Arsenic Trioxide Affects the Trace Element Balance in Tissues in Infected and Healthy Mice Differently

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Abstract. *Background: Acquired infections are common in cancer patients. As_2O_3 treatment and infections affect the body's trace element balance. However, it is unknown whether concomitant infections cause adverse element interactions that endanger the safety and therapeutic effect of As_2O_3. Materials and Methods: Coxsackievirus B3-infected mice were treated with 1.0 mg As_2O_3/kg bw for 3, 5 or 7 days. Arsenic, magnesium, iron, copper, zinc and selenium were measured (ICP-MS) in serum, heart, lung, liver, pancreas, kidney, intestine and brain. Virus in serum was followed by RT-PCR. Results: The infection increased As in all organs except the intestine, whereas selenium concentration decreased in all organs except the heart and brain. The infection markedly reduced magnesium in the heart. Conclusion: As_2O_3 treatment results in pronounced differences in trace elements between healthy and infected individuals. This finding is important to consider, regarding treatment safety and efficacy, when As_2O_3 therapy is used in the clinical setting.*

Cancer disease and associated host defence reactions greatly influence metabolism, probably affecting the body's trace element balance. In addition to the cancer-induced adverse effects on metabolism, it is important in the clinical setting to consider the high risk for community-acquired infections in immunocompromised leukaemia patients. Patients with acute leukaemia are not only prone to contracting bacterial and fungal infections because of prolonged neutropenia secondary to marrow infiltration (1), but viral infections, such as influenza and picorna viruses (*e.g.* the studied coxsackievirus B3 infection), are also common in immunocompromised leukaemia patients (2). Moreover, reactivation of varicella zoster infection has sporadically been reported during As_2O_3 treatment regimens in leukaemia patients (3, 4).

Enteroviruses (picorna virus family) are common human infections that, in most cases, pass unrecognised or cause only minor illness of the upper respiratory or gastrointestinal tract. However, these viruses have been estimated to cause more than 10 million symptomatic infections each year in the USA alone, and coxsackievirus B3 (CVB3) is one of the most common viral species (5). The murine infectious model of CVB3 shows a disease development similar to that in humans, where target organs (*e.g.* the pancreas, heart and brain) are infected and associated inflammatory lesions are induced by mobilised immune cells (6, 7).

Trace elements are essential for most physiological processes, including the function of the immune system (8) and the development and healing of inflammatory tissue lesions (9). For example, selenium (Se) has been shown to have a positive effect on the outcome of cancer (10) and infectious diseases (11), whereas a deficiency in zinc (Zn) may negatively affect the immune system and cell growth (8). In addition, arsenic (As) has, despite its toxic potential, been hypothesised to be an essential element with a physiological role (12). Arsenic has historically been used as a therapeutic drug in the treatment of various diseases, including psoriasis, syphilis and breast cancer. Today, arsenic trioxide (As_2O_3) is used as an effective and safe drug in acute promyelocytic leukaemia (APL) and clinical trials on the effect of As_2O_3 in several other types of cancer (*e.g.* myeloma, lymphoma) are underway (13).

Interestingly, serum levels of Se and Zn, two of the trace elements important for immune function, were found to be lower in leukaemia patients than in healthy controls, which may partly be explained by malnutrition and the occurrence of infections in these patients (14). During all infections, when host defence reactions are initiated, the metabolism of trace elements in the body is changed (15). Changes in essential elements (iron (Fe), copper (Cu) and Zn are often referred to

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as part of the acute-phase reaction, indicating that these elements are involved in the host resistance to infection (9, 15). A changed balance of trace elements has been shown to occur in CVB3 and in bacterial *Chlamidophyla pneumoniae* infections (16, 17). In addition, an infection *per se* (including CVB3) can affect the gastrointestinal uptake of both essential and non-essential trace elements (18), which potentially could affect the therapeutic effect of As_2O_3. Because Se and Zn were found to decrease in tissues during As_2O_3 treatment in healthy mice (19), it is tempting to suggest that As_2O_3 treatment affects trace elements differently in health and disease, and that a concomitant infection may cause adverse effects or even jeopardise the efficacy of the therapy.

