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An approach to the arsenic status in cardiovascular tissues of patients with coronary heart disease

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Abstract

Among non-cancer effects of arsenic, cardiovascular diseases have been well documented; however, few are known about the arsenic fate in cardiovascular tissues. We studied the analytic bioinorganic arsenic behaviour in cardiovascular tissues from an arsenic exposure coronary heart disease patient group from Antofagasta-Chile against a small unexposed arsenic coronary heart patient group. Total arsenic concentrations were measured in pieces of cardiovascular tissues of the arsenic-exposed and unexposed coronary heart patient groups by hydride generation atomic absorption spectrometry (HG-AAS); speciation analysis was made by high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS). Pieces of auricle (AU), mammary artery (MAM), saphenous vein (SAP) and fat residuals (FAT) were considered in this study. The arsenic concentrations in AU and MAM tissues were significantly different between both groups of patients. Also, it was demonstrated that the AU is an 'As^{3+} target tissue.' Otherwise, linking of the total concentrations of arsenic with conditional variables and variables related to medical geology factors allowed us to infer that the latter are more important for the cardiovascular risk of arsenic exposure in the Antofagasta region. Knowledge of total arsenic and the prevalence of the trivalent ion (As^{3+}) in the AU of patients could contribute to understanding the effect of arsenic on cardiovascular diseases.

Keywords

arsenic exposure, arsenic water pollution, arsenic cardiovascular effects, cardiovascular tissues, arsenic speciation, arsenic medical geology

Introduction

Arsenic is present in the coastal (Andean mountain) upper highlands ecosystem in northern Chile due to the volcanic nature of the land, mineralogy of their copper minerals and the mining exploitation of the same ones.[1,2] In this ecosystem, the Loa river basin is vitally important for their environmental sustainability, because it is the source of water consumption of the main cities and villages of the region of Antofagasta; however, the waters of this dry land river are enriched in arsenic and boron.[3,4] Besides the anthropogenic impacts, in some geological areas of the planet the adverse effects of arsenic exposure on human beings are a truth public health problem,[5-7] which is the aim of the Medical Geology that is the science that deals with the impact of geologic materials and processes on animal and human health.[8,9]

To overcome the effect of arsenic on water quality, arsenic removal plants have been in operation since 1972, for the principal cities of Antofagasta. Despite this, the effect of arsenic on human health has been

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noticeable in relation to cancer effects[10-12] and the incidence on the cardiovascular disease (CVD), which is recognized as typical non-cancer effects due to arsenic exposure.[13-17]

The average concentration of arsenic in the drinking water of Antofagasta was approximately 850 μg/L during 1958–1970,[16] but after installation of arsenic-reduction plants the arsenic concentration in drinking water of the principal cities of the Antofagasta region decreased about 50 μg/L, which is the maximum level of arsenic in water recommended by the Chilean government. However, there are still towns that consume drinkable water with arsenic levels over 50 parts per billion (ppb).[18] Recently, the efficiency of arsenic-removal plants in the Antofagasta region has improved, providing drinking water with arsenic concentrations less than 50 μg/L.

The chronic impact of arsenic in the Antofagasta region produces cancer and non-cancer outcomes. Cancer effects[11,19] are similar to those described in other countries affected by arsenic in the environment[7] in what the lung cancer risk is similar whether arsenic is ingested or inhaled.[20] In spite of this, overall increase in mortality due to arsenic in drinking water in the population of Antofagasta Region in Chile is greater than ever found for mortality from any other environmental exposure in any other population in the world, even though decreases in arsenic exposure began around 1971 due to arsenic plants removal installation.[11,16,19]

Typical non-cancer diseases affect the health and quality of life of people due to environmental arsenic exposure.[14,17,21-23] On the other hand, factors of risk like smoking, serum high levels of low-density lipoprotein (LDL cholesterol) and high blood pressure levels have been associated cooperatively with the coronary heart disease (CHD) or cardiovascular atherosclerotic disease in humans. Besides, actually it is becoming increasingly evident that low or moderate level exposure to arsenic is widely prevalent[20,21,24] for cardiovascular risk also. Therefore, due to the urgent need to understand how environmental exposure to arsenic affects cardiovascular function and disease, it is necessary to obtain more direct evidence about the behavior and fate of arsenic in cardiovascular tissues.

In healthy humans exposed to trace amounts of arsenic, the highest concentrations are found in tissues rich in sulfhydril groups (e.g., skin, hair, nails). However, little is known about the total concentration of arsenic in the organs of individuals unexposed to trace amounts of arsenic.[25] Comparative studies of trace elements and heavy metals in cardiovascular tissues are yet difficult due to the lack of knowledge about 'normal' values and due to the even scarce knowledge on the arsenic speciation in human tissues.[25,26]

In this work we report the concentrations of total arsenic in cardiovascular tissues, obtained by heart surgery of a group of patients with coronary heart disease subjected to chronic arsenic exposure in the Antofagasta region, and in the same cardiovascular tissues from a small group of coronary heart disease patients subjected to the same surgery type come of regions of Chile without drinking water arsenic exposure. The cardiovascular tissues were single samples of auricle (AU), mammary artery (MAM) and saphenous vein (SAP), and the pooled fat sample of each patient (FAT) from these same cardiovascular tissues. Also, we report the results of arsenic speciation in the cardiovascular tissues of four patients with coronary heart disease, i.e. the distribution of arsenic species (As^{5+}, As^{3+} and their metabolites) in three patients from the arsenic exposure group and one from the unexposed group. Knowledge of the total arsenic levels and the distribution of the main arsenic species present in these tissues could aid understanding of the long-term effect of arsenic on vascular and cardiovascular diseases.

Materials and methods

This work was carried out according to the Helsinki II declaration, with the consent and authorization of the Antofagasta Clinic. It was approved by the Committee of Ethics of the University of Antofagasta (CBIC REV 1 / 2005).

