Ann Hematol (2014) 93:1839–1843
DOI 10.1007/s00277-014-2124-y

ORIGINAL ARTICLE

Adding ascorbic acid to arsenic trioxide produces limited benefit in patients with acute myeloid leukemia excluding acute promyelocytic leukemia

Ibrahin Aldoss · Lisa Mark · Janice Vrona · Laleh Ramezani · Ilene Weitz · Ann M. Mohrbacher · Dan Douer

Received: 16 April 2014 / Accepted: 27 May 2014 / Published online: 7 June 2014

Abstract Arsenic trioxide (ATO) is highly effective in acute promyelocytic leukemia (APL), but despite its multiple mechanism of action, it has no activity in acute myeloid leukemia (AML) that excludes APL (non-APL AML). Ascorbic acid (AA) and ATO induces apoptosis in AML cell lines by depleting intracellular glutathione and generation of reactive oxygen species. In this study, we evaluated the effect of ATO plus AA in patients with non-APL AML. The study enrolled patient aged 18 or older with relapsed or refractory AML (non-APL) after conventional chemotherapy or previously untreated patients 55 years or older who were unfit for standard induction chemotherapy for AML. Intravenous ATO (0.25 mg/kg/day over 1–4 h) was given with intravenous AA (1 g/day over 30 min after ATO) for 5 days a week for 5 weeks (25 doses). Eleven AML patients were enrolled, including six previously untreated elderly patients aged 66–84 years in whom five had antecedent hematological disorder (ADH). Among 10 evaluable patients, one achieved a CR one a CRi and 4 patients had disappearance of blasts from peripheral blood and bone marrow. Five of the six responders were seen in previously untreated elderly patients. ATO related toxicity was mild. The combination of ATO and AA has limited clinical meaningful antileukemia activity in patients with non-APL AML.

I. Aldoss · L. Mark · J. Vrona · L. Ramezani · I. Weitz · A. M. Mohrbacher
Division of Hematology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

D. Douer (✉)
Leukemia Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, Weill Cornell Medical College, 1275 York Ave, New York, NY 10065, USA
e-mail: douerd@mskcc.org

Keywords AML · Arsenic trioxide · Ascorbic acid

Introduction

Single agent arsenic trioxide (ATO) is a highly effective drug in patients with acute promyelocytic leukemia (APL) which induces complete remissions (CR) in 90 % of relapsed [1, 2], and in almost all newly diagnosed patients [3]; in contrast to ATRA, ATO alone is capable of curing APL [4]. ATO acts by degrading the PML-RARα fusion protein, the product of the APL specific t(15:17) translocation, but it was also shown to induce apoptosis by mechanism independent of PML-RARα or PML expression [5]. In APL and non-APL cell lines, ATO, in clinically achievable serum concentrations, induced apoptosis by various mechanisms such as the intracellular bcl2 or bcl-XL, mitochondrial trans-membrane potential disruption, cytosolic cytochrome c accumulation, caspases activation, and intracellular reduced glutathione (GSH) modulation [6–12]. However, despite its multiple mechanisms of action and very high clinical activity in APL, single agent ATO has been shown to have no activity in non-APL AML patients [13–15].

One mechanism of modulating ATO activity involves reactive oxygen species (ROS) such as hydrogen peroxide and organic peroxides leading to an apoptotic effect by decreasing mitochondrial membrane potential, release of cytochrome c, and activating caspases [11]. ROS levels are affected by intracellular reduced glutathione (GSH) that is a substrate of glutathione peroxidases in a reaction that produces oxidized glutathione (GSSG) and neutralizes ROS. As a result, high levels of GSH decrease ROS lowering the cellular apoptotic activity of ATO. GSH can also decrease arsenic activity by formation of transient $As(GS)_3$ molecules through the activity of glutathione transferase (GSTπ) [16–18]. In vitro, non-APL

