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Use of arsenic trioxide in acute promyelocytic leukemia has led to investigations of the drug in multiple myeloma.

Trials of Arsenic Trioxide in Multiple Myeloma

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Background: Several reports on the use of arsenic trioxide (ATO), mainly in acute promyelocytic leukemia, have led to a renewed interest in ATO in the management of malignancies, especially those of hematologic origin such as multiple myeloma (MM). MM remains an incurable disease, with median survival rates of 4-6 years. Thus, newer treatments with good safety profiles are needed to improve the quality of responses, prolong progression, and increase overall survival.

Method: The current state of the art regarding the role of ATO in the management of MM and the rationale for this consideration is reviewed.

Results: Preclinical evidence suggests that one of the mechanisms in which ATO exerts its antimyeloma effect is by immunologic mechanisms. One such mechanism appears to be achieved by a marked increase in lymphokine-activated killer (LAK)-mediated killing and up-modulation of CD38 and CD54, two molecules involved in cell-cell interactions. Two phase II trials have shown that the drug appears to be clinically effective.

Conclusions: With the improved understanding of the interaction between the myeloma cell and its microenvironment as well as the cytokine support system, it does not appear that any particular agent will be able to control the disease permanently. In the clinical arena, the next generation of studies will be designed to combine different molecules to take advantage of non-overlapping toxicities and to compromise the extensive and redundant myeloma support system, thus controlling the disease in a chronic phase.

Introduction

The use of arsenic trioxide (ATO) as a medicinal agent dates back 2000 years. In the United States, however, clinical use of ATO declined in the early 1970s with the advent of cytotoxic chemotherapy and also due to the arsenic poisoning that developed with long-term use. The drug has remained an important agent in traditional medicine in some areas, especially in central and southern Asia. Several reports from China supporting the use of ATO in acute promyelocytic leukemia

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(APL) have led to a renewed interest for investigating the drug in the management of malignancies of hematologic origin, including multiple myeloma.¹

Multiple myeloma (MM) remains an incurable disease, with a median survival duration of 4 to 6 years, even with aggressive, high-dose chemotherapy, bone marrow transplantation, and intensive supportive care. Additionally, MM is primarily a disease of the elderly, many of whom cannot tolerate aggressive chemotherapy. Thus, newer treatments with good safety profiles are needed to improve the quality of responses, prolong time to disease progression, and extend overall survival.

The pathophysiology of MM is complex, involving many pathways and interactions among cytokines, adhesion molecules, angiogenesis, and mechanisms of resistance that, taken together, provide multiple targets for novel therapeutic modalities.² Preclinical evidence suggests an immunologic mechanism acts directly by inducing apoptosis in the malignant plasma cells and/or the stroma, as well as on myeloma cells. One of the attractive mechanisms is achieved by a marked increase in lymphokine-activated killer (LAK)-mediated killing and up-modulation of CD38 and CD54, two molecules involved in cell-cell interactions.³ ATO as a single agent or in combination with other compounds provides a novel therapy for lymphoproliferative disorders.⁴

Pharmacology

Organic arsenic compounds are significantly more toxic than inorganic arsenicals because of high binding affinity to vicinal SH group-containing proteins.⁵ Organic arsenicals such as melarsoprol, although more potent than ATO in inducing apoptosis in NB4 cells, are clinically too toxic to be used in the treatment of APL.⁶ Most previous studies that investigated the cellular and biochemical effects of ATO used concentrations greater than 5 $\mu\text{mol/L}$, often 50 $\mu\text{mol/L}$, and the relevance to therapeutic levels (1 to 2 $\mu\text{mol/L}$) remains to be determined. These high concentrations may initiate gene transcription by altering the phosphorylation state of signal transduction proteins such as tyrosine kinases.⁷ ATO affects phosphorylation by activating specific kinases, inhibiting thiol-dependent phosphatases or interfering with phosphotransferase reactions.⁸ The protective effects of thiols, such as glutathione, cysteine,⁹ and dithiols, such as dithiothreitol, against the toxic effects of ATO suggests that ATO toxicity results from forming reversible bonds with the thiol groups of regulatory proteins.

Arsenic trioxide is eliminated by many routes (urine, feces, sweat, milk, hair, skin, and lungs), although

most is ultimately excreted in urine. After oral ingestion, the half-life of the urinary excretion is 3 to 5 days. Preliminary pharmacokinetic data have been reported by the Shanghai group, which used an arsenic solution infused at a dose of 10 mg intravenously over 4 hours on a daily schedule. The reported mean maximum plasma concentration (CP_{max}) was $4.7 \pm 1.85 \mu\text{mol/L}$, the mean half-life was 0.89 ± 0.29 hours, and the mean plasma half-life was 12.13 ± 3.31 hours.¹⁰ Serial plasma arsenic concentrations were re-measured in 5 patients after at least 1 month of therapy, and the results were similar to those on the initial day of treatment. In a dose-ranging study in solid tumor malignancies,¹ an ATO solution was infused at a dose of 0.15 mg/kg intravenously over 1 to 2 hours on a daily $\times 5$ schedule. The CP_{max} ranged between 0.33 and 2.9 $\mu\text{mol/L}$, the mean half-life was approximately 4 hours, and the mean γ half-life was approximately 115 ± 28 hours.

