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Phase II study of arsenic trioxide and ascorbic acid for relapsed or refractory lymphoid malignancies: A Wisconsin Oncology Network Study

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Abstract

Arsenic trioxide (As₂O₃) has established clinical activity in acute promyelocytic leukemia and has preclinical data suggesting activity in lymphoid malignancies. Cell death from As₂O₃ may be the result of oxidative stress. Agents which deplete intracellular glutathione, such as ascorbic acid (AA), may potentiate arsenic-mediated apoptosis. This multi-institution phase II study investigated a novel dosing schedule of As₂O₃ and AA in patients with relapsed or refractory lymphoid malignancies. Patients received As₂O₃ 0.25 mg/kg IV and AA 1,000 mg IV for 5 consecutive days during the first week of each cycle followed by twice weekly infusions during weeks 2 through 6. Cycles were repeated every 8 weeks. The primary end point was objective response. In a subset of patients, sequential levels of intracellular glutathione and measures of Bcl-2 and Bax gene expression were evaluated in peripheral blood mononuclear cells during treatment. Seventeen patients were enrolled between March 2002 and February 2004. The median age was 71, and the majority of enrolled patients had non-Hodgkin's lymphoma (12/17). Sixteen patients were evaluable, and one patient with mantle cell lymphoma achieved an unconfirmed complete response after 5 cycles of therapy for an overall response rate of 6%. The trial, which had been designed as a two-stage study, was closed after the first stage analysis due to lack of activity. Hematologic toxicities were the most commonly reported events in this heavily pretreated population, and comprised the majority of grade 3 and 4 toxicities. Intracellular depletion of glutathione was not consistently observed during treatment. As₂O₃ and AA in this novel dosing strategy was generally well tolerated but had limited activity in patients with relapsed and refractory lymphoid malignancies.

Keywords

arsenic trioxide; ascorbic acid; lymphoma

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Introduction

Arsenic trioxide (As_2O_3) has demonstrated impressive single agent activity in acute promyelocytic leukemia (APL), [1-4] and clinical activity has also been shown in the treatment of multiple myeloma and myelodysplastic syndromes. [5-11] Several pre-clinical studies have suggested activity of As_2O_3 in lymphoid malignancies. [12-16] The mechanism of action is unclear, and As_2O_3 may have different mechanisms at different dosing levels and in different tumor types. In APL, As_2O_3 at low doses appears to induce differentiation of leukemic blasts. [17] In other malignancies, As_2O_3 in high doses appears to cause cell death by inducing apoptosis. [17,18] As_2O_3 -induced apoptosis is a hydrogen peroxide (H_2O_2)-mediated process, and the sensitivity of tumor cells to As_2O_3 is correlated with intracellular levels of H_2O_2 and the activity of enzymes involved in H_2O_2 metabolism, mainly catalase and glutathione peroxidase. [19,20] In preclinical models, depletion of intracellular glutathione with agents such as ascorbic acid (AA) enhances As_2O_3 -mediated apoptosis. [21-25]

Based upon the preclinical data, we hypothesized that As_2O_3 plus AA would be effective therapy for relapsed lymphoid malignancies. The majority of clinical experience with As_2O_3 has employed daily infusions of As_2O_3 given for prolonged periods of at least several weeks. In the interest of exploring a more convenient administration schedule, we devised a dosing strategy based upon the principle of a loading dose followed by twice weekly infusions. Described herein are the results of a multi-center, prospective clinical trial investigating a regimen of As_2O_3 and AA administered in a novel dosing schedule for the treatment of relapsed and refractory lymphoid malignancies.

Patients and methods

Study design

This study was designed as a multi-center phase II trial of As_2O_3 and AA in patients with relapsed or refractory lymphoid malignancies. The study design called for one cohort of patients with a mix of histologies. The study protocol was conducted at the University of Wisconsin Comprehensive Cancer Center and participating institutions in the Wisconsin Oncology Network (WON). The Institutional Review Board at each participating center approved the study and all patients signed an informed consent document describing the investigational nature of the protocol treatment.

Patient selection

Patients were eligible for this study if they had histologically-confirmed diagnoses of relapsed or refractory non-Hodgkin lymphoma (all subtypes eligible), Hodgkin lymphoma, chronic lymphocytic leukemia (CLL), or other lymphoid neoplasms. Relapsed disease was defined as disease progression after having at least a partial response to the most recent systemic therapy. Refractory disease was defined as having had less than a partial response to the most recently administered systemic therapy. Patients were required to have measurable/evaluable disease, prior treatment with at least one systemic therapy, and an ECOG performance status ≤ 3 . Prior to study enrollment, patients were required to be at least 4 weeks from their last systemic therapy and 2 weeks from their last radiotherapy treatment.