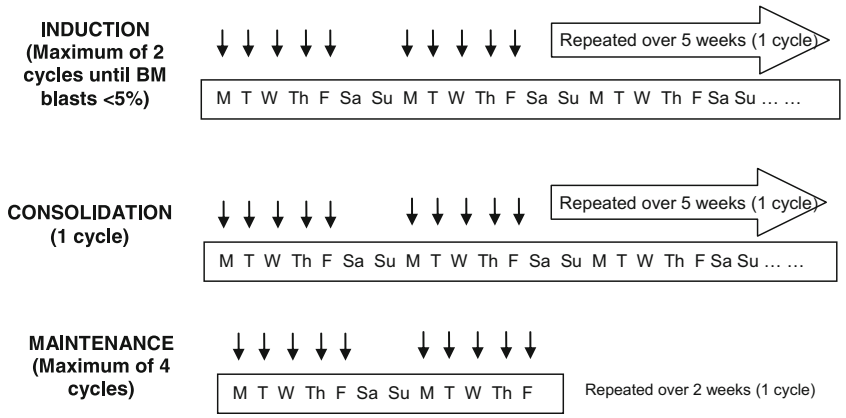

Fig. 1 Treatment schedule

cell lines that are unresponsive to ATO have high intracellular GSH levels, but they became sensitive to apoptotic effect of ATO, after reducing the intracellular GSH and higher levels of ROS [11, 16–19]. A clinically practical and safe drug that reduced intracellular GSH levels is vitamin C, [ascorbic acid (AA)]. Although often considered as an ant-antioxidant, AA can also act as an oxidizing agent, particularly in the presence of compounds that increase the production of ROS [19–21]. Dai et al. have shown in the HL60 cell non-APL AML cell line, that AA plus ATO significantly increased apoptosis compared to ATO or AA alone in association with concomitant intracellular GSH level reduction. Interestingly, AA did not enhance ATO induced apoptosis in colonies cultured of normal human hematopoietic progenitor cells [11]. Similarly, Grad et al. reported in multiple myeloma cell lines that clinically relevant doses of AA decrease GSH levels, increase ROS production and that the combination of AA plus ATO significantly increased apoptosis compared to ATO alone. This preclinical information led us to perform a small clinical trial in patients with non-APL AML to evaluate if ATO would gain antileukemia activity when combined with AA [19].

Patients and methods

This is a single center, open-label, phase II study evaluated the activity and safety of ATO and AA in non-APL AML (ClinicalTrials.gov Identifier NCT00184054). The study was approved by the Institutional Review Board of University of Southern California, and a written informed consent was obtained from all patients before study entry.

Patients

Adults with non-APL AML were enrolled at the University of Southern California (USC) Hospitals (Los Angeles County-USC Medical Center and USC/Norris Comprehensive Cancer Center) between December 2002 and June 2008. Inclusion criteria included relapsed or refractory patients after conventional chemotherapy, aged 18 or above, or previously untreated patients 55 years or older who were unfit for standard induction chemotherapy. Patients with antecedent hematological disorders (ADH) were included. Other criteria were Karnofsky performance status ≥50, creatinine <1.5× upper limit of normal (ULN), transaminases <2.5× ULN, serum total bilirubin <3 mg/dL, and absolute QT interval <460 msec. Serum K^+ and Mg^{++} concentrations had to be ≥4.0 mEq/L and ≥1.8 mg/dL, respectively. Patients previously treated with arsenics or chronic myelogenous leukemia in blastic crisis were excluded.

Study protocol

Intravenous ATO was given at 0.25 mg/kg/d over 1–4 h followed by 1,000 mg intravenous AA, over 30 min, for 5 days a week (5 days on/2 days off) for 5 weeks (one cycle=25 doses). These doses were based on a phase I/II trial of ATO plus AA in patients with multiple myeloma [22]. Responding patients (CR or CRi) received an additional consolidation

Table 1 Patient characteristics

Patients (*n*)	11
Median age (range), years	66 (36–84)
Previously untreated (>60 years)	6
With AHD	5
Previously treated	5
Relapse	4
Refractory	1
Patients with prior regimens	
0	6
1	1
2	3
3	1