Sample populations

The samples of cardiovascular tissues of the arsenic-exposed group were taken up from 215 patients subjected to heart surgery in the Antofagasta Clinic, who have lived in the region of Antofagasta for at least 5 years. The group of samples of coronary heart disease arsenic-unexposed patients were obtained from 25 heart patients operated in the Hospital of the Catholic University in Santiago, who come from regions of Chile where problems have not been informed by the arsenic presence in human consumption water sources; given their smallest size regarding the exposed group to arsenic, we avoid in this work to use the term 'control population.' Pieces of AU, MAM

Table 1. Demographic questionnaire data for the arsenic (As) exposure[a] and unexposed[b] coronary heart disease patients groups

Coronary heart disease patients	As exposure group (215)	As unexposed group (25)
Age		
Mean	57	60
Median	56	59
Min–Max	33–78	38–78
Variables influenced by medical geology factors	As exposure group, %	As unexposed group, %
BA	72.1	-
NBA	21.9	-
CN	29.3	-
RS	25.1	-
OC	40.0	-
WA	47.9	-
WCh	22.8	-
OW	12.1	-
NMW	13.5	-
Conditional variables	As exposure group, %	As unexposed group, %
S	64.7	60.0
NS	31.2	40.0
W	46.0	56.0
NW	48.4	44.0
L	18.6	-
WL	77.7	100
D	73.5	32.0
WD	20.9	68.0
F	14.0	16.0
M	81.4	84.0

BA: patients born in the Antofagasta region and who have always resided in this region, NBA: patients not born in the Antofagasta region but have lived there at least 5 years, CN: inhabitants patients of the centre-north zone of Antofagasta city, RS: patients living in the south zone of Antofagasta city, OC: patients of other cities of the Antofagasta region, WA: patients that worked in Antofagasta city, WCh: patients that worked in the mines of Chuquicamata, OW: patients that worked in other copper mining locations, thermoelectric power plants and saltpeter mining, NMW: patients that worked in different roles to mining and power generation, S: patients who smoked, NS: patients who did not smoke, W: patients who consumed wine moderately, NW: patients who did not consume wine, L: patients with leukomelanosis, WL: patients without leukomelanosis, D: dyslipidemic patients, WD: patients without dyslipidemia, M: male patients, F: female patients.

[a] Arsenic exposure coronary heart patient group. All the questions were not answered by the patients of this group.

[b] Arsenic unexposed coronary heart patient group.

and SAP were collected between the years 1995 and 2000. Besides, the fatty residuals of each sample of cardiovascular tissue by patient were joined, homogenized and considered as pooled samples (FAT). The patients had been subjected to heart surgery due to arterial thrombosis and to each one of them was consulted a voluntary questionnaire (Table 1).

Manipulations and procedures for sample preparation were made in a 'clean laboratory' inside a laminar flow hood (Labconco, Purifier Class II, Kansas Missouri, USA) using inert devices (e.g. plastic and titanium knives, agate grinding mortar) and scalpels, scissors and forceps of surgical stainless steel. After removing the titanium clasp from the tissues, the samples were rinsed with deionised water and then were stored at –20°C before use. Dry/wet weight factors were obtained according to the UNEP protocol for biological tissues.[27]

Mineralization of sample tissues for determination of total arsenic contents

To assure the complete destruction of the fatty tissues, the sample mineralization was carried out in Teflon reactor bombs heated in a homemade refractory oven with internal temperature sensor and external control, according to a two-step mineralization procedure.[28] About 0.5–1.0 g of sample was digested in the Teflon

reactor bomb with 10 mL of concentrated HNO_3, 2 mL of concentrated $HClO_4$ and 2 mL of 2% m/v $Na_2S_2O_8$. Samples were pre-digested overnight at room temperature and the reactor bombs heated to 150°C for 2 hours in the refractory oven. After cooling, 0.5 mL of concentrated H_2SO_4 was added and the digested sample heated in an aluminium heating plate from ambient until 300°C in a timing of 85 min at semi-refluxing into a 50 mL glass Erlenmeyer flask, the final volume was 2 mL approximately. The digested sample was diluted to 10 or 25 mL with 0.5 M HCl and the total arsenic was determined by HG-AAS. Deionised water was used for making up to the chosen volumes when the arsenic measurements were done by inductively coupled plasma mass spectrometry (ICP-MS).

Materials, reagents and standards

HNO_3 and H_2SO_4 were Suprapur grade (Merck); $HClO_4$ and $Na_2S_2O_8$ were INSTRA grade (J. T. Baker). $NaBH_4$, NaOH, (NH_4) H_2PO_4, H_3PO_4 and NH_3 were Merck p.a. Each stock solution of arsenic species compounds containing 1.000 g/L of As were prepared by dissolving the respective amount of the following reagent in water: inorganic As(III) and As(V) standards from sodium arsenite and sodium arsenate (Sigma Aldrich, St Quintin, Fallavier, France); dimethylarsinic acid (DMA; Merck), monometilarsonic acid (MMA;-Chem Service, West Chester PA, USA); arsenobetaine (AsB) and arsenocholine (AsC; Tri Chemical Laboratory Inc. Japan); stock solutions were kept at 4°C in the dark. Experimental solutions were prepared daily and diluted with water to the final concentration. The standard reference materials (SRMs) DORM-1 (dogfish muscle), DORM-2 (dogfish muscle), TORT-1 (lobster hepatopancreas) and LUTS-1 (non-defatted lobster hepatopancreas) from the National Research Council Canada (NRCC) were used to validate the total arsenic determinations.

The methanol–water solutions were prepared with deionised water (Milli-Q Ultrapure water systems, Millipore, Billerica MA, USA) and HPLC-grade methanol (Merck). Sonication of samples was done in a focused ultrasonic bath (Bandelin Sonopuls HD-2200, Fungilab SA, Barcelona, Spain). Solvent evaporation of extracts was carried out in a rotavapor Univapo100H-Unijet II (UNIEQUIP, New York, USA).