Clinical Efficacy in the Management of Acute Progranulocytic Leukemia

The revitalization of the arsenicals as medicinal agents followed the dramatic clinical response noted in patients with APL reported by Shen et al.¹⁰ In 1997, a study on the use of ATO in heavily pretreated patients with relapsed APL was initiated in the United States.¹¹ Of the 12 patients treated with daily infusions of 0.15 mg/kg of ATO to a maximum of 60 doses or until all leukemic cells in bone marrow were eliminated, 11 (92%) achieved complete remission with good tolerability of the medication and no evidence of cumulative toxicity. Adverse events were generally manageable and reversible and did not require interruption of therapy.

A subsequent multicenter trial in the United States, enrolling 40 patients with relapsed APL, 19 of whom had experienced two or more relapses, reported similar results. The complete response rate was 85%, the 18-month relapse-free rate was 56%, and the overall survival rate was 66%.¹² Following therapy with ATO, 86% of patients achieved molecular remission, as measured by a negative reverse transcriptase-polymerase chain reaction (RT-PCR) test for the PML-RAR α transcript.

Clinical Efficacy in the Management of Multiple Myeloma: Preclinical and Clinical Evidence

Laboratory Evidence

Arsenic trioxide holds therapeutic promise in the treatment of MM. The *in vitro* sensitivity of cultured MM cell lines to concentrations of 1 to 2 μm of ATO

and the preliminary reports of response in patients with MM have prompted investigations of its effectiveness in this disease.¹³⁻¹⁵

Antiangiogenic Activity

In addition to the direct and indirect effects of ATO on the malignant plasma cell clone and its microenvironment, acute promyelocytic leukemia was noted to inhibit tumor angiogenesis through direct and indirect mechanisms. ATO inhibits vascular endothelial growth factor (VEGF) production by a leukemic cell line and induces apoptosis and capillary tubule formation of human umbilical vein endothelial cells.¹⁶ In vivo data in a murine fibrosarcoma model show that ATO causes vascular shutdown, leading to tumor necrosis.¹⁷ Although these studies were performed at concentrations higher than what has been achieved with the 0.25-mg/kg dosage, current studies are evaluating the feasibility of increasing the dose of ATO to allow for similar in vivo concentrations.

Evidence for Direct Antimyeloma Activity

ATO induces growth inhibition and apoptosis in a number of malignant hematopoietic cell lines, including MM cell lines and freshly isolated human MM cells.^{13,14,18,19} These effects appear to be preferential for MM cells as they are greatly reduced in normal myeloid cells isolated from the bone marrow.¹³ Exogenous interleukin-6 (IL-6) does not overcome arsenic-induced growth inhibition or apoptosis.^{13,19,20} Inhibition of proliferation has been associated with induction of the p21 cyclin-dependent kinase inhibitor protein and apoptosis. Apoptosis occurs through a mechanism that involves collapse of the mitochondrial transmembrane potential, increased caspase-3 activity, and possibly the down-regulation of Bcl-2.^{14,18,19}

Direct Immunologic Mechanisms

Arsenic trioxide also induces antitumor activity through immunologic mechanisms.³ The exposure of human myeloma-like cell lines and freshly isolated MM cells to ATO resulted in increased killing mediated by LAK cells, possibly through the up-regulation of the CD38/CD31 and CD11a/CD54 receptor-ligand systems, which increase recognition, adhesion, and lysis.³ Treatment of both effector (LAK) and target (MM) cells with ATO selectively up-regulated the expression of adhesion molecules involved in cell-cell interactions (CD38, CD54) and their ligands (CD31, CD11a) on MM and LAK cells, respectively. Blocking these cell-surface molecules with antibody inhibited cytotoxicity, suggesting increased adhesion as a mechanism for the cytotoxic activity. These findings suggest that ATO may

be of clinical benefit in the potentiation of a specific immune response against myeloma cells.

At least two apoptotic signaling pathways exist, one of which is mediated by c-Jun NH₂-terminal kinases (JNKs) and is unaffected by IL-6.²¹ This pathway is involved in apoptosis induced by irradiation and ATO.^{21,22} The second mechanism, used by dexamethasone, is JNK-independent. It is associated with the down-regulation of MAPK and P70 and is inhibited by IL-6.²¹ Thus, an agent such as ATO that down-regulates Bcl-2 and induces apoptosis through an IL-6-insensitive apoptotic pathway might have additive or even synergistic effects with dexamethasone. A phase II study of ATO in combination with dexamethasone for patients with recurrent or refractory stage II or III MM is underway.