AHD antecedent hematological disorder

Table 2 Individual patient responses to ATO+AA

Patient ID #	Age (gender)	Previous condition	AHD	Karyotype	Response	Blast cell counts (%)		Number of cycles to response	Total number of cycles	Duration of response (months)
						Before treatment PB blasts	After treatment			
1	67 (M)	Untreated	PV	Der(14)t(1;14)	↓ PB blasts	69	4	1	1	0.5
7	74 (F)	Untreated	CMML	N/A	↓ PB blasts	40	<2	1	2	2
11	73 (M)	Untreated	–	Diploid	↓ PB blasts	70	0	1	2	4
						BM blasts				
6	66 (M)	Untreated	CLL	Del 5 complex	BM response	20	<5	1	2	4
5	84 (M)	Untreated	MDS	t(9;15)	CCR	50	<5	2	4	6
8	66 (M)	Early relapse	–	Complex	CCRi	15	<5	1	2	6

AHD antecedent hematological disorders, *PV* polycythemia vera, *CMML* chronic myelomonocytic leukemia, *CLL* chronic lymphocytic leukemia, MDS myelodysplastic syndrome, *PB* peripheral blood, *BM* bone marrow, *CCR* complete cytogenetic remission, *CCRi* complete cytogenetic remission without normal platelet count, *N/A* not applicable

cycle of 25 doses followed by maintenance including ATO+AA 2 weeks of every month for four cycles. Refer to Fig. 1.

Results and discussion

Patient characteristics (Table 1)

Among the total of 11 AML patients with median age of 66 years, six were previously untreated older adults (ages 66–84 years) and considered clinically unfit for intensive chemotherapy. Five of them had an antecedent hematological disorder. Five other patients had relapsed/refractory disease after chemotherapy with a median of 2 lines of previous therapy. The study was closed prematurely after enrolling 11 patients due to slow accrual over a period of 6 years.

Response

One patient could not be evaluated as treatment was discontinued early for prolonged absolute QT interval. Characteristics of each responder are shown in Table 2. Among 10 evaluable patients, one patient achieved CR and one a CRi, and in 4 patients, the blast in the peripheral blood (PB) or bone marrow dropped below 5 %. Five of the responders were previously untreated while four of five relapsed/refractory patients progressed on ATO+AA. The duration of response in the two patients with CR or CRi was 4–6 months while duration of response was shorter in those who only had a reduction in blasts, between 0.5 and 4 months. The median overall survival for all evaluable patients was 11 months.

Toxicity

In general, the treatment was well tolerated with very few grade 3 or 4 adverse effects and no deaths on study (refer to Table 3). In one patient, treatment was discontinued early for prolonged absolute QT interval. The most common grade 3 toxicity was infection though possibly related to the leukemia. The treatment was given in an outpatient setting and patients were hospitalized only for complications such as infections.

Similar to our study, all studies in non-APL patients dosed ATO at 0.25 mg/kg, which is higher than the APL dose. Palmar at al. reported no response in all 11 elderly non-APL AML patients including 7 who were previously untreated

Table 3 Treatment-related adverse events

Adverse event	Grade			
	1	2	3	3
Infection	–	2	6	–
Nausea/vomiting	4	1	–	–
Headache	4	1	–	–
Edema	1	1	–	–
Fatigue	1	2	–	–
Rash	–	2	–	–
Anorexia	2	–	1	–
Neuropathy	–	–	1	–
Liver	–	–	1	–
"Differentiation syndrome"	–	–	1[a]	–
QT >460 msec[b]	1	–	1	–

[a] Shortness of breath with hypoxia responding to steroids during hyperleukocytosis

[b] Absolute QT interval on EKG

[13]. In MDS, two large multicenter trials from USA and Europe conducted using single agent ATO 0.25 mg/kg at different dosing schedules and reported minimal response [14, 15].