One of the most severe side-effects of As_2O_3 treatment in cancer patients is a prolonged QT interval (a measure of the time between the start of the Q wave and the end of the T wave) on the electrocardiogram (ECG). The risk for this prolongation appears to be closely related to decreasing blood concentrations of the electrolyte magnesium (Mg) (20). In healthy mice, it has been shown that As_2O_3 treatment does not influence Mg, neither in serum nor in the heart (19). Mg also remained unaffected in serum during viral and bacterial infection (16, 21). Whether this is also valid in As_2O_3-treated and concomitantly infected individuals remains unknown.

Research has shown that As_2O_3 treatment and infections can affect the trace element balance in the body. However, it is not known whether trace element changes during As_2O_3 therapy are influenced by a concomitant immune activation and/or infection, *i.e.* whether responses to the As_2O_3 therapy are different in healthy individuals as compared with infected ones. The aim of this study was therefore to study the effects of supplementation of a clinically relevant dose of As_2O_3 on essential trace elements (Cu, Fe, Mg, Se and Zn) in serum, heart, lung, liver, pancreas, kidney, intestine and brain in CVB3-infected and non-infected 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 weight (19.7±0.7g) and housed at 23±1˚C (relative humidity 50±2%) on a 12-h light/dark cycle behind hygienic barriers (TouchSLIMLine, Tecniplast, Scanbur BK A/S, Lellinge, Denmark). Water and regular chow diet (Labfor R36; Lantmännen, Sweden) were supplied *ad libitum*. Food and water were analysed for As content. Infected and non-infected 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.

Virus. A myocarditic strain of CVB3 was used (11, 22). The virus was propagated as described elsewhere (17) and a stock solution of 10^7-10^8 plaque-forming units (pfu)/ml was diluted with sterile saline (NaCl) to obtain 1×10^4 pfu/ml.

Infection and experimental design. The study included two groups of mice (CVB3-infected and non-infected mice) treated daily with As_2O_3 for 3, 5 or 7 days. On day 0 of the infection, mice were inoculated intraperitoneally (*i.p.*) with 0.2 ml containing approximately $2x10^3$ pfu of CVB3 virus, or were sham-inoculated with the same volume of NaCl. At the same time, the mice received their first dose of As_2O_3, *i.e.* approximately 1 mg As_2O_3 /kg bw in 0.2 ml NaCl *i.p.* The mice were then treated once daily with As_2O_3 throughout the 7 days of the study.

Tissue sampling. Infected mice (n=6 per group) were sacrificed on each of days 3, 5 and 7 of the infection. Sham-inoculated mice were concomitantly sacrificed (n=2 at each time point) to serve as healthy controls. The mice were euthanised with Fluothane (Baxter Medical, Kista, Sweden). The thoracic cavity was opened and blood was collected from the heart using a heparinised syringe. The heart, lung, liver, pancreas, kidney, spleen 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 at –20˚C until measurement of viral titres and trace element concentration.

Detection of CVB3 RNA in serum. Total nucleic acid from serum was isolated using the NucliSens® easyMAG (Biomérieux, Boxtel, the Netherlands). Viral equivalents were quantified with real-time RT-PCR, targeting a conserved region of the 5'UTR of the enterovirus genome, as described (23) and modified (24) elsewhere. The reaction was further adjusted by using 2.5U of r*Tth* (instead of 1.5U) and by running the reaction in a total volume of 25 μl (instead of 50 μl). To avoid possible PCR inhibitors in serum, all samples were run undiluted and diluted 1:10 in DEPC-treated H_2O. Negative and positive PCR controls were included in each PCR run. CVB3 genome equivalents were estimated by interpolation in a standard curve obtained using a dilution series of a CVB3 cDNA-containing plasmid covering the amplified region. The number of genome equivalents is related to the number of viral particles. The concentration per μl serum is obtained by multiplying the number of genome equivalents by a factor derived from the measured RNA yield, volume of serum processed and the amount of RNA extract used in the PCR.

