Sequential effects of daily arsenic trioxide treatment on essential and nonessential trace elements in tissues in mice

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Despite arsenic's (As) toxic potential, arsenic trioxide (As_2O_3) is used as a safe and effective treatment in acute promyelocytic leukaemia. However, it is unknown whether such therapy influences the balance of other trace elements in the body. In this study, mice were treated intraperitoneally daily with 1.0 mg As_2O_3/kg bw for 3, 5 or 7 days. As, and seven essential and nonessential trace elements with the potential to interact with As, were measured through inductively coupled plasma-mass spectrometry in serum, heart, lung, liver, pancreas, kidney, intestine and brain. As_2O_3 supplementation increased As in all target tissues on day 3, thereafter reaching an almost steady state. The major findings in other elements were a sequential decrease in serum zinc (on day 7 by 64%; $P<0.001$), and a decrease in selenium in the pancreas on day 3 (9%; $P<0.05$), in the intestine on day 3 (30%; $P<0.001$) and finally, in the brain on days 5 (12%; $P<0.05$) and 7 (15%; $P<0.01$). Changes in magnesium, iron, copper, cadmium and mercury were minor and inconsistent. This study suggests that supplementation with other trace elements may be beneficial when As_2O_3 treatment regimens are used in the clinic. *Anti-Cancer Drugs* **19:812–818 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

Anti-Cancer Drugs 2008, **19**:812–818

Keywords: acute promyelocytic leukaemia treatment, arsenic trioxide, serum, tissues, trace elements

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Received 10 June 2008 **Revised form accepted** 11 June 2008

Introduction

Arsenic (As) is considered as a highly toxic trace element that may cause cancer, cardiovascular disease and diabetes [1]. Despite this potential danger, arsenic trioxide (As_2O_3) has been used as a therapeutic drug for more than 2000 years in the treatment of psoriasis, syphilis, rheumatosis and several other diseases [2]. Today As_2O_3 is approved by the US Food and Drug Administration for the treatment of relapsing and refractory acute promyelocytic leukaemia (APL). In addition, clinical studies on the efficacy of As_2O_3 in several other malignancies (such as myelodysplastic syndromes, multiple myeloma and non-Hodgkin's lymphoma) are under way [2]. Trisenox (Cephalon) is the commercial name for As_2O_3, where the drug is used in daily doses of 0.15 mg/kg bw for a maximum of 60 days for the induction treatment of APL. The complete remission rate in relapsing APL treated with As_2O_3 is as high as 80–90% [3], but the mechanisms underlying these effects remain poorly defined.

As_2O_3 is efficiently absorbed (> 80%) from the gastrointestinal tract into the bloodstream, partly accumulating in erythrocytes [4]. In the body As is rapidly metabolized and most of the administered dose is excreted within 24 h [5]. At short time intervals after As exposure, the target organs with the highest concentrations are the liver, kidneys, bile, intestinal mucosa and lungs [5]. However, despite the toxic potential of arsenic, it has been hypothesized that it is an essential element with a physiological role [6].

A physiological balance among trace elements is essential for human health and is reflected by the fact that 1/3 of all mammalian proteins are metal-binding proteins [4]. Metallothioneins (MTs) are inducible metal-binding proteins involved in homeostasis and detoxification of different metals [7]. MT preferentially binds cadmium (Cd) and zinc (Zn), but also has the capacity to bind As, copper (Cu) and mercury (Hg) [8–11]. Both As and Cd are known inducers of MTs [11,12]. Consequently, potentially toxic elements (such as As and Cd) can, if they are present in sufficient amounts, compete or interfere with essential elements, such as Cu, iron (Fe), selenium (Se) and Zn [4,13].

Se is an essential trace element important for a properly functioning immune system [4]. More than 40 years ago, it was shown that As enhanced gastrointestinal excretion of Se when both substances were given simultaneously [14]. It is generally accepted that uptake of one of these elements causes release, redistribution or elimination of the other element by urinary, biliary and/or expiratory routes [15]. Moreover, the cancer-protecting effects of Se have been shown to be abolished by Se-antagonistic elements, including As, lead (Pb) and Cd [16].