Determination of total arsenic

Total arsenic contents were measured by hydride generation atomic absorption spectrometry (HG-AAS). Otherwise, total arsenic concentrations in methanol–water extracts and in their digested residues were measured by ICP-MS. Multiple standard addition calibration was used for HG-AAS. For ICP-MS measurements, $^{75}As(V)$ standard solution was used for calibration and control with ^{72}Ge (10 μg/L) as internal standard.

Instrumentation and chromatographic materials

HG-AAS measurements for total arsenic concentrations were done on GBC 909 PBT equipment coupled with a GBC HG-3000 hydride generator with an electrothermal mantle GBC EHG-3000 (Australia) and an arsenic hollow cathode-boosted discharge lamp (BDL) from Photron (Australia). An ICP-MS HP-4500 (Yokogawa Analytical System, Tokyo, Japan) was used for the detection of arsenic after separation of arsenic species using HPLC. A Babington glass nebulizer coupled to a Scott double-pass spray chamber was employed, and single ion monitoring at m/z of ^{75}As was used to collect the data. Signal quantification was done in the peak area mode.

Fractionation of arsenic in cardiovascular tissues

Cardiovascular tissues of three patients of the arsenic-exposed and one of the unexposed groups were treated for the arsenic speciation study according to Shibata and Morita.[29] Hence, 0.5–1.0 g of cardiovascular tissues previously dried at 60°C was placed in plastic centrifuge tubes with 10 mL of 1:1 methanol–water (v/v) and then shaken mechanically for 3 hours. The tubes were maintained at 55°C for 10 hours and finally left in an ultrasonic focalized bath for 5 min. Samples treated this way was centrifuged for 15 min at 6000 rpm and the extract removed. Insoluble residue was re-extracted using 5 mL of the same methanol–water mixture under the same conditions and the solid residual was extracted with 9:1 methanol–water v/v solution using the procedure described above. The 1:1 and 9:1 methanol–water extracts were separately dried by rotary evaporation at 40°C with a flow of ultra-pure N_2, and then separately dissolved in an appropriate volume of deionizer water and frozen to –20°C before analysis. Triplicate extracts were prepared from each one of the cardiovascular tissue sample from the four patients in whom the arsenic speciation was studied, and each one of the reference material LUTS-1, DORM-1, DORM-2 and TORT-1 for quality control measurements.

Table 2. Instrumental conditions applied in the analytical measurements of arsenic

HG-AAS	
$NaBH_4$ concentration	3%
HCl concentration	3 M
$NaBH_4$ and HCl flow rate	2.0 mL/min
Sample flow rate reaction coil	0.8 mL/min
Length	25 cm
Measurement mode	High peak
ICP-MS	
RF Power	Forward: 1350 W Reflected: 2.2 W
Argon flow rate	Coolant: 14 L/min Nebulizer: 1.0 L/min Auxiliary: 0.9 L/min Peak area, ^{75}As
Measurement mode (integration peaks per points)	3 replicates
HPLC column	Anionic PRP X-100
Mobile phase	$(NH4)_2HPO_4$, pH 6.0 Gradient mode (%) Solution A: 5 mM; Solution B: 25 mM 0-15 min (A: 100-0 and B: 0-100) 15-25 min (A: 0-100 and B: 100-0) Conditioning: 25-30 min (A: 100 and B: 0)
Flow rate	1.5 mL/min
Sample injection	100 μL

Abbreviations: HG-AAS: hydride generation atomic absorption spectrometry, HPLC: high performance liquid chromatography, ICP-MS: inductively coupled plasma mass spectrometry, RF: radio frequency.

HPLC-ICP-MS for measurement of arsenic speciation

A PRP-X100 analytical coupled with a guard anion-exchange column (Hamilton, Reindeer, Nevada, USA) were used for HPLC-ICP-MS hyphenated analysis. For the HPLC chromatographic separations, 100 μL of samples were introduced through a 0.45-μm nylon syringe filter into the injection valve Rheodyne 9125 (USA) and then pumped into the HPLC system (Milton Roy LDC Division, Florida, USA); air was removed from the buffers by argon degassing for 15 min and the buffer filtered before injection. The column effluent was directly introduced into the nebulizer rod of the ICP-MS equipment by a polytetrafluoroethylene capillary tube of 250 mm × 0.5 mm (id). The potential interference during the ion intensity measurement of the ^{75}As m/z by ICP-MS due to the possible formation of $^{40}Ar^{35}Cl$ was monitored and the eventual interference was corrected by the correction factor consider in the software of the instrument; however, the chloride concentrations in the analyzed fractions were low. Peaks were integrated using the software ICP-MS Lab and Captures Grams/32 (Galactic Industries Salem, New Hampshire, USA). An injection of 100 μL of 5.0 ng/mL of $^{75}As(V)$ was made along with the internal standard (^{72}Ge) before each chromatographic run in order to correct drift in the ICP-MS response. Table 2 shows the optimized instrumental parameters for the application of the HG-AAS, ICP-MS and HPLC techniques.

Analytical validation of arsenic measurements

Table 3 summarizes the analytic validation data to prove the suitability and efficiency of the described techniques for the determination of arsenic in cardiovascular tissues; i.e. total arsenic, total extracted arsenic with methanol–water 1:1 and 9:1 and the arsenic recovery experiments from the standard reference materials spiked with arsenic species frequently found in biological tissues, such as primary standard of As^{3+} and As^{5+} of sodium arsenite and sodium arsenate (Sigma Aldrich, St Quintin, Fallavier, France); DMA (Merck); MMA (Chem Service, West Chester PA, USA) AsB and arsenocholine (AsC; (Tri Chemical Laboratory Inc. Japan). The SRMs used in these quality control approaches were DORM-1, DORM-2, TORT-1 and

Table 3. Quality control and traceability for the total concentration, fractionation and speciation of arsenic

