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Selenium- and zinc-deficient cardiomyopathy in human intestinal malabsorption: preliminary results of selenium/zinc infusion

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Aims Patients with intestinal malabsorption may develop cardiac dysfunction the origin of which is often unclear. We sought to investigate the pathogenesis of dilated cardiomyopathy in human malabsorption.

Methods and results Eighteen patients with intestinal bypass as treatment for severe obesity and cardiomyopathy underwent endomyocardial biopsy. Biopsies were processed by histology, electron microscopy, polymerase chain reaction (PCR) for cardiotropic viruses, instrumental neutron activation analysis (INAA) of 33 myocardial trace elements, and assessment of glutathione peroxidase (GPX) activity and LC3-II expression. Histology and electron microscopy showed hypertrophy/degeneration of cardiomyocytes with pronounced cell autophagy and high expression of LC3-II. PCR was negative for viral genomes. INAA showed severe myocardial selenium (Se) and zinc (Zn) deficiency and reduced GPX activity vs. both patients with idiopathic dilated cardiomyopathy and normal controls. Se and Zn were added to antifailing heart therapy in 10 patients (group A1) agreeing to a control biopsy, and the response was compared with that of 8 patients (group A2) on supportive therapy alone. After 6 months, myocardial normalization of Se, Zn, LC3-II, and GPX in group A1 was associated with recovery of cardiomyocyte degeneration and autophagy, and significant improvement in cardiac dimension and function, that remained unchanged in group A2.

Conclusion A reversible Se- and Zn-deficient cardiomyopathy may occur in patients with intestinal malabsorption. It is characterized by decline of myocardial antioxidant reserve, oxidative damage of cell membranes, and enhanced cell autophagy.

Keywords Malabsorption • Cardiomyopathy • Trace elements

Introduction

Chronic intestinal malabsorption is frequently associated with disorders of cardiac rhythm and function. Although various mechanisms such as chronic anaemia, autoimmune myocarditis,[1] absorption of bacterial toxins, and loss of essential nutrients have been advocated, very often the specific pathogenetic link remains unknown. Even histological and ultrastructural analysis of myocardial tissue can be inconclusive, often showing degenerative non-specific changes. In this context the search for myocardial viral genomes by polymerase chain reaction (PCR) as well as the assessment of myocardial trace elements by instrumental neutron activation analysis (INAA) can be highly informative. In fact myocardial viral infection has an increased prevalence in patients with intestinal malabsorption,[2] while deprivation of trace elements such as selenium (Se) loss can induce a progressive heart muscle disease

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indistinguishable from idiopathic dilated cardiomyopathy.[3,4] In addition, Se and zinc (Zn) deficiency has been found to impair the immune system, increasing viral genome virulence and patients' susceptibility to infectious diseases.[5–7] Nevertheless, there is no study in the literature conducting a systematic evaluation of myocardial micronutrients in patients with chronic intestinal malabsorption and cardiomyopathy, andactual knowledge is confined to rare case reports analysing single trace elements.[8,9] Even less it is known about the mechanism through which deprivation of Se or other trace elementcan cause myocardial damage.

Conversely, identification of the cause and mechanism of cardiomyopathy has practical implications since Se administration has been shown to revert the cardiac dysfunction associated with Keshan disease.[9,10]

The aim of the present study was to analyse the structural changes and the possible mechanism of damage in dilated cardiomyopathy occurring in patients with chronic intestinal malabsorption. Preliminary results of Se/Zn administration are also reported.

Methods

Patient population

From January 1998 to December 2008 in our institution 1233 consecutive white Italian patients (666 males and 567 females, mean age 46.5 $\pm$ 15.8 years) were admitted because of chronic ($\geq$6 months) unexplained left ventricular (LV) dilatation and dysfunction unresponsive to antiheart failure therapy including digitalis, furosemide, enalapril, and carvedilol for $\geq$3 months. Among them, 32 patients had intestinal malabsorption due to intestinal bypass because of severe obesity. Eighteen of these patients (time from intervention 12.5 $\pm$ 2.2 years) gave their consent for an invasive study and constituted our patient population (group A). No patient had hypertension, drug or alcohol abuse, ischaemic heart disease, or systemic disorders. No skeletal muscle and/or additional systemic symptoms were present.

