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Randomized Placebo-Controlled Trial of 2,3-Dimercaptosuccinic Acid in Therapy of Chronic Arsenicosis Due to Drinking Arsenic-Contaminated Subsoil Water

Debendra N. Guha Mazumder; Uday C. Ghoshal; Jayanta Saha; Amal Santra; Binay K De; Amitabha Chatterjee; Subir Dutta; Carol R. Angle; Jose A. Centeno

Institute of Postgraduate Medical Education and Research, Calcutta (DNGM, UCG, JS, AS, BKD); B.C. Roy Institute of Basic Medical Sciences, University College of Medicine, Calcutta (AC, SD), India; University of Nebraska Medical Center, Omaha, Nebraska (CRA); Armed Forces Institute of Pathology, Washington, DC (JAC)

ABSTRACT

Introduction: Chronic arsenic toxicity producing various clinical manifestations is currently epidemic in West Bengal, India, Bangladesh, and other regions of the world. Animal studies have indicated that 2,3-dimercaptosuccinic acid can be used as an oral chelating agent. A prospective, double-blind, randomized controlled trial was carried out to evaluate the efficacy and safety of 2,3-dimercaptosuccinic acid for chronic arsenicosis due to drinking arsenic-contaminated ($\geq$ 50 μg/L) subsoil water in West Bengal. Method: Twenty-one consecutive patients with chronic arsenicosis were individually randomized (random number; assignment made by individual not evaluating patients) into 2 groups: 11 patients (10 male, age 25.5$\pm$8 years) received 2,3-dimercaptosuccinic acid 1400 mg/d (1000 mg/m^2) in the first week and 1050 mg/d (750 mg/m^2) during the next 2 weeks with a repeat course 3 weeks later. The other 10 patients (all male, age 32.2$\pm$9.7 years) were given placebo capsules for the same schedule. The clinical features were evaluated by an objective scoring system before and after treatment. Routine investigations

Correspondence: Prof. DN Guha Mazumder, Department of Gastroenterology, Institute of Postgraduate Medical Education and Research, 244, Acharya JC Bose Road, Calcutta 700020. Tel: 91/33-474-6586; Fax: 91/33-223-2178.

including liver function tests, arsenic concentrations in urine, hair, and nails, and skin biopsy evaluations were also completed. Results: Though there was improvement in the clinical score of 2,3-dimercaptosuccinic acid–treated patients, similar improvement was observed in the placebo-treated group. There were no statistical differences in the clinical scores between the 2 groups at the beginning and at the end of treatment. Similarly, no differences were found for the other investigated parameters. Conclusion: Under the conditions of this study, 2,3-dimercaptosuccinic acid was not effective in producing any clinical or biochemical benefit or any histopathological improvement of skin lesions in patients with chronic arsenicosis.

INTRODUCTION

In West Bengal, India, a large number of people have been affected by chronic arsenicosis due to drinking geologically contaminated subsoil water from tubewells.[1,2] Similar problems have been reported in certain other geographical areas of the world.[3-5] Chronic arsenicosis leads to reversible damage to several vital organs and is established as carcinogenic.[5-7] Despite the magnitude of this potentially fatal toxicity, there is no effective therapy for this disease; patients once affected may not recover even after remediation of the arsenic-contaminated water.[8] The need for an effective therapy for chronic arsenicosis is obvious.

2,3-Dimercaptosuccinic acid (DMSA), a chelating agent, has been used in therapy for lead and mercury poisoning in humans.[10] There are reports of its efficacy in acute arsenic poisoning in mice[11] and in chronic arsenic poisoning in rats.[12] There are no studies on use in chronic arsenic poisoning of humans although mobilization of arsenic from the human body has been shown in acute arsenic poisoning.[13] We undertook this prospective, randomized controlled trial to evaluate the efficacy and safety of DMSA in patients with chronic arsenicosis due to drinking arsenic-contaminated subsoil water.

PATIENTS AND METHODS

Twenty-one consecutive patients with chronic arsenicosis were randomized into 2 groups. Eleven patients (10 males, ages 25.5±8.0 years) received DMSA 1400 mg/d (100 mg/m^2) in 4 divided doses the first week and then 1050 mg/d (750 mg/m^2) in 3 divided doses during the next 2 weeks. The same was repeated after 3 weeks during which no drug was administered. The other 10 patients (all males, ages 32.2±9.7 years) were given placebo capsules (resembling DMSA) in the same schedule. The patients were blinded about the nature of treatment being given. The patients included in the study were selected from the arsenic clinic on the basis of history of drinking arsenic-contaminated water (50 μg/L; ≥ 0.05 mg/L) for 2 years or more and clinical symptoms and signs of chronic arsenicosis. The symptoms and signs of patients were evaluated by an objective scoring system before and after treatment. The scoring system followed is summarized in Table 1. Any possible therapy-related side effect was monitored in every patient. All the patients were kept hospitalized during the study period.