	Total As by HG-AAS	As found in MeOH-H_2O 1:1 extract by HG-AAS[a]	As found in MeOH-H_2O 1:1 extract by ICP-MS[a]
N	4	5	5
	30	5	5
	–	–	5
SRM	DORM-1	TORT-1	TORT-1
	LUTS-1	DORM-2	DORM-2
			LUTS-1
Average found concentration (μg/g)	17.2	21.2 (22.9)	21.7 (23.4)
	3.00	16.2 (16.8)	16.7 (17.1)
	–	–	2.35 (2.65)
Certified concentration (μg/g)	17,7	24.6	24.6
	2,83	18.0	18.0
	–	–	2.83
RE (%)	- 3.1	6.9	4.9
	4.6	6.7	5.0
	–	–	6.4
RSD (±%)	7.9	7.7	5.8
	2.8	8.5	6.3
	–	.	5.1
C_L (ng/mL)	0.69	0.22	0.09
	0.14	0.20	0.07
	–	–	0.05

Spiked arsenic species measurements in MeOH-H_2O 1:1 SRM extracts by HPLC-ICP-MS

SRM	N	As species[b]	Species found (μg/g)[c]	RSD (± %)	Species recovery (%)	C_L (ng / mL)
TORT-1	3	As^{3+}	Nd	–	98.6	0.02
		As^{5+}	0.33	0.09	96.5	0.03
		MMA	Nd	–	96.9	0.06
		DMA	2.04	0.9	98.3	0.05
		AsB	17.0	1.7	95.1	0.03
LUTS-1	3	As^{3+}	Nd	–	97.2	0.02
		As^{5+}	Nd	–	95.7	0.03
		MMA	Nd	–	96.5	0.06
		DMA	0.15	0.07	98.5	0.05
		AsB	1.81	0.6	95.3	0.03
DORM-2	3	As^{3+}	Nd	–	96.6	0.02
		As^{5+}	Nd	–	95.4	0.03
		MMA	Nd	–	96.9	0.06
		DMA	0.66	0.09	98.9	0.05
		AsB	15.1	0.8	95.1	0.03

Abbreviations: HG-AAS: hydride generation atomic absorption spectrometry, HPLC: high performance liquid chromatography, ICP-MS: inductively coupled plasma mass spectrometry, RE: relative error, RSD: relative standard deviation, SRM: standard reference materials.

[a] Mean of total concentrations of arsenic found in the standard reference materials are between parentheses.

[b] Species spiking of 50 ng / mL.

[c] Nd = below the detection limit (C_L).

LUTS-1 (National Research Council, Division of Chemistry, Ottawa, Canada). Detection limits were calculated in accordance with International Union of Pure and Applied Chemistry (IUPAC) criteria.[30] The results of the arsenic concentrations were accepted if the variation coefficient and the relative error tests were less to 15%, respectively.

Before being applied to the cardiovascular tissue samples, the arsenic speciation protocol was applied to standard reference material samples (Table 3).

Table 4. Statistical parameters for the total arsenic concentrations in cardiovascular tissues of arsenic exposed and unexposed coronary heart disease patient groups[a]

Cardiovascular tissues	N	Mean	SD (±)	Min–Max	Median
Auricle[b]	215	4.85	3.85	0.40–29.4	3.79
Auricle[c]	22	2.56	2.51	0.52–9.75	1.99
Mammary[b]	206	1.52	1.85	0.13–13.1	0.90
Mammary[c]	25	0.56	0.27	0.20–1.35	0.51
Saphenous[b]	199	2.97	2.95	0.11–23.8	2.13
Saphenous[c]	25	3.79	3.89	0.38–15.6	1.99
Fatty CV tissue[b]	211	1.27	1.64	0.076–14.3	0.78
Fatty CV tissue[c]	25	0.72	0.54	0.24–2.60	0.51

Abbreviation: CV, cardiovascular.
[a] Concentrations in μg/g dry weight.
[b] Arsenic exposure group.
[c] Arsenic unexposed group.

These results were very similar to that obtained in other reports.[31]

Statistics

Statistical analyses were carried out using the statistical package STATISTICA 6.1 (StatSoft, Tulsa, Oklahoma, USA); $p < 0.05$ was considered statistically significant.

Results and discussion

Demographic questionnaire

According to the definition of the science of human exposure,[32] we have assumed that in an ecosystem subject to environmental stressors coming from geological sources and anthropogenic activities, it would be possible, according to the available information, to define among the multiplicity of factors to a group of possible conditions or factors that could be considered candidate-predictors or variables that could describe the effects on humans of environmental stressors like arsenic exposure in the Antofagasta region, Chile. From this viewpoint, it is possible to discriminate subjects influenced by medical geology factors (e.g. metal- and metalloid-enriched geographical areas) and conditional variables associated with risky lifestyles such as to smoke and drinking wine, sanguinity indexes or gender that could predispose to cardiovascular disease due to the arsenic exposure. Medical geology offers the opportunity to identify and characterize links between the natural environment and human health.[8,9] Table 1 shows the results of the demographic questionnaire consulted to coronary heart disease patients of the groups chronically exposed to arsenic due to the consumption of drinking water and those coming from regions of Chile where the arsenic presence in human consumption water sources have not been reported.

Statistical treatment

Table 4 shows the statistical results of the total arsenic concentration in the cardiovascular tissues of the Antofagasta region patients' coronary heart disease group and in the group of coronary heart disease patients that come from regions of Chile where arsenic problems have not been informed in their water sources. Application of the Shapiro–Wilkinson test shows that a normal ($p = 0.05$) distribution is not followed in both groups of cardiovascular tissues, but rather the distribution of the total arsenic data biased to be lognormal, this model has been proposed for representing the frequency distribution of the concentrations of trace metals found in human tissue.[33] Application of the one-way ANOVA ($p = 0.05$) demonstrated that the arsenic concentrations in AU and MAM tissues were significantly different between both coronary patient groups, but not for SAP and FAT tissues. The highest arsenic concentrations in the cardiovascular tissues of both coronary patient groups were found in AU and SAP tissues, respectively, but the arsenic concentrations in the SAP tissue between both coronary patient groups were not significantly different. High level of arsenic in SAP and FAT tissues in the arsenic unexposed group seems to be an unexpected finding that could be the consequence of non-chronic factors of risk. Reversely in the exposed group, arsenic was more significantly only in the AUR and MAM tissues, that which could be consequence from the chronic arsenic exposure for prevalence of medical geology factors.

