the cells function as leukemic stem cells [33]. Long-term survival of patients with leukemia is dependent on the complete eradication of the leukemic stem cells by chemotherapy. It has been reported that a high stem cell frequency in AML and acute lymphoblastic leukemia predicts a poor survival rate [34,35]. We do not fully understand why AML patients with adverse cytogenetics are less responsive to ARA-C therapy than AML patients with a normal karyotype [1]. One possible explanation is that these high-risk AML patients have a higher frequency of leukemic stem. To completely eradicate the leukemic stem cells with ARA-C in the high-risk AML patients, one has to increase the clinical efficacy of the chemotherapy. One approach to accomplish this objective is to use ARA-C in combination with 3-DU, which enhances the cytotoxic action of ARA-C on AML cells [22]. Another interesting approach is to use epigenetic priming with decitabine prior to SD-ARA-C treatment of patients with AML [36]. This latter approach was used in a phase I study and produced 83% CR in patients with less-than-favorable risk of AML.

Resistance to apoptosis
The suppression of apoptosis in malignant cells can occur by epigenetic silencing. Aberrant DNA methylation was reported to silence the pro-apoptotic gene, FOXO3 [37] and to down-regulate the TRAIL pathway [38]. Treatment with decitabine can lead to the reactivation of these pro-apoptotic pathways [37,38]. An interesting approach to overcome the problem of ARA-C resistance to apoptosis is to use epigenetic priming with decitabine to reactivate the apoptotic pathway prior to the ARA-C therapy [36]. Mutations in p53 are associated with resistance to ARA-C therapy in patients with AML [39]. This drug resistance is due, in part, to interference with the normal apoptotic pathway in AML cells. Decitabine was shown to be effective against p53-null AML cells and more active than ARA-C [40]. This epigenetic agent can be used to treat

Table 1 Possible mechanisms by which leukemic stem cells survive after ARA-C treatment and approaches to optimize therapy

| Survival mechanisms                                                                 | Treatment to reduce                       | References |
|------------------------------------------------------------------------------------|-------------------------------------------|------------|
| 12-h interval between ARA-C infusions too long                                     | reduce interval                           | [16]       |
| deficiency in CdR kinase                                                           | 3-DU + ARA-C                              | [20-22]    |
| increase in dCTP                                                                   | 3-DU + ARA-C                              | [20-22]    |
| increase CR deaminase                                                             | THU + ARA-C                               | [24,25]    |
| CR deaminase inactivation of ARA-C in liver                                        | THU + ARA-C                               | [24,25]    |
| block in cell cycle progression G1 to S phase                                      | intermittent ARA-C, DAC                   | [30]       |
| high frequency of leukemic stem cells                                              | 3-DU + ARA-C, DAC, prime ARA-C            | [22,26]    |
| resistance to apoptosis                                                            | DAC prime ARA-C, DAC                      | [36,40]    |

Abbreviations: h hour, 3-DU 3-deazauridine, THU tetrahydrouridine, DAC decitabine.
AML patients that show resistance to the induction of apoptosis by ARA-C.

Conclusion
To improve the effectiveness of chemotherapy for the treatment of AML, we have to learn why ARA-C does not completely eradicate all the leukemic stem cells. In vitro tests on leukemic cells from AML patients before and after relapse can give insight on how some leukemic cells survive ARA-C therapy. Several approaches can be investigated to improve the efficacy of ARA-C (Table 1). In addition to these approaches, the long interval (4 weeks) between cycles of ARA-C therapy can permit surviving leukemic cells to expand unimpeded, which can also be a source for treatment failure. Recent results indicate that it is possible to use very low (metronomic) doses (1-3X/week) of decitabine with good responses in patients with myelodysplastic syndrome [41]. This low dose decitabine can maintain or increase self-renewal of normal hematopoietic stem cells [42]. An interesting alternative is to use non-toxic genistein to slow the progression of leukemia between cycles of ARA-C [43]. It would be of interest to perform pilot studies using novel ARA-C regimens on cohorts of high-risk AML patients that have a limited life expectancy. These pilot studies to optimize ARA-C therapy can possibly lead to a better understanding of the pharmacology of this analogue and leukemogenesis and lead to an improved survival in patients with AML.

Competing interests
The author declares that he has no competing interests.

Author's contribution
RLM wrote and approved the final manuscript.

Acknowledgement
This work was supported by the Canadian Cancer Society Research Institute grant 700795.

ADDENDUM
An excellent example of an in vitro colony assay on leukemic cells from patients to evaluate the efficacy of chemotherapy is shown in the publication by: Craddock C, Quek L, Goardon N, et al: Azacitidine fails to eradicate leukemic stem/progenitor cell populations in patients with acute myeloid leukemia and myelodysplasia. Leukemia. 2013, 27:1028-1036.