Biochemical Enhancement of the Antimyeloma Effect In Vitro

The generation of reactive oxygen species enhances ATO-induced apoptosis, but glutathione reduces this effect.^{22,23} The increased expression or activity of glutathione and glutathione-related compounds is also known to confer resistance to alkylating agents. Therefore, reducing glutathione during treatment with ATO might be expected to increase the effect of the drug.^{23,24} Indeed, adding butathione sulfoximine, which is known to deplete glutathione in vivo, increases the cytotoxic effect of ATO on MM and other tumor cell lines.^{24,25}

Ascorbic acid is known to decrease glutathione concentrations and is well tolerated in vivo.^{23,26} Ascorbic acid has been shown to decrease glutathione concentrations and significantly enhance the ATO-mediated killing of drug-resistant MM cell lines and freshly isolated MM cells.²³ Compared with refractory cells, cells from previously untreated patients are more sensitive to ATO alone, and the addition of ascorbic acid does not enhance apoptosis. Ascorbic acid and ATO, either alone or in combination, have little effect on normal bone marrow cells.

The combination of ATO and ascorbic acid has increased the survival of mice implanted with lymphoma cells, which, in vitro, had reacted to combination treatment with enhanced growth inhibition.²⁵ These data suggest that ATO in combination with ascorbic acid might be clinically useful for treating refractory MM.

Clinical Trials in Multiple Myeloma

In a phase II trial at the University of Arkansas,²⁷ the activity of ATO was evaluated in 14 patients with relapsed MM refractory to conventional salvage thera-

py. ATO was administered at a dose of 0.15 mg/kg daily for 60 days. Treatment with ATO using this dose and schedule resulted in significant responses. All of the patients had received at least one autologous bone marrow transplantation, 13 of whom received at least two transplants. Salvage therapy was given to all patients in the form of dexamethasone, cyclophosphamide, etoposide, and cisplatin (DCEP) and or thalidomide prior to initiating ATO therapy. Responses were noted in 3 patients: 1 achieved 75% reduction in the monoclonal protein, and the other two achieved 50% and 25%, respectively. Stable disease was observed in 8 patients. Although treatment was reasonably well tolerated in these patients with extensive prior therapy, 11 developed cytopenia; this was associated with infectious complications in 5 patients, and 3 developed deep vein thromboses. The results of this small trial support further investigation of this novel drug for the treatment of patients with relapsed or refractory MM.

Based on these preliminary results, a multicenter trial in the United States was initiated to confirm the role of ATO in the management of relapsed or refractory myeloma using a higher dose and shorter schedule.²⁸ This multicenter phase II trial sought to determine the response rate (SWOG criteria) to ATO and the safety of ATO in patients with relapsed or refractory MM. Patients received ATO 0.25 mg/kg intravenously, 5 days per week for 2 weeks followed by no therapy for 2 weeks, in repeated 4-week cycles. Eight relapsed patients and 16 refractory patients were treated (unpublished data, 2003). All enrolled patients received ≥ 2 prior chemotherapies, and 7 had undergone prior high-dose therapy and autologous stem cell transplantation. The median patient age was 63 years (range, 41 to 80 years), with a median of 2.4 years from the initial diagnosis. Five patients were >75 years of age. Mean baseline serum $\beta 2$ -microglobulin concentration was 4 mg/dL (range, 1.9 to 16.1 mg/dL). Three patients were not evaluable for response due to progressive disease or withdrawal of consent during cycle 1. Nine (43%) of 21 evaluable patients had an objective response as measured by a $>25\%$ decrease in serum M-protein concentrations. One patient with refractory disease had a 50% decrease in plasmacytoma size, 8 patients had stable disease, and 4 had progressive disease at the first evaluation visit. Five patients had ATO-related serious adverse events: leukopenia/anemia, anemia/thrombocytopenia, fatigue, febrile neutropenia, and pulmonary edema. Transient increases in transaminase levels occurred in 15 patients during cycle 1 (NCI-CTC grade 1 in 7 patients, grade 2 in 7 patients, and grade 3 in 1 patient) and in 4 patients during cycles 2 and 3 (grade 1/2). No patients were withdrawn and no doses were decreased due to increased transaminase levels. Fifteen patients had recurrent transient weight gain in most

cycles (9 patients had grade 1 weight gain, 5 had grade 2, and 1 had grade 4), and only 6 of these 15 patients received diuretics. Five patients, including 4 with pre-existing diabetes mellitus, had grade 2 hyperglycemia. No patient had alopecia or severe nausea. In this study of heavily pretreated patients, ATO as a single agent demonstrated activity and was well tolerated.

These data support further evaluation of ATO as a single agent for MM in early relapse. Since preclinical studies suggest that myeloma cells are sensitized by ATO to corticosteroids as well as melphalan, this would allow administering these drugs at lower dosages, reducing their toxicities and possibly improving response rate and quality. Phase II trials utilizing these combinations are underway.²⁸

Conclusions

At this stage, with our current understanding of the interaction between the myeloma cell and its microenvironment, as well as the cytokine support system, it does not appear that any one agent will be able to control MM permanently. The next generation of clinical trials will be designed to combine different molecules to take advantage of non-overlapping toxicities and synergism between those agents, thus converting the disease process into a chronic phase that hopefully does not compromise quality or length of life.

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