Assessment of trace element levels in serum and tissues. To determine the elements Mg, Fe, Cu, Zn, As and Se in serum, heart, lung, liver, pancreas, kidney, intestine and brain, the samples were treated as described elsewhere (17). The element content was determined by inductively coupled plasma-mass spectrometry (ICP-MS; Perkin-Elmer SCIEX ELAN 6000, Perkin Elmer Corp., Norwalk, CT, USA). For quality control, every eighth sample was checked against certified reference materials: Seronorm Trace Elements Human Whole Blood (batch MR4206; Sero AS), Seronorm Trace Elements Human Serum (batch JL4409; Sero AS, Billingstad, Norway), NIST bovine liver 1577a (National Institute of Standards and Technology, Gaithersburg, USA), BCR bovine muscle 184 (Community Bureau of Reference, Brussels, Belgium), or IAEA H-4 animal muscle (International Atomic Energy Agency, Analytical

Quality Control Services, Vienna, Austria). All reference material measurements for each element were within a maximum deviation of 8% from the stated value and the maximum deviation of the precision was 5%. The detection limits for the measured elements were as follows: 0.2 μg/l Mg, As, Se; 0.5 μg/l Fe; 0.3 μg/l Cu; and 0.9 μg/l Zn.

Statistical analysis. Trace element results in the different tissues of the infected and non-infected groups were compared by using the Mann-Whitney *U*-test. The number of CVB3 copies per μl serum was also compared among days 3, 5 and 7 with a non-parametric ANOVA (Kruskal-Wallis) followed by multiple comparisons.

Results

Clinical signs of infection, such as ruffled hair and inactivity, started to appear from day 2 of the infection. By day 3, all infected mice had developed clinical signs of disease. All infected mice were found positive for viral RNA in serum, where viral titres gradually decreased until day 7 of infection (Table I). No visible signs of adverse reactions because of the As_2O_3 treatment were observed; exposure from food and water was low and negligible (19).

Trace elements in serum (Table II). Concentrations of all trace elements in serum in the two groups were affected differently by As_2O_3 treatment. Infected mice, in comparison with non-infected mice, had an increase in Mg on days 3 (8%; $p<0.05$) and 5 (41%; $p<0.01$); an increase in Cu on days 3 (54%; $p<0.01$), 5 (99%; $p<0.01$) and 7 (85%; $p<0.01$); and finally, an increase in As on days 3 (78%; $p<0.01$) and 5 (100%; $p<0.01$). Other major differences between the two groups were increases in Fe (93%; $p<0.05$) and Zn (122%; $p<0.01$) on day 3 and a decrease in Se on days 3 (14%; $p<0.01$) and 5 (16%; $p<0.01$) in the infected group.

Trace elements in the heart (Table III). In CVB3-infected mice, the As_2O_3 treatment resulted in a decrease in Mg (11%; $p<0.01$) on day 7, an increase in Zn on days 3 (10%; $p<0.01$) and 5 (63%; $p<0.01$), and an increase in As on days 3 (18%; $p<0.05$) and 7 (50%; $p<0.01$).

Trace elements in the lung (Table IV). In CVB3-infected mice, the As_2O_3 treatment resulted in an increase in Mg (14%; $p<0.05$) on day 3; in Cu on days 3 (24%; $p<0.05$) and 5 (15%; $p<0.01$); in Zn (21%; $p<0.01$) on day 3; and in As on days 3 (35%; $p<0.05$), 5 (57%; $p<0.01$) and 7 (49%; $p<0.01$). On the contrary, there was an infection-induced decrease in Se (17%) on days 3 ($p<0.05$) and 7 ($p<0.01$).

Trace elements in the liver (Table V). In CVB3-infected mice, the As_2O_3 treatment led to an increase in Fe on days 3 (17%; $p<0.05$) and 7 (81%; $p<0.01$); and a decrease in Se on days 3 (21%; $p<0.01$), 5 (29%; $p<0.01$) and 7 (15%; $p<0.01$). Moreover, infected mice showed a decrease in Mg on days 3 (20%; $p<0.01$) and 5 (12%; $p<0.01$), but an increase in Zn (50%; $p<0.01$) and As (37%; $p<0.01$) on day 7.

Table I. *Number of Coxsackievirus B3 (CVB3) copies per μl serum x1000 on days 3, 5 and 7 after infection.*

Organ	Day 3	Day 5	Day 7
Serum	280 (210)	39 (61)	0.01 (0.01)**

n=6 in each group. Data are expressed as mean and standard deviation. Asterisks denote a significant difference in viral load compared with day 3 of the infection (**$p<0.01$).