Cardiac studies

All patients underwent both non-invasive and invasive cardiac studies including LV endomyocardial biopsy, performed using a Bipal bioptome (Cordis Corporation, NJ, USA).

The LV approach was preferred to the right ventricular approach in order to minimize sampling error as the right ventricle usually appeared normal or barely affected. Four to six LV samples were drawn, processed for histology, immunohistochemistry, and electron microscopy, and the results were read by a pathologist blinded to the clinical data.[11] Additional samples were snap-frozen in liquid nitrogen for molecular biology studies, trace element assessment, LC3 protein evaluation, and determination of glutathione peroxidase (GPX) activity. LV endomyocardial biopsies from 15 sex- and age-matched patients with idiopathic dilated cardiomyopathy, no myocardial viral genomes or histological evidence of inflammation, with a comparable degree of LV compromise were used as controls. Additional non-dilated cardiomyopathy controls were papillary muscle surgical biopsies from 15 sex- and age-matched patients with mitral stenosis and normal LV undergoing mitral valve replacement. The latter endomyocardial fragments, although not obtained from healthy individuals, were derived from a non-overloaded chamber and were considered the nearest samples to a normal endomyocardial tissue.[12] Blood samples were collected from patients and controls, and stored at -80°C as whole blood, serum, and plasma samples. The study project received approval from our Ethics Committee.

Histopathological studies

Multiple 5 μm thick sections were stained with haematoxylin–eosin, Miller's elastic Van Gieson, and Masson's trichrome, and examined by light microscopy. In all patients, immunohistochemistry for the characterization of inflammatory infiltrates (CD45, CD43, CD45RO, CD20, CD68, CD4, and CD8) was performed.

The presence of apoptotic cardiomyocytes was evaluated by means of *in situ* ligation of hairpin probes with single-base 3′ overhangs.[13]

Morphometry using a grid and point counting system with 42 sampling points[11] (105844, Wild Heerbrugg Instruments, Gals, Switzerland) was applied on histological slides stained with Masson's trichrome at $\times$ 400 magnification to assess the myocardial volume composition (percentage area occupied by myocytes, extent of fibrosis defined as interstitial, perivascular, and replacement type, and other components, i.e. vessels and interstitial cells). In addition, the endocardial thickness was computed and the percent age of fibrous tissue, elastic fibres, and smooth muscle cells was evaluated in elastic Van Gieson-stained sections. Finally the cardiomyocyte diameter across the nucleus in 50–100 cells cut transversely was computed. Nikon Nis Elements BR software was utilized.

LC3 protein assessment

Autophagy is an intracellular degradation process that is characterized by the formation of double-membrane vesicles, known as autophagosomes, which sequester cytoplasm. It has been shown to be involved in cell growth, survival, development, and death, and its dysregulation has been linked to many human pathologies, including heart diseases. In mammalian cells, autophagy is a largely unknown process, and only recently mammalian homologues of yeast genes have been discovered and used as molecular markers for autophagy. LC3, one of the homologues of yeast ATG8, is modified during the autophagic process. The C-terminal region of LC3 is cleaved, generating a soluble form known as LC3-I, which, in turn, is lipidated, generating an LC3–phospolipid conjugate (LC3-II). LC3-II localizes to autophagosomes and autolysosome membranes. Therefore, the amount of LC3-II in mammalian cells is regarded as a good marker for the formation of autophagosomes.[14] Thus, we used this marker to test and quantify the induction of the autophagic process in the myocardium of patients with idiopathic and malabsorption-associated cardiomyopathy (see Supplementary material online).

Molecular biology studies

PCR and reverse transcription–PCR (RT–PCR) analysis was performed on two frozen endomyocardial tissue samples to search for the most common DNA and RNA cardiotropic viruses as described.[15]

Assessment of myocardial trace elements

The metal content of biopsies and sera was determined by INAA.[16]

The following elements were analysed: silver (Ag), arsenic (As), gold (Au), barium (Ba), calcium (Ca), cadmium (Cd), cerium (Ce), cobalt (Co), chromium (Cr), caesium (Cs), europium (Eu), iron (Fe), hafnium (Hf), mercury (Hg), iridium (Ir), lanthanum (La), lutetium (Lu), molybdenum (Mo), nickel (Ni), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), samarium (Sm), tin (Sn), strontium (Sr), tantalum (Ta), terbium (Tb), thorium (th), uranium (U), ytterbium (Yb), zinc (Zn), and zirconium (Zr).

