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LETTER TO THE EDITOR

Arsenic trioxide/ascorbic acid therapy in patients with refractory metastatic colorectal carcinoma: A clinical experience

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Abstract

Arsenic trioxide (As_2O_3) has demonstrated effectiveness in treating acute promyelocytic leukemia (APL). Therefore the FDA has approved it to treat APL. In patients with refractory metastatic colorectal carcinoma (CRC), we assessed the efficacy and toxicity of As_2O_3/AA (ascorbic acid) as the outcome of this trial. Five patients with refractory metastatic CRC who failed all previous standard chemotherapy were enrolled in this study. They were treated with 0.25 mg/kg body weight/day As_2O_3 and 1000 mg/day of ascorbic acid for 5 days a week for 5 weeks. Each treatment cycle extended for 7 weeks with 5 weeks of treatment and 2 weeks of rest. All the patients developed moderate to severe toxic side effects to arsenic trioxide/AA therapy and therefore the study was discontinued. No CR (complete remission) or PR (partial remission) was observed. CT scans demonstrated stable or progressive disease. Three of the five patients died within 2 to 5 months after cessation of the therapy. None of the deaths could be related to this clinical trial. Two years of follow-up study showed that two patients were alive with stable disease. Under the current treatment regimen all patients developed moderate to severe side effects with no clinically measurable activity. As an alternate, efforts may be made to reduce the dose and arsenic trioxide may be combined with other standard regimen in reversing the chemo resistance.

To the Editor

A majority of colorectal cancer patients become refractory to standard chemotherapy. It results in treatment failure and eventual death. Therefore, identification of new agents with better anti tumor activity or ability to delay the onset or reverse resistance development remains the highest priority for clinical investigators in treating refractory tumor.

Recently arsenic trioxide (As_2O_3) has been demonstrated to have clinical efficacy over Acute Promyelocytic Leukemia (APL) [1]. As a single agent it has induced complete remission (CR) with minimal side effects [2]. In the USA, CR was observed in 80 to 90% of APL patients treated with As_2O_3 [3]. Based on these studies the US Food Drug Administration approved As_2O_3 for the treatment of APL.

Studies with human colon cancer cell lines, HT29 (5-FU sensitive), HT29FU (5-FU resistant developed from the sensitive variant) and SW480 (innately 5-FU resistant) showed that 1 μm concentration of As_2O_3 was effective in killing these cells. It also down regulated the expression of thymidylate synthase (TS) (Subbarayan et al., unpublished observations). One of the reasons proposed for the resistance to 5-FU is increased expression of TS in tumor cells [4]. It is possible to sensitize the cells by reducing the expression of TS. Therefore, any agent that could down regulate TS has a potential to reverse the resistance to 5-FU.

Success of As_2O_3 in bringing about CR in APL patients, our *in vitro* experience on colon cancer cells HT29, HT29FU, SW480 and efficacy of As_2O_3/AA in other pre-clinical and clinical trials [5] provided the rationale for this study. In this clinical trial we assessed the toxicity and efficacy of As_2O_3/AA in patients with refractory metastatic CRC. We also determined its effect on the expression of thymidylate synthase *in vivo*. Here we report the outcome of a phase II clinical trial using As_2O_3/AA therapy in patients with refractory metastatic

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colorectal carcinoma and its effect on the expression of TS mRNA in leukocytes.

Patient and methods

Patients with advanced refractory metastatic colon cancer were eligible for this study. Failure to respond to two different standard chemotherapy regimens was considered refractory. Patients must be 18 years and older. The clinical criteria included histologically confirmed stage IV colon cancer, demonstrated disease progression, bi-dimensionally measurable disease and life expectancy of more than two months. Patients should have ECOG performance status of 0–2. White blood cell count of $>3\,000$ cells/mm^3 and platelet count of $>100\,000$ cells/mm^3 were considered to be within acceptable limits. If patients' initial WBC count is $<3\,000$ but $>1\,000$/mm^3 and/or platelets are $<100\,000$ but $>50\,000$/mm^3 secondary to documented bone marrow involvement, the patient will also be eligible for the study. Patients should have adequate renal function as documented by serum creatine, bilurubin level of less than 2 and SGOT level of less than four times the normal limit. Serum magnesium concentration of 1.4–2.0 mEq/L and potassium of 3.5 to 5.0 mMol/L was considered acceptable.