Other study criteria included adequate hematologic parameters ($\text{ANC} \geq 1,000/\text{mm}^3$, platelet count $\geq 50,000/\text{mm}^3$), adequate liver function (total bilirubin $\leq 2.0 \text{ mg/dL}$, $\text{AST} \leq 2.5 \times \text{ULN}$), and adequate renal function (creatinine clearance $> 50 \text{ mL/min}$). Cytopenias were not exclusionary if related to splenomegaly or disease replacement of bone marrow. Patients were excluded if they were pregnant or breast-feeding, had pre-existing grade ≥ 3 neuropathy, had a history of seizures or active CNS disease involvement, had an active second malignancy or

active and uncontrolled infection (including HIV infection). Other exclusionary criteria included a history of ventricular dysrhythmias, significant underlying cardiac dysfunction, or baseline QT_c interval prolongation (≥ 0.48 sec). There were no exclusions for the number of prior treatments received.

Study treatment

Patients were treated with As₂O₃ 0.25 mg/kg IV infused over 1 hour followed by AA 1000 mg IV infused over 15 minutes. The AA infusion was administered within 30 minutes after completion of the As₂O₃ infusion. A treatment cycle was defined as 8 weeks, consisting of 6 weeks of treatment followed by a 2 week recovery period. During the first week of each cycle, patients received infusions of As₂O₃ and AA for 5 consecutive days. During weeks 2 through 6, patients received the same doses of As₂O₃ and AA twice weekly. Cycles were repeated until there was evidence of a complete response, partial response with a stable disease status on 2 consecutive cycles, disease progression or no evidence of response, unacceptable toxicity, or patient/physician preference for discontinuation. Restaging occurred after each treatment cycle. Patients achieving only stable disease at reassessment were not eligible to continue therapy.

Assessment of response

Measurements of response in cases of non-Hodgkin and Hodgkin lymphoma were defined according to criteria established by the International Working Group.[26] Assessment of response in cases of CLL used NCIWG-defined criteria.[27]

Assessment of safety

Given the concern for cardiac conduction problems with As₂O₃ based on prior reports of heart block and ventricular dysrhythmias, patients were monitored with weekly electrocardiograms throughout the course of treatment, with treatment held for dysrhythmias or prolongation of the QT_c interval. In addition, weekly serum magnesium and potassium levels were evaluated during treatment, and complete serum chemistries were repeated prior to each cycle of treatment. Other toxicities were assessed prior to each treatment cycle using the Common Terminology Criteria for Adverse Events, version 2.0.

Statistical considerations

The primary endpoint of this protocol was objective response (complete and partial responses) to treatment with As₂O₃ and AA. Treatment failure was defined as stable disease (SD), progressive disease (PD), or death from any cause. Secondary end points included treatment-related toxicity, progression-free survival (PFS), and overall survival (OS). PFS was defined as the time from study entry until death from any cause or PD. OS was defined as the time from study entry until death from any cause. A two-stage design for patient accrual was planned to allow for early termination for lack of efficacy. The protocol was designed to initially accrue 15 evaluable patients, with plans to accrue an additional 16 evaluable patients should there be one or more responses observed in the first cohort of patients. This procedure tests the null hypothesis that the true response rate is at most 10% versus the alternative hypothesis that it is at least 25% with a significance level of 8.3% and a power of 83%.

Correlative Studies

Previous observations in preclinical models have suggested that depletion of intracellular glutathione with AA enhances As₂O₃ mediated apoptosis, with proposed reduction in Bcl-2 and amplification of Bax expression. In order to test this hypothesis, serial levels of intracellular glutathione and measurements of Bcl-2 and Bax expression were evaluated in a subset of patients during treatment. Due to logistical issues with sample collection and processing, only

patients enrolled at the University of Wisconsin site participated in the correlative study portion of the protocol.

