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Trace element changes in sclerotic heart valves from patients are expressed in their blood

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Abstract

The pathogenesis of some heart diseases has been associated with changes in the balance of certain trace elements. However, whether blood trace element changes exist that are related to changes in the cardiovascular system are, in most cases, unknown. In this study, blood trace element levels were analysed in 46 patients with non-rheumatic aortic valve sclerosis that were previously shown to have a disturbed trace element balance in their valve tissue, including 11/15 elements. Results showed significant changes of blood levels of 8/15 trace elements in these patients when compared with blood levels in 46 healthy controls. Of these elements, Cd and Mg were the only elements that increased in both blood and valves. Cu and Se were increased in blood but decreased in valves, whereas Co and Zn were decreased in blood but increased in valves. Several elements (As, Ca, Fe, Pb, and V) were unchanged in blood although changed in valves. Although Mn and Hg showed changes in blood, this was not evident in the valves. Al and Ag were the only elements that did not change in both blood and valves. Significant covariation in blood and valve levels was only observed for Al and Pb. The recorded pattern of trace element changes indicates a complex competition/exchange between body compartments in this disease, where the increased blood Cu/Zn ratio suggests an ongoing infectious/inflammatory process.

Introduction

Aortic valve sclerosis, or calcification of the aortic valve, is the main cause of aortic stenosis, a condition often requiring surgical valve replacement. Although its pathogenesis may be partly different from that of atherosclerosis (Olsson *et al.* 1994; Otto *et al.* 1994), growing evidence indicates that valve calcification involves inflammation comparable with that seen in atherosclerosis (Mohler *et al.* 1999). Moreover, the inflammatory infiltrate includes activated HLA–DR expressing macrophages (human T-cells and B-cells), suggesting an ongoing antigen-stimulated process (Olsson *et al.* 1994; Otto *et al.* 1994). In earlier studies, we have found nucleotide sequences of *C. pneumoniae* (*Cp*) in a large proportion of sclerotic aortic valves (Nyström *et al.* 1997, 2003). For its growth, *Cp* is known to depend on iron (Fe) (Al-Younes 2001) and can persist for a long time in host tissues where it can cause chronic infection and inflammation (Grayston 2000). Whether *Cp* is the cause of the inflammatory lesion in aortic valve sclerosis in *Cp*-positive patients is unknown, however.

In a recent study on 15 trace elements in the sclerotic aortic valves from 46 collected patients undergoing aortic valve replacement surgery, changed concentrations were observed for several of the ele-

ments when compared with forensic autopsy cases with no known heart disease (Nyström *et al.* 2002). Several of these elements, including copper (Cu), zinc (Zn), and selenium (Se), are important nutrients for the immune system. Other trace elements, including magnesium (Mg) and calcium (Ca), are associated with disease complications, such as arrhythmias and calcification (Fields 1999; Freidank *et al.* 2001; Stallion *et al.* 1994; Altura & Altura 1991; Beisel 1998). However, little is known about the trace element flux between plasma and infected and/or inflamed body tissues and whether observed changes in plasma reflect changes in the target tissues of disease. A few studies in infection and in toxin-induced shock show that determination of some of these trace elements in plasma may be usable to indicate target tissue involvement and progression of the disease (Funseth *et al.* 2000; Ilbäck *et al.* 2003a,b).

The aim of this study was to investigate whether the previously published trace element changes in the valves of 46 patients with advanced aortic sclerosis (Nyström *et al.* 2002) were reflected in their blood. For this purpose the same trace elements were measured in blood from these 46 patients, in addition to blood from 46 healthy volunteers with no known heart disease.

Materials and methods

Patients and controls

Between 1997 and 1999, aortic valve tissue and serum samples were taken from 46 consecutive patients (20 women and 26 men, aged 34–83 years, mean age 73 years) undergoing open-heart surgery for the replacement of stenotic aortic valves because of aortic sclerosis as previously described (Nyström *et al.* 2002). Fifteen forensic autopsy controls (6 women and 9 men, aged 17–88 years, mean age 60 years) without known heart valve disease served as controls for the valve study (Nyström *et al.* 2002, 2003).

Because no serum samples were available from the autopsy controls, plasma samples from 46 healthy volunteers (20 women aged 22–77 years, mean age 47 years and 26 men aged 30–69 years, mean age 51 years), collected during 1997–1998, served as controls. This control group, not undergoing any medical therapy or medication, was selected from 250 healthy volunteers, the majority of whom were employees of Uppsala University or the University Hospital in Uppsala. Volunteer plasma samples used in this study were sex- and age-matched as far as possible. Unfortunately, a good age-match could not be achieved because of the relatively high mean age of the patients in this study.