Patients should not have received any form of chemo- and/or radio- therapy for four weeks prior to the start of this study. The patients should be free of any toxic side effects from prior treatment. During the participation in this clinical trial the enrolled patients were excluded from receiving any other form of therapy.

Metastases of tumor to CNS, history of prior neurological disorders (grade 3 or higher by the NCI Common Toxicity Criteria, in particular seizure disorders) were also a reason for exclusion from this study. Pregnant women were also excluded and patients of childbearing age were advised against pregnancy. Prior to enrollment, all patients were counseled about investigational nature of the treatment and a written informed consent was obtained as per the institutional review board (IRB approval No. #01/576B) guidelines.

Treatment Scheme

Each patient was scheduled to receive an intravenous dose of 0.25 mg/kg/day As_2O_3 as Trisenox (Cell Therapeutics Inc, USA) injected over a period 1–4 hours daily, for 5 consecutive days of the week for 5 weeks. Within 30 min after the administration of As_2O_3, 1000 mg of ascorbic acid was administered intravenously over 15 to 30 min period. A two-week rest period was allowed between each cycle. Therefore, each treatment cycle consisted of 5 weeks of therapy and 2 weeks of rest. Three additional cycles of treatment at the same doses were permitted, if tolerated by the patient. Treatment can be discontinued due to 1) unacceptable toxicity, 2) no clinical benefit or 3) at patient's request. No other chemotherapeutic treatment (except for steroids) was allowed within 2 weeks of administration of As_2O_3.

Evaluation of toxicity and disease progression

Disease progression and response was evaluated using the new international criteria proposed by the Response Evaluation Criteria in solid Tumors (RECIST) committee [6]. EKG was obtained bi-weekly during the first week of treatment, then weekly, and as clinically indicated. History and physical examination were performed weekly. Hematology and chemistry profiles were done biweekly. Pre and post treatment carcinoembryonic antigen (CEA) levels were also monitored. CT scan of the affected area was performed at the beginning and at the end of each cycle of the therapy. End of therapy evaluation included history and physical exam, hematology and chemistry profiles, CEA measurement, EKG and urinalysis, imaging and diagnostic studies (if indicated) and tumor response assessments. To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed 4 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 6 weeks.

Correlative laboratory studies to analyze the effect of arsenic trioxide on the expression of TS mRNA

Peripheral blood samples were collected from the study participants prior to and 24 hours post arsenic administration. White blood cells were isolated from the peripheral blood as described previously [7]. Total RNA from WBC was isolated using Tri-Reagent (Sigma, USA). The sequences of primers used to quantitate TS was TS 5' Primer A–(24 mer) 5′-GGGCAGATCCAACACATCCTCCGC-3′ and TS Rev. Primer (20 mer)-5′-GCCCAAGTCCCCTTCTTCTC-3′ that synthesized a 294 bp long product. β-Actin F-(25 mer) 5′-TCACCCACACTGTGCCCATCTACGA-3′ and β-Actin R-(25 mer)-5′-CAGCGGAACCGCTCATTGCCAATGG-3′ synthesized a 295 bp fragment which also served as the internal control. These primers spanned the exon-intron junction so that genomic DNA was not amplified.

The list of specialty chemicals used, details of cDNA synthesis by reverse transcription and RT-PCR procedure are as described in Sarkar et al. [8]. Each reaction was performed at two different dilutions of the template. The mean normalized expression (MNE) was calculated as detailed in Muller et al. [9].