Quantitative glutathione levels and gene expression of Bcl-2 and Bax in circulating peripheral blood mononuclear cells (PBMC) were assessed prior to therapy and at 5 hours post-therapy during the first cycle of treatment. Glutathione activity in PBMC's were measured using the standard Tietze assay, as previously described.[28] Briefly, PBMC's were sonicated and resuspended, and glutathione activity was determined by reduction of DTNB (5-5' dithiobis [2-nitrobenzoic]) as measured spectrophotometrically at $\lambda 412$. Cells were counted and activity was normalized to 1×10^6 cells. RNA was extracted by standard methods and stored at -80°C until analysis. The RNA was quantified via NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE.). Twelve microliters of RNA extracted was reverse transcribed using random primers following the manufacturer's directions. The final cDNA volume was 24 μL . Bcl-2 and Bax gene expression analysis was performed on the Bio-Rad MyIQ real-time thermocycler with the iCycler parameters as follows: $95^\circ\text{C}/2 \text{ min} \times 1 \text{ cycle}$, $95^\circ\text{C}/30 \text{ sec}$, $61.7^\circ\text{C}/1 \text{ min}$, $72^\circ\text{C}/30 \text{ sec}$ for 50 cycle s, $60^\circ\text{C}/7 \text{ sec} + 0.5^\circ\text{C} \times 70 \text{ cycles}$. The resulting data were analyzed with the Eragen MultiCode RTx Analysis Software v1.0.14. The Bcl-2 assay was validated using cDNA obtained from the DU145 prostate cancer cell line. The total cDNA concentration was determined spectrophotometrically, and the standard curve was generated by amplifying in 39 ng-2500 ng of total DNA on 4 days over a period of 4 weeks. The standard curve is linear from 39-2500 ng, with an intra-day variability of 3.536% and an inter-day variability over 4 weeks of 3.26%. The Bax assay was also validated using cDNA obtained from the DU145 prostate cancer cell line. The total cDNA concentration was determined spectrophotometrically and the standard curve was generated by amplifying in 6.25 ng-100 ng of total DNA on 6 days over a period of 6 weeks. The standard curve is linear from 6.25-100 ng, with an intra-day variability of 14.14% and an inter-day variability over 6 weeks of 2.93%.

Results

Patient enrollment

Seventeen patients were enrolled from 5 institutions between March 27, 2002, and February 20, 2004. Of the 17 patients enrolled, 1 patient died 3 days following enrollment from progressive lymphoma before receiving any protocol treatment, and was subsequently excluded from analysis. Although the protocol met the predetermined criteria for continuation beyond first stage accrual with 1 objective response observed, the modest efficacy led to waning enthusiasm for the study and agreement amongst the investigators to close the trial early.

Patient characteristics

Baseline demographic and clinical characteristics of 16 evaluable patients are shown in Table 1. Patients ranged in age from 37 to 88 years, with a median age of 71. Twelve of the 16 patients had an ECOG PS ≤ 1 , and none of the patients had an ECOG performance status > 2 . Various lymphoid malignancies were represented, with follicular lymphoma (3 patients), diffuse large B cell lymphoma (3 patients), mantle cell lymphoma (3 patients), and CLL (4 patients) representing the most common histological subtypes. Patients were heavily pre-treated, with all patients having received ≥ 2 systemic therapies at enrollment. Of the 11 patients with NHL, 8 patients had an International Prognostic Index score ≥ 3 . [29]

Disease response

Of the 16 evaluable patients, 8 patients did not complete cycle one, 5 due to evidence of PD during cycle 1 and 3 due to toxicity. Of the 8 patients who completed at least 1 cycle of treatment, all had evidence of PD following cycle 1 except for 2 patients. One patient with marginal zone lymphoma completed three cycles of treatment before experiencing PD. The

treating center had misinterpreted the criteria for protocol continuation, and this patient had received 2 cycles of therapy with evidence of SD before demonstrating PD following cycle 3. One patient with mantle cell lymphoma completed five cycles of treatment and achieved an unconfirmed CR (CRu), with a response duration exceeding 12 months (Table 2). The overall response rate was 6% (1/16) with a 95% confidence interval of (0%-30%).

Survival

Fourteen of the 16 evaluable patients have died, and 2 patients with follicular lymphoma are alive at the date of last contact. The median PFS was 1.8 months (95% CI 0.6-5.7), and the median OS was 7.6 months (95% CI 3.7-∞). The 1-year survival rate was 37.5% (95% CI of 19.9%-70.6%).

Toxicity assessment

Sixteen patients were assessable for toxicity, with a total of 14 cycles completed. Toxicities were modest given the heavily pre-treated nature of this patient population, with the majority of grade ≥ 3 toxicities comprised of hematologic toxicities. Grade 3-5 toxicities are shown in Table 3. One patient with known coronary artery disease experienced grade 5 heart failure after suffering a myocardial infarction 3 days into treatment. One patient discontinued treatment during cycle 1 for a declining performance status. One patient was required to discontinue treatment during cycle 1 for recurrent grade 4 hyperglycemia. Non-hematologic toxicities were generally mild, with no electrolytic imbalances or changes in renal function reported as $>$ grade 2 in severity. Gastrointestinal side effects were tolerable, with only 1 patient experiencing grade 3 stomatitis. One event of grade 3 serum transaminase elevation was reported, which was reversible with a break from therapy. Six grade 1 or 2 infections were reported, 4 of which were related to herpes zoster. Two patients experienced grade 1 QT_c interval prolongation on electrocardiogram evaluation, which was reversible in each case with a break from therapy. Both patients experienced the grade 1 QT_c interval prolongation during cycle 1 on days 4 and 7, respectively.