The detailed methodology is reported in the Supplementary material online.

Determination of glutathione peroxidase activity

Se-dependent GPX activity was measured spectrophotometrically in myocardial tissue following the decrease in absorbance at 340 nm, using H_2O_2 as substrate.[17] Data are expressed as nanomoles of NADPH consumed per minute per milligram of protein.

Treatment and follow-up

Patients with malabsorption who were prepared to undergo an invasive follow-up including a control endomyocardial biopsy were treated with Se/Zn infusion (Addamel N 10 mL i.v. corresponding to Se 300 μg and Zn 13.6 mg every day for 1 week and every week for 6 months) in addition to chronic (>6 months) supportive therapy including digitalis, diuretics, angiotensin-converting enzyme (ACE) inhibitors, and carvedilol (group A1, $n = 10$). Eight patients who declined an additional invasive study were treated only with anti-heart failure therapy (group A2, $n = 8$) and constitute our control population. Clinical assessment, resting electrocardiogram (ECG) and two-dimensional echocardiography were performed at baseline, weekly during the first month, and every 4 weeks for the remaining 5 months. In all members of group A1, cardiac catheterization, angiography, and endomyocardial biopsy were performed at 6 months. The New York Heart Association (NYHA) class was used to assess functional capacity and was determined by means of a questionnaire.

Echocardiographic studies were performed with an Agilent Sonos 5500 (Hewlett-Packard, Palo Alto, CA, USA). Patients were imaged and data were analysed offline by a single senior echocardiographer blinded to the treatment groups. Echocardiographic parameters were determined according to established criteria. In particular, the ejection fraction (EF) was calculated in the apical four- and two-chamber views from three separate cardiac cycles using the modified Simpson's method.

Statistical analysis

Continuous variables are presented as the mean ± SD (standard deviation). Categorical variables are presented as absolute frequencies or percentages. Between-group comparisons of variables showing normal distribution and homogeneous variance (as assessed by Levene's test) were performed with one-way analysis of variance (ANOVA); in the case of between-group significant differences on one-way ANOVA, a post-hoc analysis was performed with the Scheffé test. Between-group

Table 1 Comparative echocardiographic, morphometric, trace element, glutatione peroxidase activity, and LC3-II data among patients with malabsorption-associated cardiomyopathy (group A) idiopathic dilated cardiomyopathy (group B), and non-dilated cardiomyopathy controls (group C)

Variables[a]	Group A ($n = 18$)	Group B ($n = 15$)	Group C ($n = 15$)	*P*-values
Age, years	57.5 ± 6.2	54.8 ± 7.9	50.7 ± 6.5	NS
Sex				NS
Male	11 (61%)	9 (60%)	8 (53%)	
Female	7 (39%)	6 (40%)	7 (67%)	
ECG findings				
Sinus rhythm	12 (67%)	11 (73%)	15 (100%)	NS
Atrial fibrillation	6 (33%)	4 (27%)	0	<0.001 A vs. C, <0.001 B vs. C
Heart rate	101.7 ± 9.4	103.3 ± 5.4	77.0 ± 7.3	<0.001 A vs. C, <0.001 B vs. C
Left bundle branch block	4 (22%)	4 (27%)	0	<0.001 A vs. C, <0.001 B vs. C
Repolarization abnormalities	16 (89%)	12 (80%)	2 (13%)	<0.001 A vs. C, <0.001 B vs. C
Echocardiography				
LVEDD, mm	67 ± 5.1	68.2 ± 5.7	47.2 ± 3.4	<0.001 A vs. C, <0.001 B vs. C
EF, %	27.6 ± 5.9	27.8 ± 3.9	61.0 ± 4.9	<0.001 A vs. C, <0.001 B vs. C
Morphometry				
Total fibrosis,%	18.0 ± 8.2	19.7 ± 5.3	3.3 ± 1.3	<0.001 A vs. C, <0.001 B vs C
Cardiomyocytes, %	81.2 ± 8.1	79.8 ± 5.7	96.1 ± 1.5	<0.05 A vs. C, <0.05 B vs. C
Other components, %	0.8 ± 0.5	0.7 ± 0.4	0.6 ± 0 .2	NS
Endocardial thickness, μm	38.4 ± 2.1	36.9 ± 7.1	12.5 ± 2.8	<0.001 A vs. C, <0.001 B vs. C
Cardiomyocyte diameter, μm	31.8 ± 3.9	30.9 ± 2.6	12.0 ± 1.3	<0.001 A vs. C, <0.001 B vs. C
Cardiomyocyte apoptosis	2084 ± 593/10^6	1989 ± 593/10^6	20 ± 10/10^6	<0.001 A vs. C, <0.001 B vs. C
Myocardial Se content (ng Se/g tissue)	172.0 ± 34.3	342.2 ± 26.5	411.5 ± 67.0	<0.001 A vs B, <0.001 A vs. C
Serum Se content, μg/L	71.9 ± 5.5	127.1 ± 18.3	146.4 ± 19.4	<0.001 A vs. B, <0.001 A vs. C
Myocardial Zn content (ng Zn/g tissue)	11 639.3 ± 2949.0	17 160.0 ± 3595.1	19 790.0 ± 6692.7	<0.001 A vs. C, <0.001 A vs. B
Serum Zn content, μg/L	748.0 ± 122.5	1131.4 ± 227.4	1260.3 ± 536.1	<0.050 A vs. C, <0.050 A vs. B
GPX activity (nmol/NADPH/mg protein)	6.2 ± 1.8	11.4 ± 2.6	13.2 ± 2.1	<0.001 A vs. C, <0.050 A vs. B
LC3-II (arbitrary densitometric units)	130 ± 24	75 ± 15	29 ± 16	<0.001 A vs. C