The total arsenic concentration in cardiovascular tissues of coronary heart disease patients of the arsenic-exposed group were associated to all demographic questionnaire data, for which the variables described in Table 1 were considered, and the total arsenic concentrations in the cardiovascular tissues of unexposed coronary heart disease patients group were associated with the conditional variables, i.e. patients who smoked (S), patients who did not smoke (NS); patients who consumed moderately wine (W), patients who did not consume wine (NW); patients with dyslipidemia (D), patients without dyslipidemia (WD); male patients (M) and female patients (F). When the arsenic concentrations were associated with

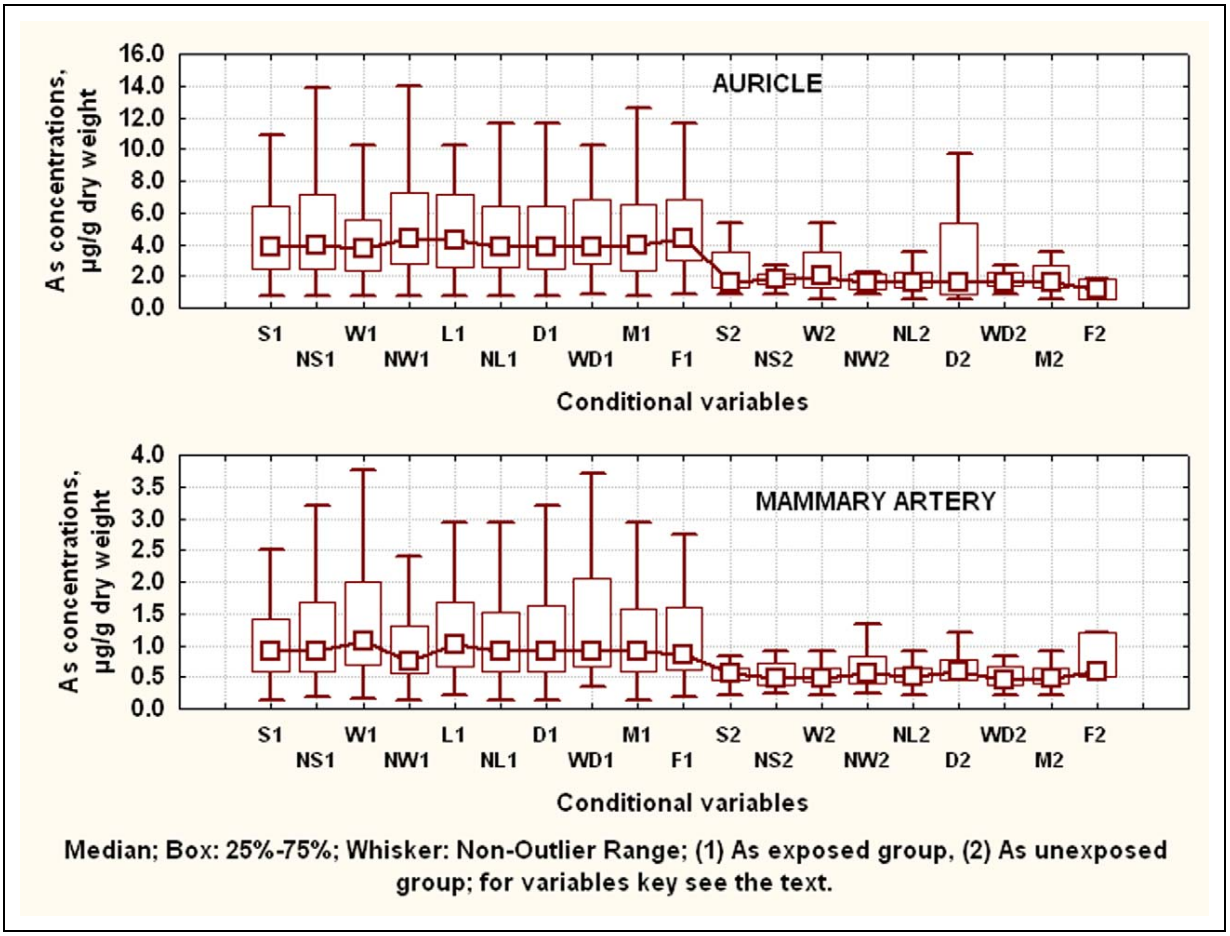

Figure 1. Box plots of the arsenic concentrations in auricle and mammary artery according to the demographic conditional variables of the arsenic-exposed and unexposed coronary patient groups.

the variables influenced fundamentally by medical geology factors, the application of the one-way ANOVA ($p = 0.05$) to the arsenic concentrations for AU, MAM and SAP of the arsenic-exposed group demonstrated that significant differences do not exist among the arsenic concentrations inside oneself type of cardiovascular tissue. Therefore, it seems to be that the arsenic concentrations in the cardiovascular tissues of the coronary heart disease patients from the Antofagasta region-exposed group would be fundamentally dependent of medical geology factor, as where they live and work. There were significant differences in the arsenic concentrations in the pooled fat cardiovascular samples (FAT) of the patients that belong to RS medical geology factor, respective of BA, CN, OC, WCh and NMW medical geology factor patients. The same was found for patients who work in Antofagasta city (WA) respective of patients NMW (for abbreviations see Table I). RS correspond to coronary heart disease patients that live near to copper smelting and cement facilities.

The associative linking among arsenic concentrations in the cardiovascular tissues of the arsenic-exposed and unexposed group with conditional variables (Table 1) allowed by means of one-way ANOVA analysis ($p = 0.05$) to identify statistical difference between the arsenic concentrations in the cardiovascular tissue of both groups of coronary heart disease patients. For AU, the differences were significant for the variables NS, NW, NL, D and M; in MAM, the differences of arsenic concentration between both groups were significant for W, NL, D and M; in SAP, the differences in arsenic concentration between both groups were significant alone for W and M. For FAT, they were no significant differences between the arsenic concentrations from the arsenic-exposed and unexposed groups. In this way, the box plots of Figure 1 show the arsenic concentrations profiles for

cardiovascular tissues translated according to the conditional variables of both groups. The plateau of the medians of the arsenic concentrations in AU and MAM tissues of the arsenic-exposed group is approximately twofold than the arsenic concentrations in the same tissues of the unexposed group.