Received: 20 July 2013 Accepted: 31 July 2013
Published: 6 August 2013

References
1. Löwenberg B, Downing JR, Bumet A: Acute myeloid leukemia. N Engl J Med 1999, 341:1051–1062.
2. Momparler RL: A model for the chemotherapy of acute leukemia with 1-beta-D-arabinofuranosylcytosine. Cancer Res 1974, 34:1775–1787.
3. Reese ND, Schiller GJ: High-dose cytarabine (HD araC) in the treatment of leukemias; a review. Curr Hematol Malig Rep 2013, 8:141–148.
4. Rudnick SA, Cadman EC, Capizzi RL, et al: High dose cytosine arabinoside (HDARAC) in refractory acute leukemia. Cancer 1979, 44:1189–1193.
5. Early AP, Preiler HD, Slocum H, et al: A pilot study of high-dose 1-beta-D-arabinofuranosylcytosine for acute leukemia and refractory lymphoma: clinical response and pharmacology. Cancer Res 1962, 42:1387–1394.
6. Herzog RH, Wolff SN, Lazarus HM, et al: High-dose cytosine arabinoside therapy for refractory leukemia. Blood 1983, 62:361–369.
7. Herzog RH, Hines JD, Herzog GP, et al: Cerbellar toxicity with high-dose cytosine arabinoside. J Clin Oncol 1987, 5:927–932.
8. Lauzon GJ, Paterson AR, Belch AW: Formation of 1-beta-D-arabinofuranosylcytosine diphosphate choline in neoplastic and normal cells. Cancer Res 1978, 38:1791–1795.
9. Clark WM: Efficacy of citocline as an acute stroke treatment. Expert Opin Pharmacother 2009, 10:839–846.
10. Bustamante A, Giralt D, Garcia-Bonilla L, et al: Citocline in pre-clinical animal models of stroke: a meta-analysis shows the optimal neuroprotective profile and the missing steps for jumping into a stroke clinical trial. J Neurochem 2012, 122:317–325.
11. Löwenberg B, Pabst T, Vellega E, et al: Cytarabine dose for acute myeloid leukemia. N Engl J Med 2011, 364:1027–1036.
12. Löwenberg B: Sense and nonsense of high-dose cytarabine for acute myeloid leukemia. Blood 2013, 121:26–27.
13. Plunkett W, Lillemark JO, Adams TM, et al: Saturation of 1-beta-D-arabinofuranosylcytosine 5-triphosphate accumulation in leukemia cells during high-dose 1-beta-D-arabinofuranosylcytosine therapy. Cancer Res 1987, 47:3005–3011.
14. Capizzi RL, Yang JL, Cheng E, et al: Alteration of the pharmacokinetics of high-dose ara-C by its metabolite, high ara-U in patients with acute leukemia. J Clin Oncol 1983, 1:763–771.
15. Raza A, Preiler HD, Day R, et al: Direct relationship between remission duration in acute myeloid leukemia and cell cycle kinetics: a leukemia intergroup study. Blood 1990, 76:1291–1297.
16. Leclerc JM, Momparler RL: Importance of the interval between exposures to cytosine arabinoside on its cytotoxic action on HL-60 myeloid leukemia cells. Cancer Treatment Rep 1984, 68:1143–1148.
17. Momparler RL, Onetto-Pothier N: Drug resistance to cytosine arabinoside. In Resistance to Antineoplastic Drugs. Edited by Kessel D. Boca Raton: CRC Press, 1988:33–367.
18. Flasshove M, Strumberg D, Ayscue L, et al: Structural analysis of the deoxycytidine kinase gene in patients with acute myeloid leukemia and resistance to cytosine arabinoside. Leukemia 1994, 8:780–785.
19. Onetto N, Momparler RL, Momparler LF, et al: In vitro tests to evaluate the response to therapy of acute leukemia with cytosine arabinoside or 5-aza-deoxycytidine. Semin Oncol 1987, 14:231–237.
20. Raynal NJ, Momparler LF, Rice GD, et al: 3-Deazauridine enhances the antileukemic action of 5-aza-2-deoxycytidine and targets drug-resistance due to deficiency in deoxycytidine kinase. Leuk Res 2011, 35:110–118.
21. Momparler RL, Momparler LF: Chemotherapy of L1210 and L1210/ARA-C leukemia with 5-aza-2-deoxycytidine and 3-deazadene. Cancer Chemother Pharmacol 1989, 25:51–54.
22. Momparler RL, Bouffard DY, Momparler LF, et al: Enhancement of anti-neoplastic activity of cytosine arabinoside against human HL-60 myeloid leukemia cells by 3-deazadene. Int J Cancer 1991, 49:573–576.
23. Momparler RL, Chu MY, Fischer GA: Studies on a new mechanism of resistance of L5178Y murine leukemia cells to cytosine arabinoside. Biochem Biophys Acta 1968, 161:481–493.
24. Eblokopoulos N, Momparler RL: Drug resistance to 5-aza-2-deoxycytidine, 2,2-difluorodeoxycytidine and cytosine arabinoside conferred by retroviral-mediated transfer of human cytidine deaminase cDNA into murine cells. Cancer Chemother Pharmacol 1998, 42:373–378.
25. Lachmann N, Brenning S, Phaltane R, et al: Myeloprotection by cytidine deaminase gene transfer in antileukemic therapy. Neoplasia 2013, 15:239–248.
26. Kees W, Chan K, Budman DR, et al: Effect of tetrahydrouridine on the clinical pharmacology of 1-beta-D-arabinofuranosylcytosine when both drugs are co-administered over three hours. Cancer Res 1988, 48:1337–1342.
27. Ebrahim Q, Mahfouz RZ, Nig KP, et al: High cytidine deaminase expression in the liver provides sanctuary for cancer cells from decitabine treatment effects. Oncotarget 2012, 3:1137–1145.
28. Ho DH: Distribution of kinase and deaminase of 1-beta-D-arabinofuranosylcytosine in tissues of man and mouse. Cancer Res 1973, 33:2816–2820.
29. Gacinti C, Giordano A: RB and cell cycle progression. Oncogene 2006, 25:5220–5227.
30. Momparler RL, Côte S, Momparler LF: Epigenetic action of decitabine (5-aza-2'-deoxycytidine) is more effective against acute myeloid leukemia than cytotoxic action of cytarabine (ARA-C). Leuk Res 2013, 37:980–984.
31. Momparler RL, Momparler LF, Samson J: Comparison of the antileukemic activity of 5-aza-2'-deoxycytidine, 1-ß-D-arabinofuranosyl-cytosine and 5-azacytidine against L1210 leukemia. Leuk Res 1984, 8:1043–1049.