ECG, electrocardiogram; EF, ejection fraction; GPX, glutathione peroxidase; LVEDD, left ventricular end-diastolic diameter; NS, non-significant.
[a]Continuous variables are presented as mean ± SD. Categorical variables are presented as number and percentages.

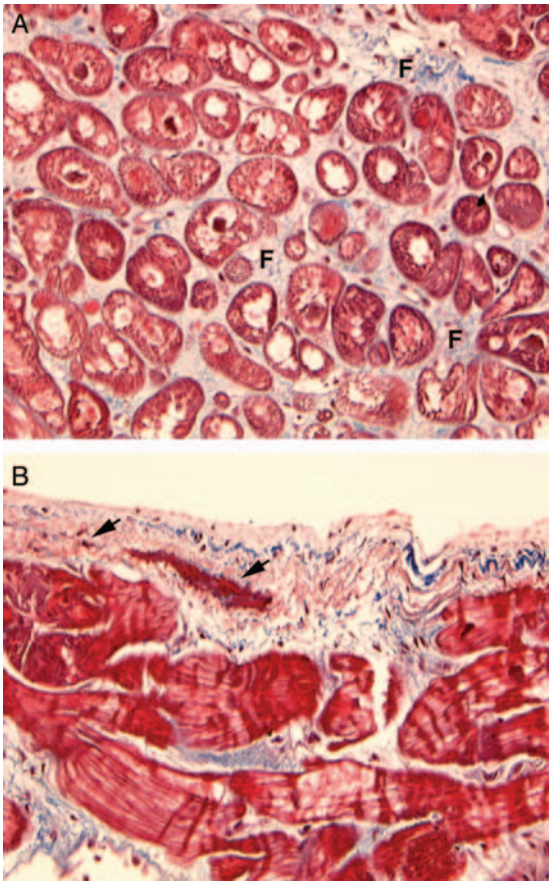

Figure 1 Myocardial histological changes in patients with malabsorption cardiomyopathy. (*A*) Hypertrophy with degeneration of cardiomyocytes associated with interstitial and focal replacement fibrosis (F). (B) A thickened endocardium with clear hypertrophy and proliferation of smooth muscle cells (arrows). Masson trichrome, magnification $\times 200$.

comparisons of variables not showing normal distribution were performed with the Kruskal–Wallis test; in the case of overall between-group significant differences with the Kruskal–Wallis test, direct comparisons were performed with the Mann–Whitney test. The categorical variables were compared by χ^2 test or Fisher's exact probability test (in the case of two-by-two contingency tables with an expected cell count of <5). In the case of multiple comparison, Bonferroni's correction was applied to control the experimental type I error probability. Changes of variables before and after treatment were analysed with the paired *t*-test or the signed rank test in the case of non-normality. A two-tailed *P*-value ≤ 0.05 was considered statistically significant.