The above results permit inferring that the influence of the medical geology factors are more determinative than conditional variables for the arsenic enrichment in human cardiovascular tissues; for another part, heart AU and MAM appear as the principal fate of arsenic in the exposed group. Arterial vessels have already been proposed as biomarkers for the exposure to other heavy metals;[34] however, in this case the AU and MAM are not routinely available samples and thus difficultly they could be considered as biomarkers.

Statistical multivariate treatment

From this point of view, we are interested in carrying out a purely phenomenological interpretation, without 'a priori' suppositions regarding the distribution of arsenic concentrations in the cardiovascular tissues in the two groups of coronary heart disease patients under study. Cluster analysis is an exploratory data analysis procedure, hence it is usually applied to data sets for which there is no a priori knowledge concerning the class membership of the samples, whose basic objective is to discover sample or variable grouping within data. There are two important issues, the way of measuring the distance between samples (metrics) and the way of measuring the distance between samples and cluster (linkage rule). We applied cluster analysis to measure the distance among the variables (metrics) considering the method of Ward with the approach of 1 – Pearson r to measure the distance between the variables and the cluster (linkage rule). On the other hand, the two-way joining plotting technique was also applied.

The dendrogram of the arsenic concentrations in the cardiovascular tissues of the arsenic exposure and unexposed groups (Figure 2a) shows the clustering of the tissues in two principal patterns. On the other hand, two-way clustering was applied to explore the bidimensional multivariate relationship of arsenic concentrations in the cardiovascular tissues linked with demographic variables (Table 1), after that the data were regrouped considering as more representative value of each one from the variables to the median of the arsenic concentrations. Figure 2b and c show the associative distribution with the demographic variables, i.e. the two-way clustering regarding the conditional variables for both coronary heart disease patients groups and the clustering of the arsenic-exposed cardiovascular tissues group regarding the arsenic concentrations, considering all demographic variables. In the first case, we can infer that arsenic appears transversally associated to AU tissue independently of conditional variables. In the second case, when all the variables were considered, the cardiovascular tissue preferred by arsenic was also the AU, but it is possible to observe that the most important associations show up among the arsenic concentrations in AU and the variables influenced by medical geology factors. These include patients with residence in other cities of the Antofagasta region (OC); patients who work in Antofagasta city (WA); patients who work in the copper mines of Chuquicamata (WCh and for some conditional variables, such as patients who do not consume wine (NW); patients with leukomelanosis (L) and female patients (F). In the unexposed arsenic group, they did not show up patient with leukomelanosis, but not all the patients of the group exposed to arsenic presented corporal stigmata.

These results allow us to conclude that the predominant destination of arsenic in the cardiovascular tissues of the coronary heart disease patients from Antofagasta region arsenic-exposed group is the AU and the MAM, that which would be other important factor of cardiovascular risk, besides the known effects on the peripheral vasculature. This way, intrinsic medical geology characteristics would be decisive for the risk of the cardiovascular health in human beings exposed to arsenic in the region of Antofagasta, Chile. These factors include exposure to arsenic and other non-essential trace elements from the water source for human consumption,[19] consumption of agricultural products cultivated in arsenic-enriched soils watered with arsenic-enriched underground waters,[35] inhalation of heavy metals from the atmosphere due to industrial activity, climatic factors and the consumption of sea foods.[36-38] The current concentrations of total arsenic in the drinking water of the city of Antofagasta after being treated in arsenic removal plants are between 15.0 ppb and 30.0 ppb; this satisfies the Chilean guide of 50 ppb but not the WHO guide of 10 ppb.

Arsenic species found in cardiovascular tissues

Table 5 shows the results of arsenic fractionation in methanol–water mixtures[34] and the ion