0959-4973 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/CAD.0b013e32830c456b

The antagonistic effect between As and Se has been suggested to originate in the formation of an As–Se compound secreted in bile [17].

The cross-protection of As against other metal toxicities, as in the case of Cd and Se, is probably a result of MT induction [4,18]. It is therefore reasonable to assume that As supplementation could lead to a changed tissue balance of these and other trace elements (both essential and nonessential). An adequate trace element homeostasis may be pivotal for proper tissue function, as well as for an optimal efficacy of the As treatment regimen being used. Consequently, the impact of As on the general trace element balance is important to consider when As is used clinically in the treatment of diseases in which host defence mechanisms partly depend on these nutrients, such as in cancer and infectious diseases where both the immune system and antioxidative mechanisms are involved.

Presently, there are no published data showing how As treatment affects the normal trace element balance in different organs. The aim of this study was therefore to investigate whether supplementation of a clinically relevant dose of As_2O_3 affects essential [magnesium (Mg), Cu, Fe, Se and Zn] and nonessential (Cd and Hg) trace elements in serum, heart, lung, liver, pancreas, kidney, intestine and brain in mice.

Materials and methods

Mice

Adult female Balb/c mice aged 8–10 weeks (Charles River, Copenhagen, Denmark) were maintained at the animal department, Biomedical Centre, Uppsala, Sweden. The mice were randomly assigned to groups of similar initial mean body weights (19.7 ± 0.7 g) and housed at 23 ± 1°C (relative humidity $50 \pm 2\%$) in a 12-h light/dark cycle within hygienic barriers (TouchSLIM-Line; Tecniplast, Scanbur BK A/S, Lellinge, Denmark). Water and regular chow diet (Labfor R36; Lantmännen, Sweden) were supplied *ad libitum*. Food and water were analyzed for As content. Control-treated and As_2O_3-treated mice were studied simultaneously.

The animal experiments described in this publication took into account all ethical aspects of the welfare of animals following the recommendations in 'Guide for the Care and Use of Laboratory Animals' of the Swedish National Board for Laboratory Animals (CFN). The study was approved (C127/4) by the local Ethical Committee for Experimental Use at the Faculty of Medicine, Uppsala University.

Arsenic test solution

Arsenic trioxide (As_2O_3) solution (10 mg/ml), obtained from Ultra Scientific, USA, was diluted in sterile physiological NaCl to a final concentration of 0.125 mg/ml. The diluted As_2O_3 test solution was analyzed by inductively coupled plasma-mass spectrometry and found to have the expected concentration.

Experimental design

The study included three groups of mice treated daily with As_2O_3 for 3, 5 or 7 days and one control group treated daily with NaCl. The mice were intraperitoneally administered 0.2 ml with approximately 1.0 mg As_2O_3/kg bw or, in the control mice, the corresponding volume of NaCl.

Tissue sampling

After start of the daily As_2O_3 supplementation, six mice from the As_2O_3 group were sacrificed on each of days 3, 5 and 7. NaCl-treated mice were concomitantly sacrificed to serve as controls ($n = 2$ on each sampling day). The mice were euthanized with Fluothane (Baxter Medical, Kista, Sweden). The thoracic cavity was opened and blood was collected from the heart using a heparinized syringe. The heart, lung, liver, pancreas, kidney and intestine were excised. Finally, the skull was opened and the brain excised. Serum was separated from whole blood by centrifugation and then stored with the organs in −20°C until trace element concentrations were analyzed.