32. Momparler RL, Samson J, Momparler LF, et al: Cell cycle effects and cellular pharmacology of 5-aza-2’-deoxycytidine. Cancer Chemother Pharmacol 1984, 13:191–194.

33. Sarry JE, Murphy K, Perry R, et al: Human acute myelogenous leukemia stem cells are rare and heterogeneous when assayed in NOD/SCID/IL2Rγc-deficient mice. J Clin Invest 2011, 121:384–395.

34. van Rhenen A, Feller N, Kelder A, et al: High stem cell frequency in acute myeloid leukemia at diagnosis predicts high minimal residual disease and poor survival. Clin Cancer Res 2005, 11:6520–6527.

35. Morisot S, Wayne AS, Bohana-Kanshan Q, et al: High frequencies of leukemia stem cells in poor-outcome childhood precursor-B acute lymphoblastic leukemias. Leukemia 2010, 24:1859–1866.

36. Scandura JM, Roboz GJ, Moh M, et al: Phase 1 study of epigenetic priming with decitabine prior to standard induction chemotherapy for patients with AML. Blood 2011, 118:1472–1480.

37. Thépot S, Laine E, Cluzeau T, et al: Hypomethylating agents reactivate FOXO3A in acute myeloid leukemia. Cell Cycle 2011, 10:2323–2330.

38. Scandura JM, Roboz GJ, Moh M, et al: Phase 1 study of epigenetic priming with decitabine prior to standard induction chemotherapy for patients with AML. Blood 2011, 118:1472–1480.

39. Thépot S, Laine E, Cluzeau T, et al: Hypomethylating agents reactivate FOXO3A in acute myeloid leukemia. Cell Cycle 2011, 10:2323–2330.

40. Scandura JM, Roboz GJ, Moh M, et al: Phase 1 study of epigenetic priming with decitabine prior to standard induction chemotherapy for patients with AML. Blood 2011, 118:1472–1480.

41. Mahfouz RZ, Englehaupt R, Juersivich JA, et al: Non-Cytotoxic Differentiation Therapy Based On Mechanism of Disease Produces Complete Remission in Myelodysplastic Syndromes (MDS) with High Risk Cytogenetics. Abstract No. 1696; 54th ASH Annual Meeting 2012. https://ash.confex.com/ash/2012/webprogram/Paper51351.html.

42. Saunthararajah Y, Triozzi P, Rini B, et al: p53-Independent, normal stem cell sparing epigenetic differentiation therapy for myeloid and other malignancies. Semin Oncol 2012, 39:107–108.

43. Raynal NJ, Charbonneau M, Momparler LF, et al: Synergistic effect of 5-aza-2’-deoxycytidine and genistein in combination against leukemia. Oncol Res 2008, 17:223–230.

doi:10.1186/2162-3619-2-20
Cite this article as: Momparler: Optimization of cytarabine (ARA-C) therapy for acute myeloid leukemia. Experimental Hematology & Oncology 2013 2:20.