Ethics

The Research Ethics Committee of the Faculty of Medicine, Uppsala University, approved this study (D. No. 97 078). The investigation conforms to the principles outlined in the Declaration of Helsinki.

Tissue sampling

During the operation, the to-be-replaced aortic valve was excised in toto and placed in an empty sterile plastic tube. Sterile surgical instruments of stainless steel were used. The tubes were immediately transferred to the laboratory and frozen at −150 °C. The samples were subsequently placed in a freezer at −70 °C. The samples for analysis were excised from the soft parts of the valve and stored for later analysis of trace element content.

Serum/plasma sampling

The patient blood specimens were immediately sent to the laboratory, centrifuged and serum was obtained. In the controls, plasma samples were available. The sera and plasmas were kept frozen at −20 °C and thawed only once. It is known that Zn concentrations may be somewhat higher in serum than in plasma (Smith *et al.* 1985). However, such concentration differences do not influence the conclusions arrived at in this study.

Determination of trace elements

The contents of the trace elements aluminum (Al), arsenic (As), cadmium (Cd), calcium (Ca), cobolt (Co), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), mercury (Hg), selenium (Se), silver (Ag), vanadium (V), and zinc (Zn) were measured in the heart valve tissue and in the serum/plasma samples by inductively coupled plasma mass spectrometry (ICP-MS) (Nyström *et al.* 2002).

Statistical analyses

The Mann Whitney U-test was used to compare differences in single trace elements between control and patient groups. Univariate associations were examined using Spearman's rank correlation.

Table 1. Concentration of trace elements in heart valve tissue from patients operated on for stenotic heart valves (n=46) and from forensic medicine controls (n=15) (Nyström-Rosander *et al.* 2002).

Trace element (ng/g wet weight)	Trace element concentration			
	Controls	Patients	P-value	Change
	Mean ± SD	Mean ± SD		
Aluminum (Al)	501 ± 141	551 ± 272	(>0.05)	⇔
Arsenic (As)	17.5 ± 5.24	94.4 ± 43.3	<0.001	⇑
Cadmium (Cd)	26.3 ± 7.16	40.1 ± 22.9	<0.05	⇑
Calcium (Ca)*	145 ± 52	103352 ± 55470	<0.001	⇑
Cobalt (Co)	13.6 ± 3.89	146 ± 62.6	<0.001	⇑
Copper (Cu)	1490 ± 319	826 ± 282	<0.001	⇓
Iron (Fe)*	17.6 ± 8.2	356 ± 189	<0.001	⇑
Lead (Pb)	78.7 ± 28.3	587 ± 513	<0.001	⇑
Magnesium (Mg)*	61.8 ± 11.2	1250 ± 635	<0.001	⇑
Manganese (Mn)	70.8 ± 26.7	60.6 ± 18.3	(>0.05)	⇔
Mercury (Hg)	1.16 ± 0.42	1.65 ± 1.17	(>0.05)	⇔
Selenium (Se)	194 ± 52.6	167 ± 32.6	<0.05	⇓
Silver (Ag)	4.17 ± 1.03	4.37 ± 2.43	(>0.05)	⇔
Vanadium (V)	70.1 ± 26.7	40.4 ± 24.6	<0.001	⇓
Zinc (Zn)*	5.13 ± 1.65	54.9 ± 26.4	<0.001	⇑

Note: Data are expressed as mean values ± standard deviations. Significant differences between the operated patients and forensic medicine controls are indicated with p-values. Asterisks (*) indicate that concentrations are expressed as μg/g wet weight

Results

In an earlier study on trace elements in sclerotic heart valves, in which the present aortic heart valves were used (Nyström *et al.* 2002), we found that the disease was associated with a pronounced imbalance in several trace elements (Table 1). Some of the elements are of well-known importance for cardiovascular and immune function, whereas others are of hitherto unknown significance.

The present study with the above patients showed that concentrations in blood of several of these trace elements were also changed. For some of the elements, changes in blood were in the same direction as changes in the valves, whereas for others there were no corresponding changes in blood, or changes occurred in reverse directions in valves and blood (Table 1). Since Zn concentrations may be slightly higher in serum than in plasma (Smith *et al.* 1985), the Cu/Zn ratio was also used in the comparisons. The Cu/Zn ratio was markedly elevated in the patient sera (Cu/Zn, pat: 1.71 ± 0.45, Cu/Zn, ctr: 1.36 ± 0.42; P<0.001).