Assessment of trace elements in tissue samples, food and drinking water

All samples were treated as described previously [19] and the content of Mg, Fe, Cu, Zn, As, Se, Cd and Hg was measured by inductively coupled plasma-mass spectrometry (Perkin-Elmer SCIEX Elan 6000; Perkin Elmer Corp., Norwalk, Connecticut, USA). Quality control was assessed with the following certified reference materials: Seronorm Trace Elements Human Whole Blood (batch MR4206; Sero AS, Billingstad, Norway), Seronorm Trace Elements Human Serum (batch JL4409; Sero AS), NIST bovine liver 1577a (National Institute of Standards and Technology, Gaithersburg, USA), BCR bovine muscle 184 and pig kidney 186 (Community bureau of reference, Brussels, Belgium) and IAEA H-4 animal muscle (International Atomic Energy Agency, Analytical Quality Control Services, Vienna, Austria). For quality control, every eighth sample was a reference material. All reference material measurements for each element were within 8% variation of the stated value and the maximum deviation of the precision was 5%. The detection limits for the measured elements were as follows: Mg, As, Se (0.2 μg/l), Cd (0.01 μg/l), Fe (0.5 μg/l), Cu (0.3 μg/l), Zn (0.9 μg/l) and Hg (0.05 μg/l).

Statistical analysis

Because the experimental design involves one control group and three treated groups, an analysis of variance (ANOVA) was applied. For the primary analysis of variance, a one-way ANOVA was performed to determine

whether there were any differences between the four independent groups. In the case of rejecting the null hypothesis in the ANOVA, Dunnett's method for multiple comparisons was adopted to establish which of the groups differed significantly. In some cases (Cd in the heart, Hg in the liver, Hg in the kidney and Mg in the brain) the assumption of homogeneity in group variances was not fulfilled. The Kruskal–Wallis, nonparametric ANOVA followed by multiple comparisons was performed in these cases.

Results

The As_2O_3-treated mice did not display any visible clinical symptoms of adverse reactions (such as reduced weight gain, inactivity and ruffled hair) because of the treatment. The exposure to As from food and water was 87 μg/kg and 0.3 μg/l, respectively. Thus, the exposure in untreated mice was low and comparable to the human situation [20].

Trace element results in serum, heart, lung, liver, pancreas, kidney, intestine and brain are shown in Tables 1–8. These data on the As concentration show that As on day 7 after initiation of the As_2O_3 treatment had reached almost steady-state levels in most of the studied organs. Moreover, the concentration of As after the As_2O_3 treatment was greatly increased ($P < 0.001$) in all organs and on all time points studied when compared with controls.

Trace elements in serum and tissues

Serum

The most pronounced effect in serum of the As_2O_3 treatment was a sequential decrease in Zn (Table 1). Zn decreased by 64% on day 7 ($P < 0.001$). In addition, the Cd concentration showed an initial decrease of 50% ($P < 0.05$) on day 3. There was also a nonsignificant decrease (36%) in Fe on day 7. Serum concentrations of Mg, Cu, Se and Hg were not affected by the As_2O_3 treatment.

Heart

As_2O_3 treatment did not significantly affect the trace element balance in the heart (Table 2). However, Hg showed an increasing trend over time with a nonsignificant increase (44%) on day 7.

Lung

In the lung there were no trace element changes, except for the supplemented As (Table 3).

Liver

All studied trace elements in the liver, except for the supplemented As, remained unaffected by the As_2O_3 treatment (Table 4).

Table 1 Concentrations (μg/l wet weight) of selected trace elements in serum of female Balb/c control mice and in As_2O_3-treated mice on days 3, 5 and 7

	Trace element concentration (μg/l wet weight) in serum			
Trace element	Controls	Day 3	Day 5	Day 7
Magnesium (Mg)	17 600 (1900)	17 800 (1100)	16 500 (700)	18 400 (1100)
Iron (Fe)	3300 (1000)	3000 (900)	3500 (700)	2100 (1100)
Copper (Cu)	365 (34)	380 (32)	345 (31)	354 (40)
Zinc (Zn)	839 (205)	667 (169)	669 (111)	299 (98)***
Arsenic (As)	1.9 (0.6)	8.0 (1.3)***	6.4 (1.7)***	9.8 (4.6)***
Selenium (Se)	247 (24)	247 (19)	222 (16)	267 (29)
Cadmium (Cd)	2.6 (1.0)	1.3 (0.3)*	1.9 (0.2)	2.0 (0.9)
Mercury (Hg)	0.5 (0.2)	0.5 (0.1)	0.5 (0.1)	0.5 (0.2)

$n = 6$ in each group.
Data expressed as mean (standard deviation).
Asterisk denotes a significant difference (*$P < 0.05$; ***$P < 0.001$) between untreated and As_2O_3-treated mice.