Trace elements in the pancreas (Table VI). The pancreas was the organ with the most pronounced differences between the groups. In CVB3-infected mice, the As_2O_3 treatment resulted in an increase in Fe (71%; $p<0.01$) on day 7 and in As on days 3 (551%; $p<0.01$), 5 (269%; $p<0.01$) and 7 (61%; $p<0.01$). A concomitant infection-induced decrease was found in Mg on days 3 (82%; $p<0.01$) and 5 (74%; $p<0.01$); in Cu on day 7 (37%; $p<0.01$); in Zn on days 3 (63%; $p<0.01$), 5 (56%; $p<0.01$) and 7 (48%; $p<0.01$); and in Se on day 5 (9%; $p<0.05$).

Especially noteworthy was the finding that the As concentration in infected mice peaked on day 3 and thereafter gradually declined, whereas in non-infected mice the lowest As concentration was found on day 3 followed by a gradual increase until day 7.

Trace elements in the kidney (Table VII). In CVB3-infected mice, the As_2O_3 treatment resulted in an increase in Zn on days 5 (157%; $p<0.05$) and 7 (15%; $p<0.05$) and in As on day 7 (27%; $p<0.05$). Other major differences between the two groups were infection-induced decreases in Mg on days 3 (12%; $p<0.05$) and 5 (11%; $p<0.01$); in Fe on day 7 (14%; $p<0.05$); and in Se (11%; $p<0.01$) on days 5 and 7.

Trace elements in the intestine (Table VIII). All trace elements in the intestine decreased in the infected group except for Zn, which increased by 13% ($p<0.05$) on day 7 of the As_2O_3 treatment. The most prominent infection-induced decrease was found in Cu on days 3 (27%; $p<0.05$) and 5 (50%; $p<0.01$); in As on day 5 (32%; $p<0.05$); and in Se on day 5 (37%; $p<0.01$). Furthermore, decreases were observed in Mg on day 5 (14%; $p<0.01$) and in Fe on day 3 (22%; $p<0.05$).

Trace elements in the brain (Table IX). The As_2O_3 treatment in CVB3-infected mice resulted in an increase in Mg on day 3 (5%; $p<0.05$); in Fe on day 3 (11%; $p<0.01$); in Zn on day 7 (7%; $p<0.01$); and in As on day 7 (73%; $p<0.01$).

Table II. *Concentrations (μg/l wet weight) of selected elements in serum on days 3, 5 and 7.*

Element	Element concentration (μg/l wet weight) in serum					
	Day 3		Day 5		Day 7	
	As	As+inf	As	As+inf	As	As+inf
Magnesium (Mg)	17800 (1100)	19200 (900)*	16500 (700)	23200 (2700)**	18400 (1100)	20700 (2400)
Iron (Fe)	3000 (900)	5800 (2400)*	3500 (700)	4800 (2700)	2100 (1100)	2700 (900)
Copper (Cu)	380 (32)	585 (67) **	345 (31)	685 (130)**	354 (40)	654 (131)**
Zinc (Zn)	667 (169)	1480 (274)**	669 (111)	802 (222)	299 (98)	399 (145)
Arsenic (As)	8.0 (1.3)	14.2 (5.4)**	6.4 (1.7)	12.8 (2.9)**	9.8 (4.6)	13.3 (1.9)
Selenium (Se)	247 (19)	213 (15) **	222 (16)	187 (19)**	267 (29)	247 (29)

n=6 in each group. Data expressed as mean (standard deviation). Asterisks denote significant differences between the two study groups at each time point (*p<0.05, **p<0.01).