Patients who stopped drinking arsenic-contaminated water for more than 5 months before inclusion into the trial, those who smoked, drank alcohol, took hepatotoxic drugs, and were found positive for hepatitis B virus surface antigen were excluded from the study. Ages below 18 years and pregnancy were also exclusionary. Informed consent was obtained from every patient before inclusion into the study. The study was cleared by the ethical committee of the institute.

Before inclusion into the study, all the patients underwent evaluation of their hemogram, liver function, prothrombin time, blood sugar, urea, and creatinine, and routine examination of urine and stool. Abdominal ultrasonography and upper gastrointestinal endoscopy were done to look for portal hypertension. A needle liver biopsy was obtained on all patients willing to provide informed consent.

Skin was biopsied from unexposed areas by punch biopsy technique for histologic evaluation before and after treatment. Hyperkeratosis, acanthosis, papillo-

Table 1

System of Clinical Scoring of the Symptoms and Signs Before and After Therapy with DMSA and Placebo

Symptoms and Signs	None	Present Mild	Moderate	Severe
Weakness	0	1		
Cough	0	1		
Dyspnea	0	1	2	3
Rales, rhonchi	0	1		
Hepatomegaly	0	1 (14 cm span)	2 (16 cm)	3 (> 16 cm)
Splenomegaly	0	1 (2 cm)	2 (4 cm)	3 (> 4 cm)
Pigmentation	0	1 (Diffuse)	2 (Spotty)	3 (Blotchy)
Keratosis	0	1 (Thickening)	2 (Few nodules)	3 (Multiple nodules)
Flushing of face	0	1		
Conjunctivitis nonpitting	0	1		
Edema leg/hand	0	1		
Abdominal pain	0	1		
Anorexia	0	1		
Nausea	0	1		
Diarrhea	0	1		
Hearing defect	0	1		
Claudication	0	1		
Hand/leg ulcers	0	1		
Paresthesia	0	1 (Only legs)	2 (Leg + hands)	
Pallor	0	1		
Ascites	0	1		
Loss of ankle jerk	0	1		

Maximum score 33.

matosis, and parakeratosis, the characteristic features of chronic arsenic toxicity,[14] were evaluated objectively by measuring with an ocular micrometer by a pathologist unaware of the treatment category. Dysplasia was assessed visually on the basis of nuclear chromatin clumping. Parakeratosis was graded as absent, mild, moderate, and severe on the basis of visual assessment. Ocular micrometer measurement of skin histology was graded as follows: Parakeratosis: absent $\leq$ 20 μ; mild 21–41 μ; moderate 42–75 μ; marked 76–125 μ; severe > 126 μ. Acanthosis: absent < 140 μ; mild 141–195 μ; moderate 196–350 μ; severe > 351 μ. Papillomatosis: absent $\leq$ 30 μ; mild 31–40 μ; moderate 41–100 μ; severe 101 μ. Hair samples, cut from the root, and nail samples were taken for estimation of arsenic content before and after treatment.[5]

Urine samples were collected for 2 consecutive days before, and then at 48 and 72 hours after starting the drug or placebo. Aliquots of the 24-hour urines were immediately deep frozen and preserved until sent to the US Armed Forces Institute of Pathology at Washington, DC. Urine arsenic was determined by graphite furnace atomic absorption with Zeeman-background correction. The lamp excitation source consisted of a "Super lamp power supply" to provide a better sensitivity at the low level of this analyte in urine. The preparation of the samples prior to the analysis was based on an acid-induced sample digestion procedure. Each sample (1 mL) was acidified with highly purified HNO_3 (70% Ultrex quality 1 mL), placed in acid-

Table 2

Demographic, Clinical, and Laboratory Parameters in Patients Treated with DMSA and Placebo

	DMSA (n=11)	Placebo (n=10)	p Value
Age (years)	25.5±8.0	32.2±9.7	ns*
Sex (M:F)	10:1	10:0	
Clinical features:			
Pigmentation	11	10	
Keratosis	11	10	
Hepatomegaly	2	4	
Vasculopathy	4	1	
Clinical score	9.3±3.3	10.6±3.2	ns†
As mg/L in drinking water	0.66±0.39	0.65±0.34	ns*
Duration of exposure (y)	15.25±10.7	21.6±11.95	ns*
Duration of drinking As-free water before entry (months)	.54±1.37	1.67±1.22	ns*
Portal hypertension	0	0	
Liver histology (abnormal)	6/7	5/5	

*Student's *t* test; †One way ANOVA.