Table 2 Concentrations (μg/kg wet weight) of selected trace elements in the heart of female Balb/c control mice and in As_2O_3-treated mice on days 3, 5 and 7

	Trace element concentration (μg/kg wet weight) in the heart			
Trace element	Controls	Day 3	Day 5	Day 7
Magnesium (Mg)	229 000 (10 000)	226 000 (14 000)	226 000 (5000)	235 000 (10 000)
Iron (Fe)	142 000 (14 000)	145 000 (15 000)	139 000 (15 000)	144 000 (10 000)
Copper (Cu)	6800 (600)	6700 (500)	7000 (1700)	6700 (600)
Zinc (Zn)	22 300 (2700)	19 400 (1000)	20 400 (1500)	22 300 (2800)
Arsenic (As)	5.1 (1.2)	89.3 (6.5)***	124 (19)***	133 (22)***
Selenium (Se)	259 (20)	276 (51)	257 (21)	232 (21)
Cadmium (Cd)	5.1 (0.9)	4.5 (0.3)	5.1 (0.5)	4.1 (1.0)
Mercury (Hg)	1.6 (0.8)	1.9 (0.4)	2.1 (0.8)	2.3 (0.7)

$n = 6$ in each group.
Data expressed as mean (standard deviation).
Asterisk denotes a significant difference (***$P < 0.001$) between untreated and As_2O_3-treated mice.

Table 3 Concentrations (μg/kg wet weight) of selected trace elements in the lung of female Balb/c control mice and in As_2O_3-treated mice on days 3, 5 and 7

	Trace element concentration (μg/kg wet weight) in the lung			
Trace element	Controls	Day 3	Day 5	Day 7
Magnesium (Mg)	112 000 (5000)	104 000 (10 000)	113 000 (15 000)	118 000 (7000)
Iron (Fe)	132 000 (15 000)	121 000 (19 000)	131 000 (13 000)	118 000 (11 000)
Copper (Cu)	2100 (200)	2100 (300)	2000 (200)	2100 (300)
Zinc (Zn)	15 800 (1100)	15 800 (1600)	15 700 (2000)	15 500 (1700)
Arsenic (As)	4.7 (1.3)	93 (25)***	102 (12)***	124 (20)***
Selenium (Se)	1100 (200)	1200 (100)	1200 (100)	1200 (100)
Cadmium (Cd)	1.7 (0.4)	1.4 (0.3)	1.5 (0.3)	1.8 (0.3)
Mercury (Hg)	0.5 (0.1)	0.6 (0.1)	0.5 (0.1)	0.5 (0.1)

$n=6$ in each group.
Data expressed as mean (standard deviation).
Asterisk denotes a significant difference (***$P<0.001$) between untreated and As_2O_3-treated mice.

Table 4 Concentrations (μg/kg wet weight) of selected trace elements in the liver of female Balb/c control mice and in As_2O_3-treated mice on days 3, 5 and 7

	Trace element concentration (μg/kg wet weight) in the liver			
Trace element	Controls	Day 3	Day 5	Day 7
Magnesium (Mg)	245 000 (12 000)	244 000 (7000)	249 000 (7000)	250 000 (9000)
Iron (Fe)	128 000 (10 000)	127 000 (9000)	137 000 (11 000)	126 000 (6000)
Copper (Cu)	4500 (200)	4600 (100)	4500 (200)	4500 (200)
Zinc (Zn)	29 600 (1500)	30 800 (700)	30 900 (1500)	30 400 (1800)
Arsenic (As)	3.7 (0.3)	240 (20)***	296 (41)***	302 (41)***
Selenium (Se)	1400 (100)	1400 (100)	1400 (100)	1300 (100)
Cadmium (Cd)	10.6 (1.5)	9.2 (0.7)	9.6 (0.6)	10.8 (1.1)
Mercury (Hg)	0.6 (0.1)	0.5 (0.1)	0.4 (0.1)	0.4 (0.1)

$n=6$ in each group.
Data expressed as mean (standard deviation).
Asterisk denotes a significant difference (***$P<0.001$) between untreated and As_2O_3-treated mice.