In our study, ATO was clinically active in a few non-APL AML patients, when combined with intravenous AA, but the responses were mostly not complete and of short duration. It would have been interesting to know if the two complete responders were biologically different. Prior to 2008 when the study was closed, molecular testing for NPM1, FLT3, DNMT3A, IDH1, or IDH2 was not routinely done, and unfortunately, this information is not available. Although the toxicity of ATO plus AA was mild without myelosuppression, ATO treatment, even in APL, requires many weeks and months of almost daily IV treatment. The minimal meaningful clinical benefit would not justify such long time commitment and patient discomfort. We also studied in vitro non-APL AML cells freshly obtained from patients (instead of cell lines) and found that in contrast to cells lines, AA plus ATO-induced apoptosis markedly varied between patients (Douer D unpublished data).

A different approach to try to increase the activity of ATO in AML was suggested by Roboz et al. who combined ATO with low-dose cytarabine [23]. As opposed to AA, low-dose cytarabine has minimal single agent activity in AML with a reported CR rate of approximately 20 % [24]. Among 64 previously untreated elderly non-APL AML patients considered unfit for chemotherapy, induced by ATO plus low-dose cytarabine, 34 % achieved a CR including patients with post-MDS AML or unfavorable cytogenetics. The combination of ATO with low cytarabine was myelosuppressive with frequent occurrence of neutropenic fever that was not seen with ATO plus AA [23]. Unfortunately, a subsequent randomized trial in elderly AML patients reported no improvement in outcome for the addition of ATO to low-dose Ara-C [25].

Arsenical have been used for centuries in the treatment of leukemia, and the lack of activity, outside of APL, in modern studies is disappointing. The striking contrast between an almost universal response of APL patients to ATO and lack of response in other subtypes of AML highly suggests that the fusion PML-RARα gene expression is a critical factor in the sensitivity of APL cells to ATO, although other mechanisms of action may make this drug more active than all trans retinoic acid alone (ATRA) [5]. Similarly, our small study of adding AA to ATO in non-APL AML is also not encouraging since the promising preclinical activity could not be translated into a clinical meaningful treatment approach. Overall, both non-APL AML and MDS patients are unlikely to benefit from ATO alone or in combination with AA or ARA-C. It is baffling that ATO is so active in APL and has almost no activity in other AML subtypes, but unfortunately, more clinical trials in this direction would be dispensable, unless new and compelling rational is presented.

Acknowledgments The study was supported by a National Cancer Institute grant R21 CA0998650-02 and by a research grants from Cell Therapeutics Inc. and Cephalon Inc.

Statement to the effect of human studies The study was approved by the institutional review board of University of Southern California, and a written informed consent was obtained from all patients before study entry.

Conflict of interest The authors declare no conflict of interest.