In general, the trace element concentrations exhibited greater inter-individual variation among the patients than among the controls, both in valves and blood. Two of the trace elements, Al and Ag, evidenced no changes either in the sclerotic valves or in the blood.

Female patients had significantly higher concentrations of Ca (P<0.05) and Pb (p<0.05) in their sclerotic valves as compared with male patients. Moreover, a higher concentration in serum was observed in female patients in comparison with male patients for Ag (P<0.05), Cd (P<0.05), and Pb (P<0.05), but not for Ca.

In the sclerotic valves, the concentrations of four trace elements (As, Ca, Fe, and Pb) were increased, some dramatically so, whereas no changes were detected in the blood. The sclerotic valve concentration of V was decreased, but in the blood no change in the V concentration was observed.

In the blood Mn was increased by 100%, whereas Hg was decreased by 56%. In the sclerotic valves the concentrations of these trace elements were, however, unchanged.

None of the studied trace elements showed decreased concentration in both blood and sclerotic heart valves. However, two of the trace elements (Co and

Table 2. Concentration of trace elements in sera from patients operated on for stenotic heart valves (n=46) and in control plasma samples from healthy volunteers (n=46).

Trace element (ng/ml)	Trace element concentration			
	Controls	Patients	P-value	Change
	Mean ± SD	Mean ± SD		
Aluminum (Al)	1.86 ± 0.65	1.82 ± 0.91	(>0.05)	⇔
Arsenic (As)	1.78 ± 0.49	1.74 ± 0.67	(>0.05)	⇔
Cadmium (Cd)	0.25 ± 0.09	0.53 ± 0.52	<0.01	⇑
Calcium (Ca)*	102 ± 10	103 ± 28	(>0.05)	⇔
Cobalt (Co)	0.49 ± 0.10	0.45 ± 0.18	<0.05	⇓
Copper (Cu)	1107 ± 158	1258 ± 412	<0.05	⇑
Iron (Fe)	1204 ± 187	1357 ± 481	(>0.05)	⇔
Lead (Pb)	1.20 ± 0.68	1.39 ± 1.06	(>0.05)	⇔
Magnesium (Mg)*	19.7 ± 2.3	26.2 ± 7.5	<0.001	⇑
Manganese (Mn)	1.54 ± 0.31	3.14 ± 0.98	<0.001	⇑
Mercury (Hg)	1.26 ± 0.46	0.55 ± 0.25	<0.001	⇓
Selenium (Se)	81.1 ± 12.7	99.9 ± 13.8	<0.001	⇑
Silver (Ag)	0.63 ± 0.12	1.37 ± 2.45	(>0.05)	⇔
Vanadium (V)	0.074 ± 0.077	0.077 ± 0.016	(>0.05)	⇔
Zinc (Zn)*	863 ± 187	752 ± 216	<0.05	⇓

Note: Data are expressed as mean values ± standard deviations. Significant differences between the operated patients and healthy volunteers are indicated with p-values. Asterisks (*) indicate that concentrations are expressed as μg/ml.

Zn) were decreased in the blood, whereas dramatic increases of both elements were recorded in the sclerotic valves. The reverse was observed for Cu and Se, i.e. there was an increased concentration in the blood but a decreased concentration in the sclerotic heart valves. Neither of these changes showed significant co-variation between the compartments.

Two of the 15 elements (Cd and Mg) displayed increased levels in both blood and sclerotic heart valves. However, neither of these two elements indicated a significant co-variation between the compartments.

A significant co-variation in trace element changes between serum and sclerotic heart valves was only observed for Al ($r = -0.250$, $P<0.05$) and Pb ($r = 0.330$, $P<0.05$). In addition, Fe, which increased in the sclerotic valves but not significantly so in serum, also tended to show a positive correlation ($r = 0.241$, $P<0.053$) between the compartments.

Discussion

In sclerotic heart valves in comparison with normal heart valves a pronounced imbalance exists in several trace elements (Table 1) (Nyström-Rosander *et al.* 2002). The findings of the present study clearly demonstrate that the concentration of many of those elements showing changed concentration in the sclerotic valves also show changed concentration in the blood of patients with this disease, i.e., Cd, Co, Cu, Mg, Se, and Zn. Cd and Mg concentrations were increased both in blood and valves; Cu and Se concentrations were increased in blood but decreased in valves; whereas for Co and Zn, the reverse pattern was found. Although not changed in the sclerotic valves, Mn concentration was increased and Hg concentration was decreased in blood. Furthermore, blood concentrations of Ag, Cd, and Pb were significantly higher in female than in male patients, although mean Ag and Pb concentrations did not differ from those in the controls. Finally, the inter-individual differences in blood concentration, as well as in valve tissue concentration, of most of the elements were greater in the patients than in the controls, which may express various levels of inflammatory activity among the patients.