Results and discussion

There are no treatment options available for patients with refractory metastatic colorectal cancer. Of late, arsenic trioxide had been shown to cure APL [1;3]. It also showed promising results in multiple myeloma patients [5]. Preclinical studies demonstrated the efficacy of arsenic trioxide on various cultured colorectal cancer cell lines. This led us to initiate this Phase II clinical trial to assess the anti tumor activity and toxicity of arsenic trioxide in patients with refractory metastatic colorectal carcinoma.

Five patients (three males and two females) were enrolled in this clinical trial between May and October of 2002 at the Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, FL, USA. The median age was 60 that ranged from 41 to 78 years. All of them were evaluable for response and toxicity. Every one had evidence of progressive disease, as documented by CT scans prior to therapy. They had undergone prior surgeries and chemotherapies. Under this trial three patients completed at least one cycle, while two others received more than two cycles of chemotherapy.

All the patients developed moderate to severe toxic side effects. A list of toxicities encountered is detailed in Table I. The most common side effects reported were fatigue, nausea/vomiting, dehydration, neuropathy, thrombocytopenia and anemia. One of the patients developed Herpes zoster and another had a relapse of Herpes genitalis that resolved with antiviral treatment. Occurrence of herpes infection in patients under arsenic therapy had been reported. It appears that arsenic weakens immune system [10]. Due to personal reasons one patient opted out who was latter diagnosed with CNS metastasis. In four patients the CEA level remained stable while, in one it increased by 3.4 fold (Table II). Across the group, a moderate decrease in hemoglobin level, WBC and RBC counts were observed. In one patient, the platelet count doubled. One of the patients showed mild changes in QTc that eventually returned to normal. CT scan was performed at the end of the treatment cycle to evaluate disease. Due to lack of clinical response as monitored by CT scan and severe toxicity in all the five patients, this study was aborted after two cycle of treatment. Thus, arsenic trioxide/ascorbic acid therapy did not produce desirable outcome in this group of heavily pretreated patients.

Two of the participants were alive at the time of last follow-up. The remaining three died within 2 to 5 months after withdrawing from the study. None of the deaths could be attributed to arsenic trioxide administration.

Toxic side effects of arsenic trioxide were a major concern. A previous phase I study demonstrated 0.25 to 0.35 mg/kg/day to be acceptable dose [11]. In this study, each patient received five doses of arsenic trioxide/ascorbic acid a week for 5 consecutive weeks. We believe that down sizing the treatment regimen might lessen the toxicity.

The lack of clinical response and pronounced side effects in this trial is intriguing but not without precedence. A similar study involving renal cell carcinoma (RCC) [12], metastatic melanoma [13] and refractory germ cell malignancies [14] also did not produce any clinical response. It appears that solid tumors do not respond to arsenic, which affects oxidative phosphorylation pathway thereby killing the cells [2]. Most of the solid tumors are believed to be hypoxic [15]. Therefore, even if delivered, due to their anaerobic lifestyle hypoxic tumors might be least affected by arsenic. Besides, by virtue of the

Table I. Toxicity

Toxicity profile			
	Total No. of patients	Male	Female
Grade I			
Dehydration	1 (20%)	1	0
Neuropathy	1 (20%)	1	0
Grade II			
Fatigue	4 (80%)	3	1
Dehydration	1 (20%)	1	0
Grade III			
Nausea/Vomiting, diarrhea, dehydration, neuropathy, thrombocytopenia and anemia	2 (40%)	0	2

Table II. Data from some of the analyzed hematological parameters

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
	Start/End	Start/End	Start/End	Start/End	Start/End
CEA[@]	5.1/6.8	15.9/17.0	2.8/2.1	66.4/180	273/213
HGb (g/dL)	14.4/9.0	11.7/10.3	13.4/11.5	12.6/12.1	16.4/10.5
Platelet (10*3)	236/209	257/528	290/236	216/250	167/155
WBC (10*3)	5.46/3.23	6.61/2.39	9.39/7.19	8.94/6.07	5.67/4.54
RBC (10*6)	4.39/2.85	3.90/3.60	4.94/4.08	3.84/3.42	5.59/3.69