Table 3

Clinical Scores of Patients Before and After Therapy

	Before	After	p Value
DMSA n=11	9.33±3.33	6.2±2.11	0.017
Control n=10	10.6±3.20	6.7±1.70	0.003

*One way ANOVA.

washed (Ultrex solution), Teflon-based plastic vessel, capped, and digested employing a microwave digestor unit (CEM Microwave Model 2000). After digestion, each sample was reconstituted in a total volume of 4–5 mL with highly purified, distilled deionized (Millipore quality) water. Quality controls used to monitor detection limits and accuracy were based on spiked urine from National Institute of Standards and Technology, and in-house prepared spiked urine specimens. The analyte recovery on these spiked samples was between 90–100%. The detection limits of the methodology were calculated based on the standard deviation calculated from 10 consecutive blank measurements and it was estimated to be ~1–3 μg/L (ppb). The detection limit in the urine samples was calculated based on the relative standard deviation. The average of 2 values obtained from 2 days' measurements before starting the drug was taken for calculation; if one of these 2 values was less than the detection limit, the other value was taken for calculation.

The significance of the differences between the parametic data obtained in the 2 groups was calculated by student's *t* test. The clinical scores of the patients before and after therapy were compared by one-way ANOVA. The concentration of arsenic before and after therapy was compared by Wilcoxon's rank sum test, as the data were not expected to have normal distribution. For nonparametric data, Chi-square with Yates' correction, as applicable, was used.

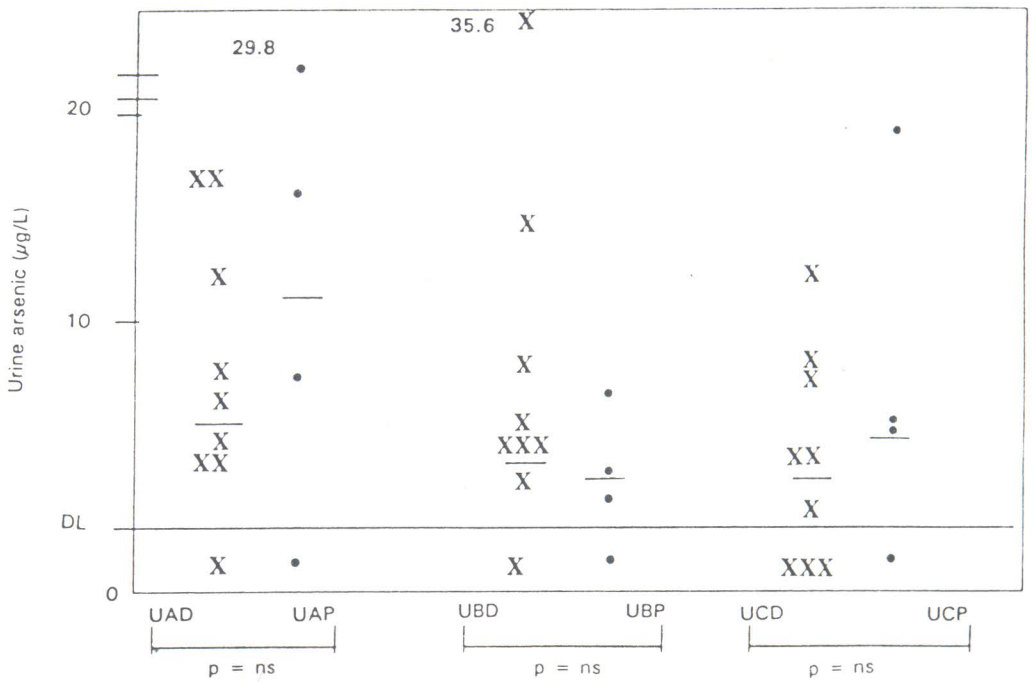

Figure 2. Clinical score of patients pre- and posttherapy with DMSA and placebo.