Table III. *Concentrations (μg/kg wet weight) of selected elements in the heart on days 3, 5 and 7.*

Element	Element concentration (μg/kg wet weight) in the heart					
	Day 3		Day 5		Day 7	
	As	As+inf	As	As+inf	As	As+inf
Magnesium (Mg)	226000 (14000)	227000 (5000)	226000 (5000)	207000 (24000)	235000 (10000)	208000 (9000)**
Iron (Fe)	145000 (15000)	135000 (7000)	139000 (15000)	139000 (16000)	144000 (10000)	132000 (11000)
Copper (Cu)	6700 (500)	7100 (600)	7000 (1700)	6900 (400)	6700 (600)	6200 (500)
Zinc (Zn)	19400 (1000)	21400 (900)**	20400 (1500)	33200 (3500)**	22300 (2800)	24500 (3600)
Arsenic (As)	89.3 (6.5)	105 (17)*	124 (19)	165 (47)	133 (22)	199 (17)**
Selenium (Se)	276 (51)	246 (11)	257 (21)	242 (18)	232 (21)	243 (17)

n=6 in each group. Data expressed as mean (standard deviation). Asterisks denote significant differences between the two study groups at each time point (*p<0.05, **p<0.01).

Table IV. *Concentrations (μg/kg wet weight) of selected elements in the lung on days 3, 5 and 7.*

Element	Element concentration (μg/kg wet weight) in the lung					
	Day 3		Day 5		Day 7	
	As	As+inf	As	As+inf	As	As+inf
Magnesium (Mg)	104000 (10000)	119000 (5000)*	113000 (15000)	118000 (7000)	118000 (7000)	116000 (5000)
Iron (Fe)	121000 (19000)	125000 (12000)	131000 (13000)	125000 (15000)	118000 (11000)	136000 (12000)
Copper (Cu)	2100 (300)	2600 (100)*	2000 (200)	2300 (100)**	2100 (300)	2100 (200)
Zinc (Zn)	15800 (1600)	19100 (800)**	15700 (2000)	17100 (400)	15500 (1700)	14600 (1000)
Arsenic (As)	93 (25)	126 (30)*	102 (12)	160 (33)**	124 (20)	185 (20)**
Selenium (Se)	1 200 (100)	1000 (100)*	1200 (100)	1100 (200)	1200 (100)	1000 (100)**

n=6 in each group. Data expressed as mean (standard deviation). Asterisks denote significant differences between the two study groups at each time point (*p<0.05, **p<0.01).

Discussion

The influence of As_2O_3 treatment on the trace element balance in healthy mice has recently been described (19). However, the pattern of changes in trace elements that is caused by As_2O_3 treatment was different when mice concomitantly harboured a common human viral (CVB3) infection. Throughout the seven days of As_2O_3 treatment, the CVB3-infected mice, in comparison with the non-infected mice, showed increased concentrations of As in all studied organs except the intestine.

Table V. *Concentrations (μg/kg wet weight) of selected elements in the liver on days 3, 5 and 7.*

Element	Element concentration (μg/kg wet weight) in the liver					
	Day 3		Day 5		Day 7	
	As	As+inf	As	As+inf	As	As+inf
Magnesium (Mg)	244000 (7000)	196000 (22000)**	249000 (7000)	219000 (16000)**	250000 (9000)	261000 (11000)
Iron (Fe)	127000 (9000)	149000 (21000)*	137000 (11000)	154000 (22000)	126000 (6000)	228000 (21000)**
Copper (Cu)	4600 (100)	4400 (400)	4500 (200)	4000 (700)	4500 (200)	4500 (200)
Zinc (Zn)	30800 (700)	38400 (6400)	30900 (1500)	35900 (7400)	30400 (1800)	45600 (15000)**
Arsenic (As)	240 (20)	248 (27)	296 (41)	316 (53)	302 (41)	414 (38)**
Selenium (Se)	1400 (100)	1100 (100)**	1 400 (100)	1000 (200)**	1300 (100)	1100 (100)**

n=6 in each group. Data expressed as mean (standard deviation). Asterisks denote significant differences between the two study groups at each time point (*p<0.05, **p<0.01).