Table 5 Concentrations (μg/kg wet weight) of selected trace elements in the pancreas of female Balb/c control mice and in As_2O_3-treated mice on days 3, 5 and 7

	Trace element concentration (μg/kg wet weight) in the pancreas			
Trace element	Controls	Day 3	Day 5	Day 7
Magnesium (Mg)	328 000 (12 000)	311 000 (30 000)	326 000 (20 000)	330 000 (9000)
Iron (Fe)	54 500 (2400)	50 400 (5100)	56 800 (9600)	53 100 (4600)
Copper (Cu)	1600 (100)	1900 (400)	1700 (400)	1600 (100)
Zinc (Zn)	32 800 (800)	37 500 (5100)	34 000 (3300)	38 400 (5800)
Arsenic (As)	3.0 (1.7)	63.0 (11.6)***	81.9 (14.6)***	84.1 (16.3)***
Selenium (Se)	461 (13)	421 (34)*	449 (19)	469 (20)
Cadmium (Cd)	5.1 (0.9)	4.2 (0.4)	5.8 (0.5)	4.7 (0.4)
Mercury (Hg)	0.4 (0.2)	0.5 (0.2)	0.7 (0.2)	0.3 (0.1)

$n=6$ in each group.
Data expressed as mean (standard deviation).
Asterisk denotes a significant difference (*$P<0.05$; ***$P<0.001$) between untreated and As_2O_3-treated mice.

Table 6 Concentrations (μg/kg wet weight) of selected trace elements in the kidney of female Balb/c control mice and in As_2O_3-treated mice on days 3, 5 and 7

	Trace element concentration (μg/kg wet weight) in the kidney			
Trace element	Controls	Day 3	Day 5	Day 7
Magnesium (Mg)	181 000 (16 000)	188 000 (10 000)	203 000 (9000)*	172 000 (17 000)
Iron (Fe)	93 000 (17 000)	81 000 (9000)	95 000 (10 000)	84 000 (10 000)
Copper (Cu)	3700 (300)	3800 (200)	4100 (200)	3500 (300)
Zinc (Zn)	16 900 (1400)	17 600 (800)	18 500 (800)	16 100 (1700)
Arsenic (As)	6.3 (1.2)	130 (12)***	160 (33)***	176 (35)***
Selenium (Se)	1800 (100)	1700 (100)	1800 (100)	1900 (100)
Cadmium (Cd)	19.3 (4.2)	16.9 (2.4)	16.9 (1.7)	18.1 (1.1)
Mercury (Hg)	0.7 (0.1)	0.6 (0.1)	0.5 (0.1)	0.6 (0.1)

$n=6$ in each group.
Data expressed as mean (standard deviation).
Asterisk denotes a significant difference (*$P<0.05$; ***$P<0.001$) between untreated and As_2O_3-treated mice.

Table 7 Concentrations (μg/kg wet weight) of selected trace elements in the intestine of female Balb/c control mice and in As_2O_3-treated mice on days 3, 5 and 7

	Trace element concentration (μg/kg wet weight) in the intestine			
Trace element	Controls	Day 3	Day 5	Day 7
Magnesium (Mg)	108 000 (9000)	103 000 (9000)	111 000 (3400)	109 000 (5200)
Iron (Fe)	16 300 (2100)	17 300 (2300)	15 400 (2000)	14 400 (1900)
Copper (Cu)	1200 (200)	1100 (200)	1200 (100)	1100 (100)
Zinc (Zn)	13 900 (1900)	14 500 (1800)	12 600 (600)	12 300 (1100)
Arsenic (As)	6.3 (1.7)	82 (16)***	101 (15)***	105 (27)***
Selenium (Se)	236 (21)	166 (26)***	237 (14)	220 (21)
Cadmium (Cd)	8.9 (2.2)	9.5 (1.8)	8.0 (1.1)	6.2 (1.7)*
Mercury (Hg)	0.9 (0.2)	0.7 (0.2)*	0.8 (0.1)	0.9 (0.2)

$n=6$ in each group.
Data expressed as mean (standard deviation).
Asterisk denotes a significant difference (*$P<0.05$; ***$P<0.001$) between untreated and As_2O_3-treated mice.