@ In some of the patients the CEA measurements were done just before the termination of the treatment. Therefore, the precise end of the treatment CEA levels may not be represented.

route of administration, hematopoietic cancer cells might be in direct contact with As_2O_3. This direct physical contact may augment the effectiveness of treatment. In contrast inner core of tumors lack blood vessels and therefore may not receive As_2O_3 / AA. Thus, non-delivery of arsenic trioxide to solid tumors may be a reason for the treatment failure. Therefore, the problem of delivery of drugs to inner core of the tumor needs to be addressed.

Arsenic affected TS transcription

The standard colorectal cancer chemotherapy regimen includes 5-FU in combination with other drugs like CPT-11, Leucovorin etc. However, a majority of patients eventually fail to respond to this line of treatment. This limits the treatment options available for refractory cases. Our aim is to find agents that could sensitize refractory CRC to 5-FU. It interferes with thymidylate synthase (TS), a key enzyme for the conversion of deoxyuridylate to deoxythymidylate. In general the TS expression RNA is found elevated in 5-FU refractory tumor samples. Lowering of the TS quantity in resistant cells restored their sensitivity to 5-FU [16] (Subbarayan et al. unpublished observations). Therefore, we quantitated TS message in the peripheral blood leukocytes in the patients following arsenic trioxide/ascorbic acid administration. The result of this study is presented in Figure 1. We found that following treatment TS mRNA expression was significantly reduced ($p = 0.03$, one-sided paired t-test) in the peripheral blood mononuclear cells (PBMC) of the four patients from whom blood samples were obtained. There is inter-patient variation in the expression of TS and in response to treatment. TS transcripts in PBMC decreased from 37% to 92% after treatment with arsenic (with a median value of 63%). In one patient who was available for post treatment evaluation, within seven days after the cessation of arsenic treatment, the TS message level was restored to 67% of the pretreatment level (Figure 1, Arrow). When TS level is reduced the tumor might have been sensitized back to 5-FU. Therefore, a combination of sub-toxic dose

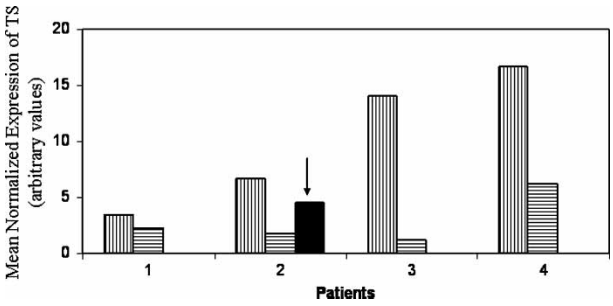

Figure 1. TS transcript level decreased with arsenic treatment. Peripheral blood samples were drawn prior to (vertical bars) and post arsenic infusion (horizontal bars) and Mean Normalized Expression of TS mRNA was measured as described in materials and methods. Real time PCR analysis of TS and β-actin expression was done as described under materials and methods. Recovery of TS during two weeks of intermission in treatment is highlighted with an arrow.

of As_2O_3 with 5-FU may restore sensitivity in refractory tumors. This strategy can form a basis for As_2O_3/5-FU combination chemotherapy.

In conclusion, since there were severe toxic side effects to 0.25 mg/kg/day dose, we suggest that a judicious combination of low or dose of As_2O_3 and a modified treatment regimen could lower TS level in tumor cells with less severe toxic side effects. It may also open a way for the use of other chemotherapy agents in combination with arsenic trioxide.

Conflict of interest statement

None declared

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Dedicated to the memory of late Dr. Benito Que.

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