Correlative study results

Of the 4 patients enrolled at the University of Wisconsin site, 3 patients participated in the correlative analysis. Among these 3 patients, 1 patient did not complete cycle 1 due to PD, and the remaining 2 patients had SD after cycle 1. Serial levels of intracellular glutathione and gene expression of Bcl-2 and Bax were measured in these 3 study patients during therapy with As₂O₃ and AA, with results shown in Table 4. Although moderate reductions in intracellular glutathione were observed in one of the patients following treatment, the 2 remaining patients had minimal to no reduction in intracellular glutathione or were observed to have an increase in intracellular glutathione with treatment. Overall, mean glutathione levels were not significantly different before and 5 hours after treatment in this small subset of patients.

Measurement of Bcl-2 gene expression demonstrated consistent reductions during treatment, as shown in Table 4. A nearly 10-fold reduction in Bcl-2 expression was observed 5 hours after treatment with As₂O₃ and AA with Bax expression remaining constant.

Discussion

Several *in vitro* studies have demonstrated activity of As₂O₃ in lymphoid malignancies. [12-15] This pre-clinical work as well as studies suggesting potentiation of As₂O₃ with AA led us to hypothesize that this combination would provide an active and relatively non-toxic combination in relapsed lymphoid malignancies. Despite prior evidence suggesting synergy between As₂O₃ and AA and showing promising activity of As₂O₃ in lymphoid malignancies,

the response rate with the regimen of As₂O₃ and AA administered in this study was only 6% and the study was closed at the first interim analysis.

Arsenic trioxide has already demonstrated activity in multiple hematologic malignancies, most importantly in the treatment of APL.[1-3] Two small US trials in relapsed APL demonstrated CR rates of 92% and 100%. [4,30] A recent publication examined the role of single agent As₂O₃ used for remission induction, consolidation, and maintenance for newly diagnosed APL. [31] The complete response rate was 86% and the 3 year estimates for EFS and OS were 75% and 86%, respectively. Although the duration of follow-up in this study is relatively short, these provocative results are comparable to those obtained with conventional chemotherapy.

Modest activity of As₂O₃ has also been reported in multiple myeloma and myelodysplastic syndromes (MDS).[5-10,32,33] Two large multicenter phase II studies have recently been published in MDS. In a US trial, hematologic improvement was noted in 34% of low risk patients and 6% of high risk patients.[5] In a European trial, which utilized a dosing strategy similar to the one in our study, hematologic response rates of 26% and 17% were observed in low risk and high risk cohorts, respectively.[6] When evaluated as a single agent in multiple myeloma, As₂O₃ produced objective responses in 2/9 (23%) patients.[9,10]

Arsenic trioxide (As₂O₃) has dual, dose-dependent mechanisms of action against malignant cell lines. At low doses (0.1-0.5 mmol/L), As₂O₃ appears to induce partial differentiation of APL cell lines and primary cultures of blasts. At higher doses (0.5-2.0 mmol/L), As₂O₃ induces apoptosis, including cell lines that overexpress MDR, MRP, Bcl-2, and Bcl-X_L. [34]

Arsenic-induced apoptosis is likely due to the production of reactive oxygen species, and resistance to oxidative damage is largely mediated by intracellular glutathione.[17,19,35] Previous experience with NB-4 cell lines (an APL cell line) have shown that these cells with low baseline levels of glutathione are exquisitely sensitive to treatment with As₂O₃, whereas HL-60 cells (non-APL acute myeloid leukemia cell line) with increased levels of glutathione are more resistant to As₂O₃. [19,20] Agents which deplete intracellular glutathione should sensitize cells to As₂O₃. A recent publication from Taiwan demonstrates that HL-60 cells quickly develop resistance to As₂O₃ by compensatory increases in intracellular glutathione, and depletion of glutathione with buthionine sulfoximine (BSO) reversed the drug resistance *in vitro*. [36] While BSO is an attractive agent for glutathione depletion, availability of this agent for clinical use is limited. [37,38]

Ascorbic acid (AA) is another agent capable of depleting intracellular glutathione. [39] AA at concentrations of 62.5 mol has been shown to lower glutathione levels in NB-4, HL-60, and su-DHL-4 (B-cell lymphoma) cell lines, and the combination of AA and As₂O₃ leads to increased apoptosis in these cell lines compared with treatment using either agent alone. [20] Other investigators have also shown potentiation of As₂O₃ mediated cytotoxicity when combined with AA. [22-25,40,41] We were unable to demonstrate a similar decline in glutathione after treatment with AA, although this may be a function of our small sample size, the limited sampling schedule, or dosing of AA that was insufficient to lower glutathione.