Table VI. *Concentrations (μg/kg wet weight) of selected elements in the pancreas on days 3, 5 and 7.*

Element	Element concentration (μg/kg wet weight) in the pancreas					
	Day 3		Day 5		Day 7	
	As	As+inf	As	As+inf	As	As+inf
Magnesium (Mg)	311000 (30000)	57400 (4600)**	326000 (20000)	85400 (31400)**	330000 (9000)	189000 (80000)
Iron (Fe)	50400 (5100)	47100 (10000)	56800 (9600)	55000 (11200)	53100 (4600)	90700 (18100)**
Copper (Cu)	1900 (400)	2000 (200)	1700 (400)	1900 (100)	1600 (100)	1000 (100)**
Zinc (Zn)	37500 (5100)	13800 (300)**	34000 (3300)	15100 (4300)**	38400 (5800)	20100 (7300)**
Arsenic (As)	63.0 (11.6)	410 (161)**	81.9 (14.6)	302 (84)**	84.1 (16.3)	135 (19)**
Selenium (Se)	421 (34)	400 (21)	449 (19)	409 (18)*	469 (20)	495 (47)

n=6 in each group. Data expressed as mean (standard deviation). Asterisks denote significant differences between the two study groups at each time point (*p<0.05, **p<0.01).

Table VII. *Concentrations (μg/kg wet weight) of selected elements in the kidney on days 3, 5 and 7.*

Element	Element concentration (μg/kg wet weight) in the kidney					
	Day 3		Day 5		Day 7	
	As	As+inf	As	As+inf	As	As+inf
Magnesium (Mg)	188000 (10000)	165000 (17000)*	203000 (9000)	180000 (9000)**	172000 (17000)	170000 (8000)
Iron (Fe)	81000 (9000)	78000 (14000)	95000 (10000)	87000 (19000)	84000 (10000)	72000 (5000)*
Copper (Cu)	3800 (200)	4000 (300)	4100 (200)	3700 (400)	3500 (300)	3100 (100)
Zinc (Zn)	17600 (800)	18100 (1600)	18500 (800)	47500 (20700)*	16100 (1700)	18500 (700)*
Arsenic (As)	130 (12)	131 (24)	160 (33)	168 (51)	176 (35)	223 (24)*
Selenium (Se)	1700 (100)	1600 (100)	1800 (100)	1600 (100)**	1900 (100)	1700 (100)**

n=6 in each group. Data expressed as mean (standard deviation). Asterisks denote significant differences between the two study groups at each time point (*p<0.05, **p<0.01).

Concomitant to the increase in As, Se decreased in all organs except the heart and brain. Furthermore, the infection caused markedly increased Zn concentrations in all organs except the pancreas. Despite an increase of Mg in serum, concentrations in the heart, liver, pancreas, kidney and intestine decreased in the infected group. Consequently, treatment with As_2O_3 results in a completely different trace element balance in health compared with disease.

During the As_2O_3 treatment period, Se decreased in both non-infected and infected mice. This element has previously

Table VIII. *Concentrations (μg/kg wet weight) of selected elements in the intestine on days 3, 5 and 7.*

Element	Element concentration (μg/kg wet weight) in the intestine					
	Day 3		Day 5		Day 7	
	As	As+inf	As	As+inf	As	As+inf
Magnesium (Mg)	103000 (9000)	107000 (11000)	111000 (3400)	95000 (9700)**	109000 (5200)	110000 (4400)
Iron (Fe)	17300 (2300)	13500 (2500)*	15400 (2000)	13000 (1900)	14400 (1900)	14000 (1500)
Copper (Cu)	1100 (200)	800 (100)*	1200 (100)	600 (100)**	1100 (100)	1000 (100)
Zinc (Zn)	14500 (1800)	13400 (1900)	12600 (600)	12500 (1500)	12300 (1100)	13900 (1100)*
Arsenic (As)	81.6 (15.9)	63.7 (12.0)	101 (15)	68.7 (23.3)*	105 (27)	99.2 (8.3)
Selenium (Se)	166 (26)	142 (13)	237 (14)	150 (17)**	220 (21)	223 (25)

n=6 in each group. Data expressed as mean (standard deviation). Asterisks denote significant differences between the two study groups at each time point (*p<0.05, **p<0.01).