Results

Patient population and cardiac studies

No cases of occupational exposure to trace elements were observed, not did any patient or control subject come a from heavily polluted geographic area. None had a recent history of a flu-like syndrome. Coronary angiography was normal in all. Clinical and echocardiographic data of the 18 patients and comparison among the three groups are shown in *Table 1*.

Histological, morphometric, and ultrastructural analysis

Histology of LV biopsy fragments from each patient ruled out specific heart muscle diseases such as myocarditis, and storage or infiltrative heart muscle diseases, whereas signs of hypertrophy with attenuation and degenerative changes of myocardial fibres were seen (*Figure 1*). No distinctive histological changes were observed between those with idiopathic dilated cardiomyopathy and malabsorption-associated cardiomyopathy (*Table 1*). In particular the total fibrosis, including the replacement, interstitial, and perivascular components, was increased, being represented mainly by the replacement type in both groups compared with controls (*Figure 1*). The endocardium was thickened because of increased fibrous tissue and hypertrophy and proliferation of smooth muscle cells (*Figure 1*). Electron microscopy showed degenerative changes to consist of loss of contractile elements, the presence of myelin bodies, and vacuolization of mitochondria with fragmentation of mitochondrial cristae in both groups (*Figure 2*). However, in patients with malabsorption-associated cardiomyopathy, remarkable evidence of cell autophagy was also detected (*Figure 3*). Apoptosis was increased to a degree comparable with those with idiopathic dilated cardiomyopathy (see *Table 1*). The presence of a consistent amount of myocardial fibrosis suggests necrosis to have an appreciable role in malabsorption cardiomyopathy.

LC3 protein assessment

LC3 assessment showed an accumulation of LC3-II in patients with dilated cardiomyopathy due to malabsorption, whereas samples from control patients or from patients with idiopathic dilated cardiomyopathy showed a lower amount of LC3-II (*Figure 2*).

Molecular biology studies

In all patients with malabsorption and with idiopathic dilated cardiomyopathy, as well as the controls, PCR and RT–PCR were negative for the presence of myocardial virus infection.

Assessment of myocardial trace elements

Patients with malabsorption showed a remarkable decrease in myocardial and serum Se and Zn concentration (*Table 1*). The results for the remaining detectable trace elements are listed in *Table 2*.

Glutathione peroxidise activity

Reduced myocardial GPX activity was evident in patients with malabsorption vs. both those with idiopathic dilated cardiomyopathy and normal controls (*Table 2*).

Treatment and follow-up

After 6 months of treatment, a normalization of serum Se/Zn levels (*Table 3*) was observed in group A1, accompanied by a reduction in heart rate, an enhancement in QRS voltages, and improvement of repolarization abnormalities. These changes were associated with