Monitoring of increased Cu and decreased Zn concentrations, as well as the increased Cu/Zn quotient having increased sensitivity, is an established tool in experimental infectious disease research to indicate the occurrence of a host response (‘acute phase

reaction') to the infectious microorganisms (Beisel 1998; Ilbäck *et al.* 1983, 2003a). The main source of the Cu is the liver and Zn is sequestered into liver (Beisel 1998) and possibly other organs and tissues. Metallothioneins (MTs) are metal binding proteins directly involved in the homeostsis of Cu, Zn, and Cd (Palmiter 1998). They are highly inducible by exposure to a wide variety of endogenous and exogenous agents, including Cd, hormones, cytokines, and infectious agents (Palmiter 1998; Funseth *et al.* 2002). The present findings of an increased blood level of Cu and a decreased level of Zn, resulting in an increased Cu/Zn ratio suggest a persistent low grade 'acute phase reaction' in patients with aortic valve sclerosis. This is supported by a recent report of moderately increased C-reactive protein levels in such patients (Galante *et al.* 2001). These results are compatible with an ongoing infectious/inflammatory process, e.g., by *C. p* (Nyström-Rosander *et al.* 1997, 2003).

In low-grade inflammatory and sclerotic tissues in atherosclerosis of the aorta (Vlad *et al.* 1994), as well as in aortic valve sclerosis (Table 1) (Nyström-Rosander *et al.* 2002), the Cu content is decreased. Because Cu is an important element in antioxidative enzymes (Barandier *et al.* 1999), Cu deficiency at the site of an inflammatory process may potentially increase tissue damage. By comparison, in studies of experimental viral myocarditis, representing the early phase of an acute inflammatory process, the tissue level of Cu was increased, while the plasma level showed an expected raised Cu/Zn quotient comparable with the present study (Funseth *et al.* 2000; Ilbäck *et al.* 2003a).

Zn is mobilised to healing wounds (Savlov *et al.* 1962). Further, it is involved in the regulation of immune function and is a nutrient required by immune cells (Driessen *et al.* 1995; Fernandes-Pol *et al.* 1979) present in these valves. Immune activation and infection, including cytokine production and release, induce metal binding proteins, such as MT (Palmiter 1998). It has been shown that coxsackievirus infection can increase MT 10-fold in the liver (Funseth *et al.* 2002) and that influenza can increase the expression of MT genes in the liver and lungs (Ghoshal *et al.* 2001). Because MT preferentially binds Zn (Nordberg & Nordberg 2000), the present 10-fold accumulation of Zn in the valves may represent over-expression of metal binding protein synthesis, caused by a long-term inflammatory/infectious process.

However, no statistical correlation was found between blood and tissue levels of Cu and Zn, either in the present study of aortic valve sclerosis or in acute viral myocarditis (Funseth *et al.* 2000). Thus, determination of blood Cu and Zn can be used to indicate ongoing infectious and inflammatory disease, but blood levels cannot be used to predict levels of these elements in inflammatory tissues.

Although the Se level was increased in blood, it was decreased in the sclerotic valves. Likewise, in acute viral myocarditis Se is decreased in the inflamed heart (Funseth *et al.* 2000). Particularly noteworthy is that Se deficiency in the food impairs immune function, resulting in selection for more virulent virus variants and more aggressive disease (Levander *et al.* 1995), whereas Se food supplementation has the opposite effect resulting in milder disease (Ilbäck *et al.* 1998). Further, Se deficiency has been linked to various other cardiovascular diseases, including Keshan and Chagas disease (Chen *et al.* 1980; Rivera *et al.* 2002; Salonen *et al.* 1982). It is well known that Se interacts with Hg and that it can modify the uptake and distribution of Hg (Wicklund-Glynn *et al.* 1993), resulting in markedly reduced Hg content in the kidneys but increased Hg content in the liver (Cikrt & Bencko 1989). In addition, Se is protective against Hg-induced damage to the kidneys and the liver (Lindh *et al.* 1996). The increased Se level and decreased Hg level in blood in the present study may therefore indicate a flux and mobilisation of stored Se as a response to an increased demand and concomitant accumulation of Hg in other compartments of the body.