Table IX. *Concentrations (μg/kg wet weight) of selected elements in the brain on days 3, 5 and 7.*

Element	Element concentration (μg/kg wet weight) in the brain					
	Day 3		Day 5		Day 7	
	As	As+inf	As	As+inf	As	As+inf
Magnesium (Mg)	150000 (5000)	157000 (3000)*	161000 (7000)	159000 (2000)	162000 (2000)	165000 (4000)
Iron (Fe)	20900 (800)	23200 (1400)**	22400 (1500)	24600 (1700)	23100 (4300)	25400 (2600)
Copper (Cu)	4200 (600)	4400 (100)	4200 (100)	4300 (300)	4300 (200)	4500 (200)
Zinc (Zn)	18600 (2500)	18800 (1100)	18600 (1400)	18600 (300)	17200 (400)	18400 (600)**
Arsenic (As)	14.0 (1.6)	18.8 (5.0)	17.6 (2.5)	31.0 (16.0)	21.1 (3.3)	36.4 (2.9)**
Selenium (Se)	224 (12)	237 (32)	218 (10)	240 (30)	211 (8)	228 (21)

n=6 in each group. Data expressed as mean (standard deviation). Asterisks denote significant differences between the two study groups at each time point (*p<0.05, **p<0.01).

been found to decrease during As_2O_3 treatment (19) and viral infection (25). However, in the present study the decrease in Se was more pronounced in infected than in non-infected mice. This is important to consider in the clinical setting where there is an increased risk for community-acquired infections in immunocompromised leukaemia patients. Adverse additive effects of As_2O_3 treatment and infections on the Se status emphasise the importance of careful monitoring and, if needed, supplementation of Se during cancer therapy. This may otherwise jeopardise the outcome of the As_2O_3 treatment in the patient. Interestingly, protective effects of Se on prostate cancer have been found to be more pronounced for advanced cancer, indicative of an effect of Se on cancer progression rather than cancer initiation (26). This observation further illuminates the importance of a careful management of trace element balance in cancer patients, particularly in cancer cases treated with drugs known to affect the balance of Se and/or other essential trace elements, especially in patients suffering from concomitant infections and/or inflammatory diseases.

Infections and immune activation are also known to change the balance of Zn (15, 27). During generalised infections, serum Zn decreases (9) and Zn deficiency is known to negatively influence the function of certain arms of the immune system (28). However, in the present study markedly increased Zn concentrations were found in all organs except the pancreas in CVB3-infected As_2O_3-treated mice. This finding is in contrast to As_2O_3 treatment in healthy mice, where serum Zn during As_2O_3 treatment markedly decreased over time (19), possibly because of an arsenic-induced increase in the metal-binding protein metallothionein (MT) (29). Consequently, depending on the health status of the individual, Zn, similarly to Se, seems to be differently affected by As_2O_3 treatment.

It has recently been reported that As_2O_3 treatment in healthy individuals did not affect Mg in serum or the heart (19). However, in the present study, the infection reduced Mg in the heart but caused a simultaneous increase of the same element in the serum. Low serum levels of Mg have

been associated with a prolongation of the QT time on the electrocardiogram (20). It has also been reported that, under certain conditions, As_2O_3 treatment can induce this kind of adverse cardiovascular effect (30). Thus, an increase in serum Mg during As_2O_3 treatment and concomitant infection might hide an actual decrease of this element in vital organs and result in an increased risk of complications. The presence of an electrolyte abnormality during As_2O_3 treatment was believed to cause one case of sudden death, which highlights the importance of a careful electrolyte management during this kind of treatment in the clinical setting (31).

Another intriguing finding was the increased retention of As in organs of infected individuals, *i.e.* the As concentration gradually increased in all organs except the intestine throughout the seven days of the As_2O_3 treatment. An increased accumulation of As in infected organs, possibly also having inflammatory lesions, implies an increased risk for adverse side-effects of the As_2O_3 treatment when patients concomitantly suffer from infectious diseases. Possibly, a lowering of the administered As_2O_3 dose to infected patients might be needed to minimise potential side-effects of the drug.

In conclusion, As_2O_3 treatment results in pronounced differences in trace element balance between healthy and infected individuals, a finding important to consider when using As_2O_3 in the clinical setting. These differences may affect the efficacy of the As_2O_3 therapy, as well as the development of treatment-related adverse effects. Thus, depending on the health status of the patient under treatment, supplementation of various specific and selectively chosen essential trace elements during As_2O_3 treatment may improve the efficacy and safety of the As_2O_3 treatment.

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