Table 8 Concentrations (μg/kg wet weight) of selected trace elements in the brain of female Balb/c control mice and in As_2O_3-treated mice on days 3, 5 and 7

	Trace element concentration (μg/kg wet weight) in the brain			
Trace element	Controls	Day 3	Day 5	Day 7
Magnesium (Mg)	159 000 (4000)	150 000 (5000)	161 000 (7000)	162 000 (2000)
Iron (Fe)	23 900 (2300)	20 900 (800)	22 400 (1500)	23 100 (4300)
Copper (Cu)	4400 (200)	4200 (600)	4200 (100)	4300 (200)
Zinc (Zn)	18 200 (1900)	18 600 (2500)	18 600 (1400)	17 200 (400)
Arsenic (As)	1.5 (0.6)	14.0 (1.6)***	17.6 (2.5)***	21.1 (3.3)***
Selenium (Se)	247 (33)	224 (12)	218 (10)*	211 (8)**
Cadmium (Cd)	3.0 (1.3)	3.4 (0.9)	2.9 (0.5)	2.9 (0.5)
Mercury (Hg)	0.6 (0.1)	0.6 (0.1)	0.6 (0.1)	0.5 (0.1)

$n=6$ in each group.
Data expressed as mean (standard deviation).
Asterisk denotes a significant difference (*$P<0.05$; **$P<0.01$; ***$P<0.001$) between nontreated and As_2O_3-treated mice.

Pancreas

As_2O_3 treatment decreased the Se concentration in the pancreas on day 3 by 9% ($P < 0.05$) (Table 5), whereas Hg tended to increase on day 5 (75%; NS). The concentration of all other studied trace elements, except for the supplemented As, remained unchanged.

Kidney

As_2O_3 treatment increased the Mg concentration in the kidney on day 5 by 12% ($P < 0.05$) (Table 6). All other studied trace elements remained unchanged in the As-treated mice, except for the supplemented As.

Intestine

In the intestine, the As_2O_3 treatment resulted in a decrease by 30% of both Se on day 3 ($P < 0.001$) and Cd on day 7 ($P < 0.05$), as well as a decrease in Hg by 22% ($P < 0.05$) on day 3 (Table 7). All other studied trace elements, except for the supplemented As, remained unchanged.

Brain

In the brain, the daily As_2O_3 treatment resulted in a decrease by time in Se, that is, Se decreased by 9% (NS), 12% ($P < 0.05$) and 15% ($P < 0.01$) on days 3, 5 and 7, respectively (Table 8). Except for the supplemented As, all other studied trace elements remained unaffected by the As_2O_3 treatment.

Discussion

The As_2O_3 dose and treatment regimen used in this experimental study were comparable to the use of As_2O_3 in the clinical setting. The mice showed no visible symptoms of adverse reactions because of the treatment. The major changes in trace element balance after As_2O_3 treatment were a sequential decrease in serum Zn and a decrease in Se in the pancreas, the intestine and the brain. Changes in Mg, Fe, Cu, Cd and Hg in investigated tissues were minor and not consistent.

The normal As levels in serum of untreated patients is < 2 μg/l, whereas the mean plasma As level in patients treated with daily doses of 50 mg/kg bw of As_4S_4 was found to be 8.6 ± 6.9 μg/l on day 5 of the treatment schedule [21]. Thus, clinical data on serum As levels correlate well with serum As levels in this study, that is, 1.9 ± 0.6 μg/l in untreated mice and 6.4 ± 1.7 μg/l in the As_2O_3-treated group after 5 days of treatment.