Tissue Ca accumulation is correlated to the histopathological severity of this and other cardiovascular diseases, such as myocarditis (Hess *et al.* 2000; Nyström *et al.* 2003; Stallion *et al.* 1994). Moreover, as in myocarditis, Fe was greatly increased in the sclerotic heart valves (Nyström-Rosander *et al.* 2000). However, blood levels of Ca and Fe were not significantly changed. Conversely, in acute myocarditis, characterized by a more pronounced acute phase reaction than aortic sclerosis, blood Fe is increased and correlates to the Fe level in the myocardial tissue (Funseth *et al.* 2000). From the demonstrated deposition of Fe in atherosclerotic lesions (Meijer *et al.* 1996; Ponraj *et al.* 1999), an 'iron hypothesis' has evolved to explain its role in atherosclerotic heart disease (DeWalk & Marx 1999). Although mechanisms are not fully understood, it has been proposed that an interaction of macrophages and endothelial cells with Fe and low-density lipoprotein is a key mechanism in the disease

process. This mechanism is also involved in valve calcification (Mohler *et al.* 1999). A likely explanation of the greatly increased tissue levels of Ca and Fe in the face of normal blood levels in the present study may be that aortic sclerosis progresses slowly over many years compatible with a long-term low-grade uptake.

There was an increase of Co in sclerotic valves and a concomitant decrease in the blood. Several studies have shown that Co in association with alcohol consumption may produce cardiomyopathy (Klatsky 2002). In addition, in the sclerotic valves, the concentrations of As and Pb were increased but no changes could be detected in the blood. Conversely, in the early phase of viral induced inflammatory heart disease (Ilbäck *et al.* 2003c) an increase in serum Pb has been observed. However, it was noteworthy that there was a positive correlation between blood Pb levels and the 20-fold increased levels in sclerotic valves. In beer drinkers disease high As intake has been associated with cardiac failure and is assumed to be the principal cause of death (Klatsky 2002). Thus, several of the trace elements that were increased in the sclerotic heart valves have been associated with cardiovascular dysfunction. This relation may indicate that a long-term inflammatory/infectious process induces accumulation of several trace metals that adversely interact with the disease process.

Mn, regarded as an antioxidant trace element (Barandier *et al.* 1999), was increased in the patients' blood but not in the sclerotic valves. Mn has been shown to dose-dependently increase its concentration in the blood after EDTA-chelating treatment (Ilbäck *et al.* 2000). However, with increasing doses of EDTA and concomitant increases in Mn concentrations, adverse effects on cardiovascular function occurred and became gradually more pronounced. At the EDTA doses at which severe cardiovascular dysfunction occurred, Cd concentration was also greatly increased. Thus, it is uncertain whether the dysfunction observed in that study was caused by Mn, Cd, or both, or some unknown factor.

The patients had increased concentrations of Cd, both in the blood and in the sclerotic valves. The Cd binding protein MT can be induced by both Cd (Brzoska 2001) and infection (Funseth *et al.* 2002; Palmiter 1998). Consequently, Cd has been shown to dose-dependently accumulate in the heart in viral myocarditis (Wicklund-Glynn *et al.* 1998) and also to adversely affect the outcome of atherosclerosis (Meijer *et al.* 1996). Therefore, the increase in blood Cd concentration may indicate immune activation and concomitant MT induction and Cd redistribution.

The patients also evidenced increased concentrations of Mg, both in the blood and in the sclerotic valves, which is not known to be associated with disease. Mg deficiency, on the other hand, may cause cardiac arrhythmia and sudden death (Altura & Altura 1991).

Noteworthy was the finding of women having higher serum Cd and Pb concentrations than men. Only four women, compared with 12 men, had previously been smokers, which rules out smoking as a source of the Cd increase in the women. Furthermore, Ca as well as Pb concentrations in the sclerotic valves were higher in women than in men. This latter finding may indicate a higher lifetime degradation rate and turnover of bone tissue in women and a concomitant flux of bone-derived trace elements to a diseased valve.

In conclusion, patients with aortic sclerosis have a disturbed trace element balance, both in blood and sclerotic valve tissue, and some of the elements showed significant co-variation between these two compartments. Changes in blood Cu and Zn concentrations are indicative of a low-grade infectious/inflammatory process. For most of the trace elements, the disease seems to be associated with a complex competition/exchange.

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