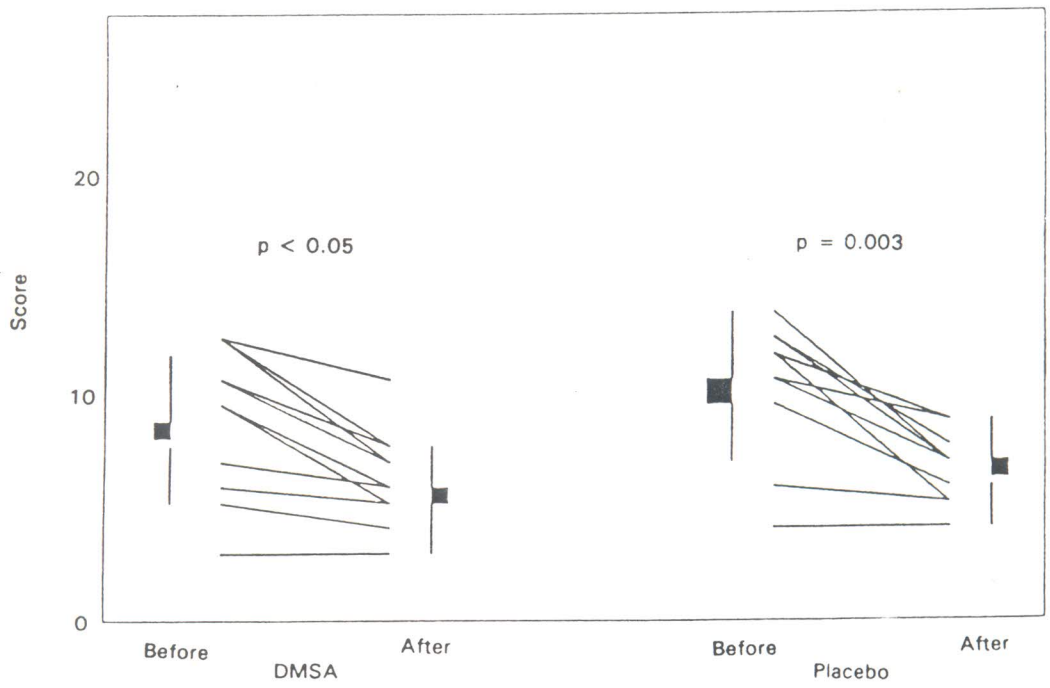

Figure 1. Urine As μg/dL in DMSA (UD) and placebo (UP) treated patients. UAD and UAP 24 hours before therapy. UBD and UBP 48 hours posttherapy. UCD and UCP 72 hours posttherapy.

Table 4

Laboratory Parameters Before and After Therapy in Patients Treated with DMSA and Placebo

	DMSA		Placebo		
	Before	After	Before	After	p Value
Liver function (n: D=11; P=10)					ns
Bilirubin (mg/dL)	0.7±0.12	0.6±0.08	0.7±0.09	0.7±0.11	
ALT (IU/L)	36±18.8	46±13	38.5±16.8	50.1±28.1	
AP (IU/L)	241.3±122.8	254.6±66.9	258.2±93.7	342.0±201.3	
Albumin (g/L)	3.8±0.5	4.0±0.6	4.0±0.8	3.9±0.6	
Urine As (μg/L)* (n: D=9; P=4)					
Before drug	7.89±5.93		17.45±11.53		ns
48 hours postdrug		8.83±11.54		3.04±2.6	
72 hours postdrug		4.56±3.88		9.20±8.56	
Hair As concentration (mg/kg) (n: D=11; P=10)	2.9±1.8	2.8±3.4	3.5±3.2	2.6±1.6	ns
As concentration in nail (mg/kg) (n: D=9; P=9)	7.5±4.9	7.3±4.9	6.9±4.8	6.5±5.5	ns

As: arsenic; D: drug (DMSA) group; P: placebo group; ALT: alanine aminotransferase; AP: serum alkaline phosphatase; DL: detection limit. *in 1 patient, 1 of the 2 24-hour urines pre-DMSA contained arsenic 202 μg/L; it was analyzed 3 times with the same result. It was thought unlikely for the endogenous arsenic level to be so high, and the second 24- hour urine arsenic (2.26 μg/L) was used in the analysis.