In humans, As is readily absorbed from the gastrointestinal tract and distributed to a number of organs, such as the liver [5], where most of its biotransformation occurs [22]. The most severe side effects of As treatment in the clinical setting seem to be a prolonged QT time of the electrocardiogram and the APL differentiation syndrome [23]. However, these adverse effects are often reversible

and rarely require discontinuation of the As_2O_3 treatment [23]. To minimize the risk of a prolonged QT interval during Trisenox treatment, it is recommended that the serum levels of Mg be carefully monitored because low serum levels of this element seem to have the potential to prolong the QT time [24]. In this study, the As_2O_3 treatment increased Mg in the kidney, whereas no effects on Mg levels were observed in serum or the heart. Thus, it seems that retention and homeostasis of Mg are not affected by the As_2O_3 treatment, at least not in healthy individuals.

It seems that the toxic/therapeutic effects of As are mediated, at least in part, by redox-sensitive proteins and enzymes [25]. An analog to α-tocopherol, the essential antioxidant component of vitamin E, was recently shown to increase the antilymphoma effects of As_2O_3 and at the same time reduce As-induced liver toxicity [26]. As generates reactive oxygen species (ROS) and free radicals, and it has been proposed that these reactive species are responsible for the stress response elicited by arsenicals [18]. ROS signaling is critical for the responses of cytokines, growth factors and activation or inactivation of transcription factors [18]. ROS induced by low levels of As can increase the transcription of NF-κB, whereas high concentrations of As inhibit the activation of NF-κB and cell proliferation, as well as cause apoptosis [18] and disturbed macrophage function [27]. Thus, both development and healing of inflammatory lesions in tissues seem to be affected by As.

The observed decrease in Se in the pancreas, intestine and brain is noteworthy in the sense that Se is an essential trace element with antioxidative properties and anticarcinogenic potential, affecting cell cycle arrest and apoptosis [28]. Evidence from other studies strongly suggests that there are biochemical interactions between As and Se in blood and the liver [4]. An in-vivo formation of metabolites with As–Se implies that ingested As targets the metabolism of Se [4]. A decreased concentration of Se in the liver and blood, but an increase in the excreted feces has been reported after simultaneous As and Se supplementation [14,29]. Thus, one possible explanation for the Se-detoxifying effect of As is the formation of an As–Se compound excreted in the bile [17]. Moreover, it seems that a chronic low-level exposure of humans to As renders part of the ingested dietary Se unavailable for tissue uptake, that is, induced Se deficiency in internal organs [4]. In this study, the As supplementation at normal physiological concentrations of Se caused a decrease of Se in the pancreas, intestine and brain, but only minor effects in the other studied organs. Such a reduction in Se during the therapeutic use of As might affect the outcome of the disease under treatment. Thus, the possible benefit of Se supplementation during concomitant therapeutic intervention with As is an intriguing question, that is, a simultaneous supplementation of Se during As_2O_3 treatment might lower the dose of As through As-detoxifying mechanisms of Se, whereas an uncontrolled reduction in Se because of the As treatment may worsen the prognosis of the disease.

Another trace element closely linked to immune integrity is Zn [30], which is intimately involved in the regulation of immune function and is crucial for optimal T-cell functioning [31]. Arsenic has been shown to induce hepatic Zn-Thionein, an event that may affect the body Zn homeostasis [32]. Moreover, Zn supplementation in mice induces MT and protects from As-induced lethality [33]. Zn in serum in this study was greatly decreased after 7 days of daily As administration in the phase of concomitantly high levels of As. Whether these changes in Se and Zn, and other relatively minor changes in trace element concentrations during As_2O_3 treatment, are also similar in individuals suffering from a concurrent disease requires further study.

In conclusion, a clinically relevant dose of As_2O_3 caused a sequential decrease in serum Zn and a decrease of Se in the pancreas, intestine and brain. These changes may affect antioxidative and immune functions when As is used in the treatment of diseases where these arms of host defence are required. It seems important to maintain the patient on an adequate Se and Zn status, both before initiating the As treatment and at specific time points during the course of more long-lasting treatment regimens. Thus, a concomitant supplementation with other trace elements during the clinical use of As_2O_3 may be beneficial. Whether the observed changes in trace elements also influence pathophysiological effects during the clinical use of As_2O_3 requires further study.

Acknowledgements

The authors thank Erland Johansson for constructive criticism of the paper. This study was supported by grants from The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS).

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