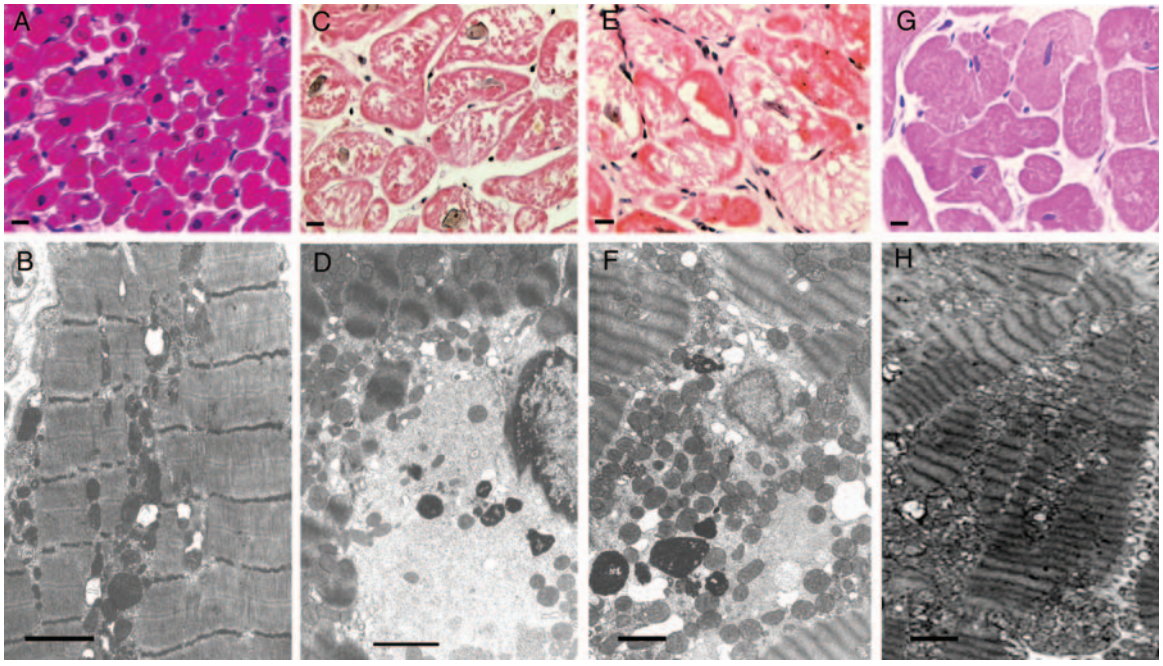

Figure 2 Histology and transmission electron microscopy (TEM) of normal (*A* and *B*), idiopathic dilated cardiomyopathy (*C* and *D*), and malabsorption-associated cardiomyopathy before (*E* and *F*) and after (*G* and *H*) Se/Zn administration. The figure shows cardiomyocyte degenerative changes that in malabsorption improved after trace element administration. Haematoxylin and eosin, ×400 (A, C, E, G). Histology scale bar = 10 μm; TEM scale bar = 2 μm.

an improvement in at least one NYHA class, a reduction in LV end-diastolic diameter, and an increase in LV EF (*Table 3*). Conversely, in group A2, serum Se/Zn levels, ECG findings, and LV dimensions and function remained unchanged (*Table 3*).

In all group A1 patients after 6 months of treatment an improvement in degenerative changes, consisting of a reduction in cardiomyocyte vacuoles due to an increase in myofibrillar content, remarkable reduction of autophagic bodies (*Figure 1*), reduction in LC3-II levels, and significant increase in myocardial Se and Zn content and GPX activity, was observed (*Table 3*).

Discussion

Dilated cardiomyopathy is a clinical entity characterized by progressive cardiac dilatation and dysfunction which may involve various aetiologies and mechanisms of damage.[18] Cardiomyopathy associated with intestinal malabsorption has been variously attributed to chronic anaemia, autoimmune myocarditis, absorption of bacterial toxins, and loss of essential nutrients.[19] In our study, neither remarkable anaemia (haemoglobin <10 g/ dL), nor myocarditis, nor detectable viral genomes were observed, while hypertrophy with degeneration and death of cardiomyocytes was associated with myocardial deficiency of Se and Zn at INAA of LV endomyocardial biopsies. Se and Zn were similarly low in the homologous serum samples; however, in accordance with previous reports,[8,20] cardiomyopathy was not associated with skeletal muscle and/or additional systemic symptoms.

Instrumental neutron activation analysis and myocardial trace elements

INAA has been shown to be the most advantageous method for quantitative determination of trace elements in human tissues owing to its high level of precision, high specificity, and high accuracy.[21]

In our study a remarkable reduction of myocardial and serum Se and Zn content was shown in patients with cardiomyopathy and intestinal malabsorption in comparison with subjects with idiopathic dilated cardiomyopathy and normal controls.

Selenium/zinc loss and myocardial antioxidant reserve

Se and Zn are incorporated in the active site of several human enzymes. In particular, Zn is an essential component of nearly 100 enzymes where it exerts a catalytic, structural, and regulatory function.[7] Its deficiency may impair gene expression and hormonal release, compromising grow and development; it may depress the immune system and favour cell apoptosis and necrosis.

Se has its own mRNA codon that specifies its insertion into proteins as selenocysteine. Selenocysteine, the 21st physiologically essential amino acid, is present at the active site of all known Se-dependent enzymes where its unique redox potential facilitates their biochemical function. Se deficiency has been associated with the development of a progressive dilated cardiomyopathy (Keshan disease) and an impairment of the immune system increasing viral genome virulence and patients' susceptibility to infections.