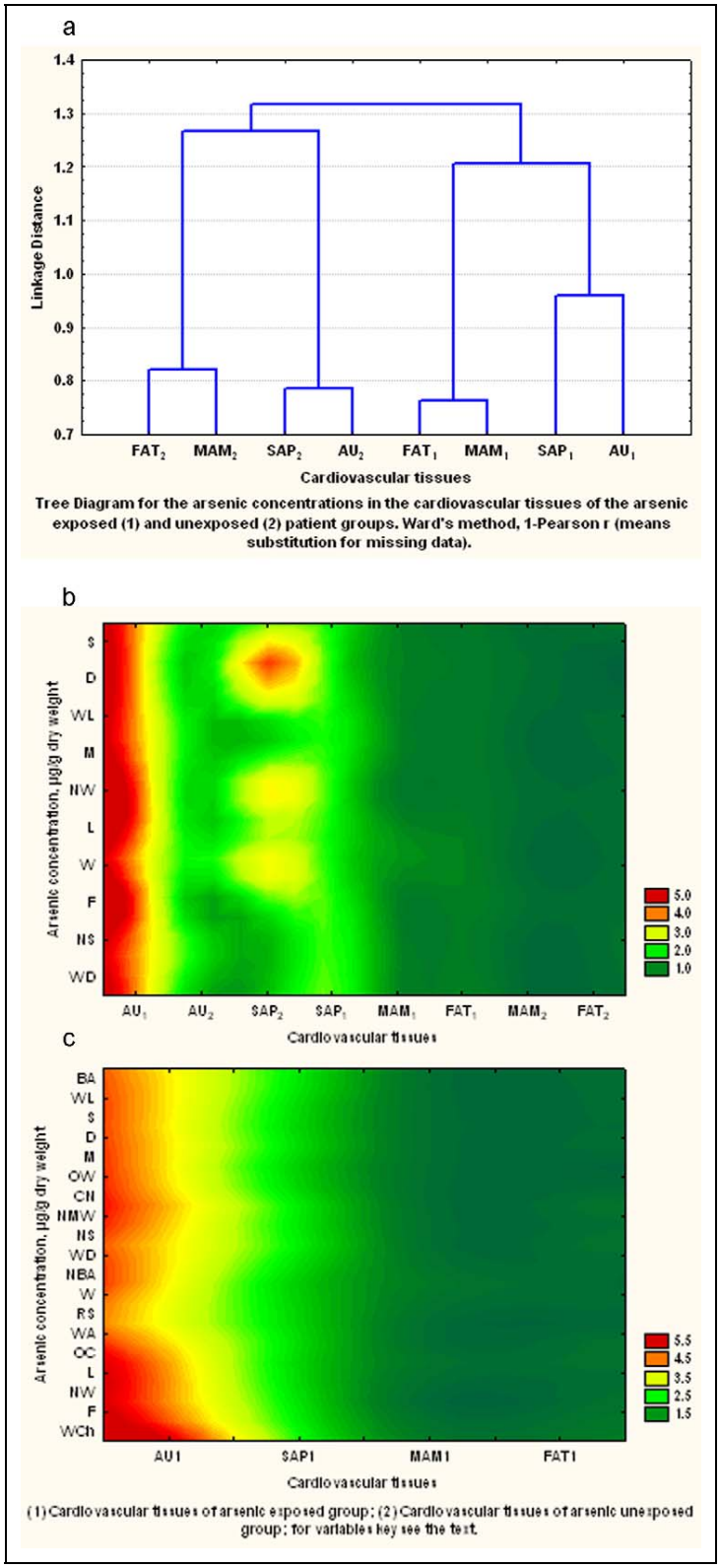

Figure 2. a, Dendrogram of the arsenic concentrations in cardiovascular tissues of arsenic-exposed and unexposed coronary heart disease patient groups. b, Two-way joining plot of arsenic concentrations with conditional variables for the cardiovascular tissue of arsenic exposed and unexposed coronary heart disease patient groups. c, Two-way joining plot of the arsenic concentrations with all demographic variables for cardiovascular tissues of arsenic exposure coronary heart disease patient group.

Table 5. Fractionation and chromatographic speciation (HPLC-ICP-MS) of arsenic in cardiovascular tissues of arsenic exposure coronary heart disease patient group from the region of Antofagasta at North of Chile

	Auricle	Mammary artery	Saphenous vein	Fat tissue
Total As (μg/g)	6.9 ±2.1	0.9 ± 0.2	4.96 ± 1.2	0.8 ± 0.2
Fractionation and chromatographic arsenic speciation[a]				
MeOH-H_2O (1:1)	70.9 ± 3.1	60.3 ± 2.7	56.2 ± 2.9	67.4 ± 2.9
As^{3+}	42.4	Nd	30.1	Nd
As^{5+}	6.8	29.8	4.4	21.5
MMA	Nd	3.8	Nd	Nd
DMA	Nd	6.2	3.1	5.8
AsB	Nd	18.8	Nd	33.9
MeOH-H_2O (9:1)	19.3 ± 2.0	26.2 ± 2.5	29.7 ± 2.9	30.3 ± 2.2
Residual As	9.1 ± 1.8	12.0 ± 1.1	12.2 ±1.3	1.9 ± 0.7

[a] Mean percentages (wet weight basis) of the tissues of three coronary heart disease patients regarding their average total arsenic concentration; Nd = below the detection limit (C_L), see Table 3.

chromatography-high performance liquid chromatography-inductively coupled plasma-mass spectrometry (IC-HPLC-ICP-MS) chromatographic speciation of the cardiovascular tissues of three patients with coronary heart disease of 37, 44 and 53 years old, who had lived in the Antofagasta region. These results show that most of the arsenic in the tissues was extracted by the 1:1 methanol–water-extracting solution; this has also been observed in other biological tissues.[31] Under these conditions, the arsenic species contained in these cells were extracted and the arsenic speciation investigated, so we can assume that at least the 1:1 methanol–water extract contains the arsenic species of the cytosol solution.

The arsenic speciation protocol described in this work was applied separately to the AU, MAM, SAP and FAT tissues of each one of the three coronary heart disease patients from the arsenic exposure group of the Antofagasta region, and to one patient with coronary heart disease who had lived in the Valparaíso region all of his life, where problems have not been informed by the arsenic presence in human consumption water sources. The arsenic speciation results are shown in Table 5. Figure 3a and b show the HPLC-ICP-MS arsenic chromatographic speciation profiles of AU and MAM cytosols of the coronary heart disease patients with the highest arsenic enrichment. The arsenic speciation chromatograms in these same types of tissues of the coronary heart disease patient of the unexposed arsenic group were similar to the previous ones, but the concentrations of the arsenic species were smaller.

The cardiovascular tissue speciation results obtained in this work shows that only arsenite (As^{3+}) and arsenate (As^{5+}) species were found in the cytosol of the AU tissue (Figure 3a). Both inorganic arsenic species were confirmed by spiking with the standard solutions of the species; arsenite was also confirmed after being separated by means of cationic chromatography using the cationic column Hamilton PRP-X 200 (chromatograms not shown), in which As^{3+} does not overlap with other arsenic species. Arsenite was also the predominant species in the cytosol of the SAP, which suggests that the arsenite could be the main arsenic species in the cytosol of this tissue, but a small amount of DMA species were also found (chromatogram not shown). In the cytosol of the MAM (Figure 3b), the major arsenic species were arsenate and AsB, DMA and MMA were only minor species; arsenite was not found. In the cytosol of the pooled fat sample of the cardiovascular tissues (FAT) AsB was the predominant species, followed by arsenate and DMA (chromatogram not shown). The arsenic species stability was monitored during sample preparation and storage.[31]