RESULTS

Demographic and clinical data of patients in each group are summarized in Table 1. There were no differences in age, sex, duration of exposure to the arsenic-contaminated water, arsenic concentration in the drinking water, duration of drinking arsenic-free water before inclusion into the study, and clinical score of symptoms and signs between patients in the drug group and in controls (Table 2).

Therapy with DMSA did not cause any significant clinical improvement as compared to patients treated with placebo (Figure 1). The clinical score improved after therapy with DMSA, with similar improvement in patients treated with placebo (Table 3).

Excretion of arsenic in urine before treatment, at 48 hours and 72 hours after treatment in DMSA and placebo group was comparable (Figure 2). There was no difference in the results of the liver function tests and arsenic concentration in hair and nails before and after treatment (Table 4). No patient developed any therapy-related side effects. The histologic abnormalities in skin biopsy did not show any difference in patients treated with DMSA and placebo before and after therapy (Table 5).

DISCUSSION

In this study, we did not find DMSA 1 g/m^2 x 1 week and 0.75 g/m^2 x 2 weeks for 2 courses at 3-week intervals to have any clinical or biochemical benefit in patients with chronic arsenicosis. To the best of our knowledge, this is the first randomized placebo-controlled trial on use of this chelating agent in therapy of chronic arsenicosis.

Table 5

Change in Histology of Skin Biopsies in Patients with Chronic Arsenicosis Before and After Therapy with DMSA and Placebo*

	DMSA (n=11)	Placebo (n=10)	p Value
Hyperkeratosis			
No change	5	6	
Upgrade	4	1	ns
Downgrade	1	0	
Parakeratosis			
No change	5	7	
Upgrade	3	0	ns
Downgrade	2	0	
Acanthosis			
No change	5	5	
Upgrade	4	1	ns
Downgrade	1	1	
Papillomatosis			
No change	5	5	
Upgrade	3	1	ns
Downgrade	2	1	
Dysplasia			
No change	7	6	
Upgrade	3	1	ns
Downgrade	0	0	

*Denotes change in relation to therapy with DMSA and placebo. No change: persists in same severity despite therapy; Upgrade: increased severity after therapy; Downgrade: decreased severity with therapy. Criteria for histologic assessment are described in text.

Toxicity of arsenic has long been of concern due to use of this heavy metal in industries. Arsenic is also used as herbicide, pesticide, and rodenticide, and as an ingredient in paints and wood preservatives. The current reports of epidemics of chronic arsenicosis from drinking arsenic-contaminated subsoil water from different parts of the world make it a matter of great concern. Various noncarcinomatous and carcinomatous manifestations of chronic arsenic toxicity are increasingly recognized.[2] Our 12-year follow-up study showed that patients with chronic arsenicosis, once affected, may not recover and some symptoms, like lung disease, may appear or progress after they stop drinking arsenic-contaminated water.[9] There is an impelling concern to define an effective pharmacotherapeutic agent to remove arsenic from human body. Unfortunately, our study conclusively shows DMSA to be ineffective therapy for chronic human arsenicosis.

In the 1940s, *DL*-2,3-dimercaptopropanol[16] was found to be an effective antidote in poisoning by trivalent arsenicals. However, the dithiols *DL*-2,3-dimercaptopropane sulfonate (DMPS)[17] and meso-2,3-dimercaptosuccinic acid (DMSA)[13] are much more soluble in water and can be administered orally. Evaluation of poisoning by lewisite in rabbits[19,20] and arsenic trioxide in mice[11,21] and guinea pigs[22] all favor treatment with DMSA over BAL. The significantly lower toxicity, the ease of administration, and the enhanced biliary clearance of arsenic all contributed to the clinical consensus that DMSA and DMPS, not BAL, are first choice of therapy for arsenic poisoning.[23] The greater intracellular distribution of DMPS makes it somewhat more efficacious but the lower toxicity of DMSA makes it preferable for widescale administration.[24] Shum and Whitehead[13] reported that treatment of an adult who had ingested 80 g methane arsenate with DMSA 30 mg/kg/d x 5d over 1 month reduced serum arsenic from 2871 μg/L to 6 μg/L. Lenz *et al.*[25] also found DMSA to be effective in man. However, Kew *et al.*[26] found no improvement in peripheral neuropathy of 4 months duration after DMPS 300 g/d x 3 weeks and DMSA 1.2 g/d x 2 weeks. There was no improvement of neuropathy following treatment with DMSA in our patients. Further, our study has conclusively shown that DMSA is globally ineffective in the therapy of chronic arsenic toxicity in man.

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