References

1. Shigeno K, Naito K, Sahara N et al (2005) Arsenic trioxide therapy in relapsed or refractory Japanese patients with acute promyelocytic leukemia: updated outcomes of the phase II study and postremission therapies. Int J Hematol 82(3):224–229
2. Soignet SL, Frankel SR, Douer D et al (2011) United States multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia. J Clin Oncol 19:3852–3860
3. Lo-Coco F, Avvisati G, Vignetti M et al (2013) Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. N Engl J Med 369:111–121
4. Mathews V, George B, Lakshmi KM et al (2006) Single-agent arsenic trioxide in the treatment of newly diagnosed acute promyelocytic leukemia: durable remissions with minimal toxicity. Blood 107: 2627–2632
5. Douer D, Tallman MS (2005) Arsenic trioxide: new clinical experience with an old medication in hematologic malignancies. J Clin Oncol 23:2396–2410
6. Zhang W, Ohnishi K, Shigeno K et al (1998) The induction of apoptosis and cell cycle arrest by arsenic trioxide in lymphoid neoplasms. Leukemia 12:1383–1391
7. Akao Y, Mizoguchi H, Kojima S et al (1998) Arsenic induces apoptosis in B-cell leukaemic cell lines in vitro: activation of caspases and down-regulation of Bcl-2 protein. Br J Hematol 102: 1055–1060
8. Akao Y, Nakagawa Y, Akiyama K (1999) Arsenic trioxide induces apoptosis in neuroblastoma cell lines through the activation of caspase 3 in vitro. FEBS Lett 455:59–62
9. Perkins C, Kim CN, Fang G, Bhalla KN (2000) Arsenic induces apoptosis of multidrug-resistant human myeloid leukemia cells that express Bcr-Abl or overexpress MDR, MRP, Bcl-2, or Bcl-x(L). Blood 95:1014–1022
10. Cai X, Shen YL, Zhu Q et al (2000) Arsenic trioxide-induced apoptosis and differentiation are associated respectively with mitochondrial transmembrane potential collapse and retinoic acid signaling pathways in acute promyelocytic leukemia. Leukemia 14: 262–270
11. Dai J, Weinberg RS, Waxman S et al (1999) Malignant cells can be sensitized to undergo growth inhibition and apoptosis by arsenic trioxide through modulation of the glutathione redox system. Blood 93:268–277
12. Jing Y, Dai J, Chalmers-Redman RME et al (1999) Arsenic trioxide selectively induces acute promyelocytic leukemia cell apoptosis via a hydrogen peroxide-dependent pathway. Blood 94:2102–2111
13. Parmar S, Rundhaugen LM, Boehlke L et al (2004) Phase II trial of arsenic trioxide in relapsed and refractory acute myeloid leukemia, secondary leukemia and/or newly diagnosed patients at least 65 years old. Leuk Res 28:909–919
14. Schiller GJ, Slack J, Hainsworth JD et al (2006) Phase II multicenter study of arsenic trioxide in patients with myelodysplastic syndromes. J Clin Oncol 24:2456–2464

15. Vey N, Bosly A, Guerci A et al (2006) Arsenic trioxide in patients with myelodysplastic syndromes: a phase II multicenter study. J Clin Oncol 24:2465–2471
16. Lee TC, Wei ML, Chang WJ et al (1989) Elevation of glutathione levels and glutathione S-transferase activity in arsenic-resistant Chinese hamster ovary cells. In Vitro Cell Dev Biol 25:442–448
17. Ochi T, Kaise T, Oya-Ohta Y (1994) Glutathione plays different roles in the induction of the cytotoxic effects of inorganic and organic arsenic compounds in cultured BALB/c 3T3 cells. Experientia 50: 115–120
18. Ochi T, Nakajima F, Sakurai T et al (1996) Dimethylarsinic acid causes apoptosis in HL-60 cells via interaction with glutathione. Arch Toxicol 70:815–821
19. Grad JM, Bahlis NJ, Reis I et al (2001) Ascorbic acid enhances arsenic trioxide-induced cytotoxicity in multiple myeloma cells. Blood 98:805–813
20. Sakagami H, Satoh K (1997) Modulating factors of radical intensity and cytotoxic activity of ascorbate. Anticancer Res 17:3513–3520
21. Vera JC, Rivas CI, Fischbarg J, Golde DW (1993) Mammalian facilitative hexose transporters mediate the transport of dehydroascorbic acid. Nature 364:79–82
22. Bahlis NJ, McCafferty-Grad J, Jordan-McMurry I et al (2002) Feasibility and correlates of arsenic trioxide combined with ascorbic acid-mediated depletion of intracellular glutathione for the treatment of relapsed/ refractory multiple myeloma. Clin Cancer Res 8:3658–3668
23. Roboz GJ, Ritchie EK, Curcio T et al (2008) Arsenic trioxide and low-dose cytarabine in older patients with untreated acute myeloid leukemia, excluding acute promyelocytic leukemia. Cancer 113: 2504–2511
24. Burnett AK, Milligan D, Prentice AG et al (2007) A comparison of low-dose cytarabine and hydroxyurea with or without all-trans retinoic acid for acute myeloid leukemia and high-risk myelodysplastic syndrome in patients not considered fit for intensive treatment. Cancer 109:1114–1124
25. Burnett AK, Hills RK, Hunter A et al (2011) The addition of arsenic trioxide to low-dose Ara-C in older patients with AML does not improve outcome. Leukemia 25:1122–1127