A modified dosing schedule was used in this study to facilitate patient compliance and limit treatment time and toxicity. One patient with mantle cell lymphoma achieved an unconfirmed CR that was durable for over 12 months. This suggests that further investigation of As₂O₃ and AA may be reasonable but will likely require a better understanding of biological features predictive of response. One study indicated As₂O₃ mediates apoptosis through inhibition of NFκB. [42] If this proves to be a major mechanism of apoptosis, then tumor types known for constitutive NFκB expression (e.g., mantle cell lymphoma) may be better targets for further study. Alternatively, it is possible that As₂O₃ does not have sufficient activity outside of APL to warrant further study and negative phase II studies have been reported in melanoma, germ

cell tumors, and acute lymphoblastic leukemia.[43-45] Inadequate dose-intensity may also be a factor, particularly as the per cycle dose of As₂O₃ 3.75 mg/kg utilized in our study is similar to the dose-intensity of As₂O₃ utilized in the consolidation phase of APL therapy (total of 3.75 mg/kg) rather than the higher dose-intensity utilized during APL induction therapy (up to 9 mg/kg). We can not discern whether the lack of activity in our study was due to a suboptimal dosing strategy, the heavily pretreated nature of our patient population, or due simply to inactivity of this combination. It is possible that alternative dosing strategies in a similar patient population could prove to be more efficacious.

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Table 1

Baseline characteristics (n=16).

Age		
	Median	71
	Range	37-88
Sex		
	Male	10 (63%)
	Female	6 (38%)
Performance status		
	0	5 (31%)
	1	7 (44%)
	2	4 (25%)
Prior systemic therapies		
	2-3	7
	4-5	4
	≥ 6	5
	Median	4
	Range	2-12
Disease status at enrollment		
	Refractory	12 (75%)
	Relapsed	4 (25%)
International prognostic index [†]		
	0-1	2 (18%)
	2-3	4 (36%)
	4-5	5 (45%)

[†] International prognostic index scoring includes only the 11 patients with NHL.

Table 2

Response rate by disease histology.

Histology	Responses	
Non-Hodgkin lymphoma		
Burkitt's	1	0/1
Follicular	3	0/3
Diffuse large cell	3	0/3
Marginal zone	1	0/1
Mantle cell	3	1/3
Chronic leukemia		
CLL	4	0/4
Hairy cell	<u>1</u>	<u>0/1</u>
	N = 16	1/16 (6%)

Table 3

Events of grade 3-5 toxicity.

Toxicity	Grade 3	Grade 4	Grade 5	Total
Anemia	6	1	--	7
Thrombocytopenia	6	1	--	7
Leukopenia	4	1	--	5
Neutropenia	1	3	--	4
Neutropenic fever	3	--	--	3
Infection [†]	3	--	--	3
Hyperglycemia	--	1	--	1
Elevated serum transaminases	1	--	--	1
Anorexia	1	--	--	1
Stomatitis	1	--	--	1
Fatigue	3	1	--	4
Pain	1	--	--	1
Dizziness/light-headedness	1	--	--	1
Dyspnea	3	--	--	3
Hypoxia	2	--	--	2
Pleural effusions	4	--	--	4
Edema	1	--	--	1
Cardiac ischemia/infarct	--	1	--	1
Heart failure	1	--	1	2

[†] Infections occurring in the absence of neutropenia.

Table 4

Intracellular glutathione levels and gene expression of Bcl-2 and Bax during treatment with arsenic trioxide and ascorbic acid.

Analyte	n	Pre (Mean $\pm$ SE)	5hr Post (Mean $\pm$ SE)
Glutathione (ng/10 ⁶ cells)	3	239 $\pm$ 280	239 $\pm$ 121
Bcl-2 (ng/ng cDNA)	3	49070 $\pm$ 32185	8924 $\pm$ 5436
Bax (ng/ng cDNA)	3	262 $\pm$ 57	329 $\pm$ 71