Pharmacodynamic study of the selenium-mediated arsenic excretion in arsenicosis patients in Bangladesh

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ABSTRACT: Since 1938 several investigators have confirmed antagonistic interaction whereby the administration of selenium mitigates arsenite toxicity in mammals. More recently a number of animal model and human studies have revealed that the bimolecular basis of this antagonism involves the glutathione mediated formation of major metabolite seleno-bis(S-glutathionyl)arsinium [(GS)₂AsSe]⁻ ion which is rapidly excreted through bile. To investigate whether the oral supplementation of sodium selenite will enhance the fecal excretion of arsenites in human, a limited pharmacodynamics study was carried out involving ten arsenicosis patients. However, data from five patients are reported here. Patients were screened for pre-existing hepatic diseases and they were excluded from the study. Subjects were selected based on the arsenic level in their household drinking water, hair, nail samples and diffuse melanosis symptoms. Patients received ⁷⁷Se-labeled sodium selenite as oral supplement, which they ingested together with their arsenic-containing drinking water. Total selenium levels increased in both urine and feces following the co-administration of sodium selenite (800 µg). The patients receiving a placebo showed no change in their selenium excretion. The increase in selenium levels in feces and arsenic to some extent after dosing agrees with our hypothesis that selenium supplementation promotes co-excretion of arsenic and selenium through formation of [(GS)₂AsSe]⁻ in the bile.

1 INTRODUCTION

Since the discovery of unsafe levels of arsenic in groundwater in Bangladesh in 1993, a number of reports have indicated that approximately 70–80 million people are being exposed to this metalloid to concentrations in drinking water that are above the World Health Organization (WHO) permissible level of 10 µg L⁻¹ from shallow aquifers contaminated by geogenic arsenic (Mukherjee & Bhattacharya, 2001; Kinniburgh & Smedley 2001; Ahmed *et al.*, 2006). Chronic exposure of humans to inorganic arsenic leads to arsenicosis with pathological symptoms including skin melanosis and keratosis, endocrine diseases and cancers of the lungs, the liver, the kidneys and the bladder (Kapaj *et al.*, 2006).

1.1 Antagonism of Arsenic and selenium

During 1930–1950, several animal model studies indicated that arsenite can detoxify selenium in form of

selenite and/or seleniferous wheat in rats, dogs, cattle (Moxon *et al.*, 1938). The therapeutic benefit of selenium for countering the toxicity of inorganic arsenic stems from a number of studies that have been carried out over the past 80 years. The oxy-anions of arsenic and selenium are arsenite and selenite – are toxic to animals if given separately. However, if both are administered together they mutually de-toxify each other. This antagonistic interaction has been linked to the *in vivo* formation of the seleno-bis(S-glutathionyl)arsinium [(GS)₂AsSe]⁻. Synchrotron-based X-ray absorption (XAS) spectroscopy has recently revealed the formation of the seleno-bis(S-glutathionyl)arsinium anion in hepatocytes of rabbits and its subsequent excretion in the bile (Gailer *et al.*, 2002, 2004). In addition, studies indicate that multidrug resistance protein 2 (MRP2) transports [(GS)₂AsSe]⁻ mediates its excretion from the liver to the bile (Carew & Leslie, 2010).

During 2006 to 2009 we have conducted a 48-week, 821 patient, randomized, double-blinded placebo controlled Phase III clinical trial in Bangladesh to

investigate the effectiveness of daily oral dose of 200 μg selenite in combating arsenic toxicity. Our study suggested that the employed selenium dose was inadequate to reduce the prevalent condition of melanosis and other arsenicosis symptoms since their arsenic intake through drinking water and food were quite high (Alauddin *et al.*, 2012). However, our recent studies which involved the administration of selenite and arsenite to rats and hamsters conclusively revealed the presence of [(GS) 2AsSe]- in the bile (George *et al.*, 2016; Ponomarenko *et al.*, 2017).

1.2 Objectives of the current study

A number of our previous studies using rabbits, rats, hamsters and human have demonstrated that the co-administration of selenite will promote the hepatobiliary co-excretion of arsenic and selenium in the form of the [(GS) 2AsSe]- ion. We hypothesize that the dietary supplementation of selenium may promote the formation of the arsenic-selenium-glutathione conjugate which is excreted in the bile and therefore increases the excretion of arsenic via feces. The present study is a follow up of the previous pharmacodynamics study involving five patients. Our objectives were to monitor the excretion of total arsenic and selenium via feces and urine in five patients that were provided with daily meals and drinking water which they would normally consume in their household. Our aim was to assess the effectiveness of a selenium supplement in the excretion of arsenic from the body of arsenicosis patients.

2 EXPERIMENTAL

2.1 Study design

This is a limited study which involved five arsenicosis patients from Laksham, Shahrasty in Bangladesh. The subjects were patients which were recruited following defined selection criteria including a) arsenic exposure based on arsenic in their drinking water source, and b) normal kidney and liver function. The study protocol was explained to all patients and signed consents was obtained from all participants. All participants stayed in a private clinic in Laksham for 10 days where they were given fixed food regimen prepared with the drinking water from their homes. On the sixth day they received either a 800 μg Se-77 labeled sodium selenite dose or a placebo, which was drinking water that did not contain selenite. The stable isotope Se-77 labelled sodium selenite was used as supplement to differentiate native selenium and selenium that was administered to patients. All urine and fecal matter were collected from all participants every day to account for all excretions. Feces were weighed, volumes of urine were measured and all excretions were recorded during the patients 10 day stay in the clinic. Data for 5th thru 8th day are presented in this paper. The urine samples and fecal matters were pooled

together separately for each day for each participant. All food samples for breakfast, lunch, dinner were weighed for each participant every day and recorded. A portion of breakfast, lunch, dinner, snacks were homogenized in distilled deionized water and aliquots were preserved in vials for arsenic and selenium analysis. All water, fruit juice, tea intake for each patient were recorded daily. All pooled feces samples were weighed and an weighed portion was homogenized in distilled deionized water and aliquots were preserved at -4°C in a freezer before further analysis in the laboratory. The aliquots of food, water, patient samples such as blood, urine and feces were transported to the USA and stored immediately in a -80°C freezer prior to analysis.