MMA and DMA are the main products of the cellular biomethylation through the conjugated effects of S-adenosylmethionine and the methyltransferase enzyme, but presently work was not discriminated against between As(III) and As(V) methylated species, the spiking additions were made with As(V)-methylated arsenicals. As(V) is the main arsenic species in surface natural waters, therefore the biotransformation of As(V) to As(III) must be a main mechanism in the organisms. By means of this mechanism, the mammals metabolize and detoxify inorganic arsenic, which involves methylation to methylarsonate and dimethylarsinate of trivalent and pentavalent arsenic, respectively.[39,40] Before

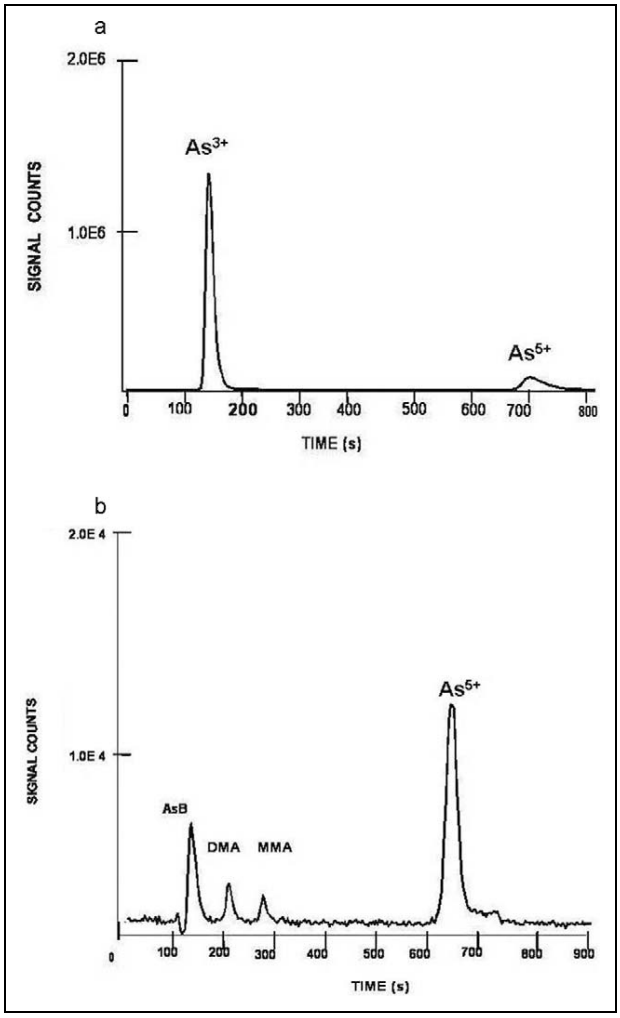

Figure 3. Ion chromatography-high performance liquid chromatography-inductively coupled plasma-mass spectrometry (IC-HPLC-ICP-MS) chromatogram profiles for auricle (a) and mammary artery (b) arsenic (As) species after 1:1 methanol–water extraction.

methylation occurs, As^{5+} must be reduced to As^{3+}, forming trivalent MMA and trivalent DMA, which are persistent metabolites more acutely toxic than the pentavalent forms and may be more toxic than the trivalent inorganic arsenic ion.[41] In vivo methylation has long been proposed as an arsenic detoxification pathway for inorganic arsenic,[40] but it has been shown that As(III)-MMA and As(III)-DMA are more toxic to cells than As^{3+} and As^{5+} ions.[42]

MMA and DMA species have been considered important in arsenic detoxification mechanisms, so it was surprising that in this work DMA and MMA species were not detected in the cytosol of AU and the concentration of DMA was very low in the cytosols of SAP and MAM (Table 5). Similar results were found in heart tissues of chickens.[43] AsB is believed to have a very low toxicity and has been found in human serum,[44] the presence of AsB was unexpected in this work; this organ arsenical species enters human beings principally from marine foods and from agricultural products coming from polluted soils and/or watered with arsenic-enriched waters.[35,44] Arsenic is primarily metabolized in the liver, and probably cardiovascular tissue does not efficiently metabolize arsenic. The inefficiency of methylation mechanisms could be due to the lack of methylating agents in cardiovascular tissues; organs not directly involved in arsenic metabolism may accumulate arsenic only when the ingested dose is high.[45]

In general, the distribution and speciation of arsenic in certain tissues of organism including the human beings is a complex mechanism not fully

elucidated. In marine fish tissues, the majority of arsenic has been also extracted in the methanol–water soluble fraction, but in the most tissues including the heart, arsenic was found as AsB.[46] The rate of elimination of arsenic in mammals varies with type of exposure, the form of arsenic, the type of tissue, the animal species and other factors such as diet.[45]

We report the total concentrations of arsenic in cardiovascular tissues from a group of arsenic-exposed patients with coronary heart disease who have lived in Antofagasta, an area at the North of Chile with arsenic chronic exposure due to drinking water, in comparison to a small group of patients with coronary heart disease who have lived in regions of Central and South of Chile where problems have not been informed by the arsenic presence in human consumption water sources. Arsenic speciation status was also investigated in the cardiovascular tissue cytosol of three coronary patients subjected to heart surgery from the arsenic-exposed group and in one coronary heart patient from the arsenic-unexposed group. Knowledge of total arsenic level concentrations and the speciation distribution of arsenic in the cytosol of cardiovascular tissues, in particular, the prevalence of As^{3+} in the AU of the arsenic exposure group, could aid to understanding the long-term effects of arsenic on cardiovascular and vascular diseases. The results of this work and recent epidemiologic knowledge[16,17,47] allow concluding that the arsenic accumulation and their speciation in cardiovascular tissues, particularly in human beings subjected to chronic arsenic exposure, they are factors of risk for their heart health. In particular, the auricle (AU) behaves as an "As^{+3}" target tissue, which is one of the most toxic arsenic species. Otherwise, linking of the total concentrations of arsenic with conditional variables and variables related to medical geology factors, allowed us to infer that the latter are more significant for the cardiovascular risk of arsenic exposure in the Antofagasta region.

Conflicts of interest

The authors have no competing financial interest.

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