2.2 Reagents and standards used for the chemical analysis of all biological tissues for total arsenic and selenium

Homogenized feces samples were oven dried at 95°C for an hour and dry weight was obtained. The dried fecal matter were digested in high purity nitric acid and diluted with deionized water to a fixed volume. Arsenic (As), selenium (Se) in urine and fecal matter were determined by the DRC-ICP-MS technique while the arsenic, selenium in food and water samples were determined by the graphite furnace atomic absorption spectroscopy (GF-AAS) (Perkin Elmer Model AAnalyst 800) with Zeeman background correction and electrodeless discharge lamps (EDL). Matrix modifier for Se analysis were prepared from a solution containing 1% (w/v) $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 2% (w/v) $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 0.1% (v/v) Triton x-100 while the matrix modifier for As analysis consisted of 1% (w/v) $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 0.1% Triton X-100. For all matrix modifiers, high purity (99.999%) $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 2% (w/v) $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (Sigma-Aldrich Co., USA) $18\text{ M}\Omega\text{cm}^{-1}$ water were used. Stock As, Se standard solutions (Perkin Elmer Co., USA) were used to calibrate AAS. The National Institute of Standards and Technology (NIST) standard reference materials (SRM 1640), served as control reference material for As, Se analysis in water and food samples. The method detection limit for As, Se analysis in water and urine was $1.0\text{ }\mu\text{g L}^{-1}$, with an RSD of 2.5%.

3 RESULTS

3.1 Fecal and urinary Se, As data

Arsenic and selenium in fecal matter from all (5) patients collected before Se dosing and after Se dosing are shown in Table 1. Patients 1, 2 and 3 received selenium supplements and patients 4 and 5 received placebo (drinking water without selenium dose) on day 6. Here we report data for day 5 thru day 8 for brief observation for the fate of selenium administered to patients.

Table 1. Daily As, Se intake and pooled fecal and urinary excretion (μg) by patients .

	Day	In As	Ex As	In Se78	Ex Se78	In Se77	Ex Se77
Pat1	5	2026	1825	39.4	53.9	0	74.6
	6	1948	1688	24.2	49.8	800	54.6
	7	2161	1221	30.7	50.1	0	9985
	8	1803	1224	19.7	46.6	0	4132
Pat2	5	1400	374	39.6	21	0	23.3
	6	1146	602	23.9	30.5	800	31.9
	7	1488	497	38.7	31.3	0	4352
	8	1401	877	19.4	69.4	0	7328
Pat3	5	1846	694	39.4	27.7	0	31.9
	6	1906	1237	26.5	38.9	800	44.6
	7	1771	391	31.9	17.2	0	4318
	8	1835	732	19.7	16.6	0	1995
Pat4	5	1804	2435	59.9	52.8	0	56.6
	6	1774	1853	35.9	52.4	0	57.6
	7	1767	1199	64.2	32.8	0	63.1
	8	1801	1958	23.8	77.9	0	88.5
Pat5	5	1344	646	38.9	25.6	0	26.6
	6	1210	852	23.4	29.4	0	33.7
	7	1416	632	37.2	25.8	0	28.7
	8	1634	867	19.4	33.9	0	46.2

Figure 1. As, Se intake and excretion (μg) for patients 1–5.

3.2 Intake and excretion of As, Se

In Table 1 total As, Se intake by a patient are calculated from the As, Se levels in their daily food intake and their drinking water consumed by each patient. The total excretion of As, Se have been determined from the As, Se levels in urine and fecal matter and the pooled amount of urine and fecal matter for each patient for each day. Patients 1–3 received Se supplementation on day 6. Patients 4 and 5 did not receive selenium supplement.

The As and Se in fecal excretion are high, especially in fecal matter for the supplement group (Table 1) as

Figure 2. Fecal excretion of total As and Se by patients.

Figure 3. Urinary excretion of total As and Se by patients.

expected. Data for only 2 pre-dose days and 2 post-dose days have been reported here for a brief overview of the pharmacodynamics study. Detailed outcome involving all ten patients and analysis of patient samples from all 10 days will be reported later. The present data indicate that (a) selenium increases in fecal matter in supplement group on the 7th and 8th days at the appropriate interval 6–12 hours after Se ingestion and (b) in every case fecal arsenic excretion increases after Se ingestion. The total amount of Se-77 excretion is much higher in all three patients in the supplement group than the intake amount. The urinary As, Se excretion increased for patient 2, 3 in the supplement group. High urinary As excretion in patient 4 might

be indicative of other physiologic condition for this patient.

4 CONCLUSIONS

The urine, fecal matter samples from the remaining 5 patients in the same batch are being analyzed and the data will be reported later in a separate paper. The current data obtained from a limited number of patients are indicative of potential pathway for removal of body burden arsenic by Se supplementation. Currently a pharmacodynamics study involving a larger number of patients is under way by our group.

CLINICAL TRIAL REGISTRY

Clinical Trial.gov Identifier: NCT02377635.

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