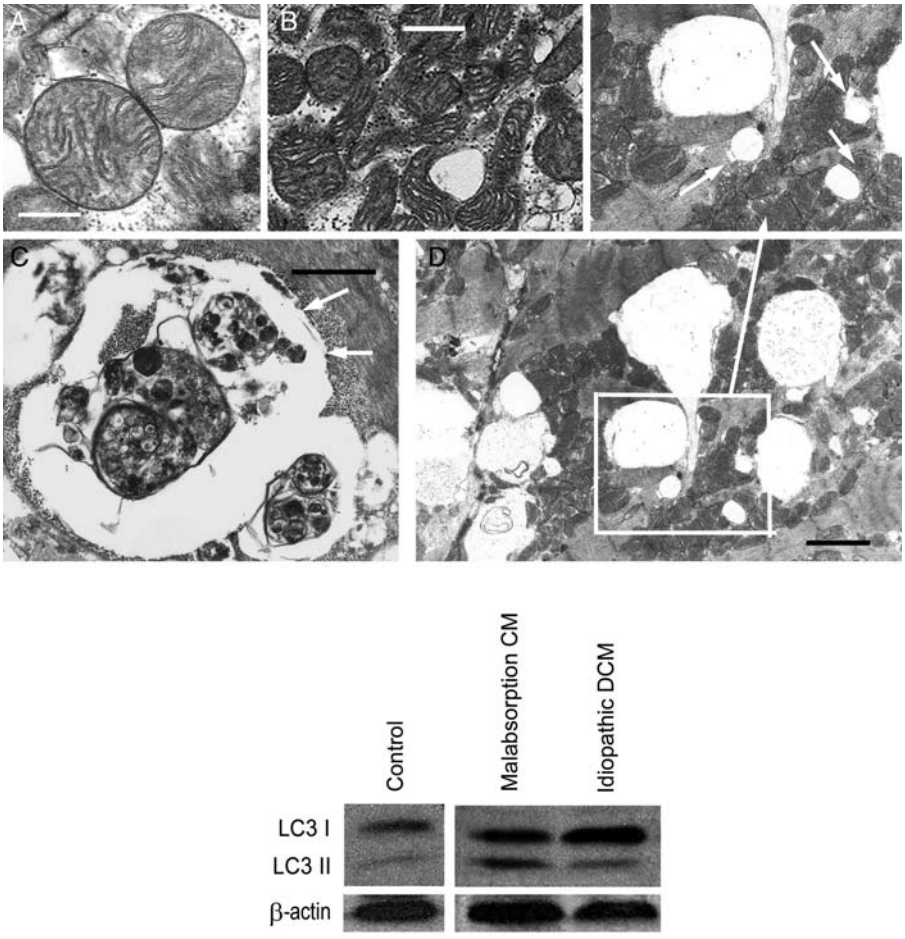

Figure 3 Ultrastructural detail of cardiomyocyte from a patient with malabsorption. (*A* and *B*) Details of mitochondria before (*A*) and after (*B*) Se/Zn supplementation. Mithochondria in *A* are swollen with a clear matrix and some membrane fragmentation (arrow); in *B* they appear to have recovered their volume and are in energized form (condensed matrix and twisted cristae). The bar represents 1 μm. In *C* and *D* several authophagosomes (arrows) are visible. The insert shows primary lysosomes close to or fusing with large phagosomes. Scale bar = 5 μm (*C*) and 2 μm (*D*). (*E*) Morphological changes are associated with increased expression of LC3-II (E). SDS–polyacrylamide gel immunoblotted for LC3 in control, malabsorption cardiomyopathy (CM), and idiopathic dilated CM (DCM). β-Actin was used as the loading control.

At the cardiac level, Zn and Se are essential for the activity of copper–zinc superoxide dismutase and GPX, respectively, enzymes that play a major role in the detoxification of cardiomyocytes from free radicals. Deficiency of both trace elements may, therefore, result in a compromise of the antioxidant property of the heart, with oxidative damage of the cell membrane and a decline of cardiac function.

In our patients, myocardial Se deficiency was accompanied by a significant reduction in GPX activity. Myocardial and serum Se were not extremely low but were significantly lower than in patients with idiopathic dilated cardiomyopathy and in controls, and were accompanied by structural changes consistent with oxidative damage. On the other hand, it is already known that there is no definite relationship between serum concentration and GPX activity. Similarly, superoxide dismutase, not tested in this study because of shortage of tissue, could have been compromised as a result of Zn deprivation con tributing to oxidative cell damage. Zn is also a structural component of metalloproteinases and its deficiency may have played a part in jeopardizing the extracellular matrix contributing to the development of cardiac dilatation and dysfunction.

The elective cardiac involvement of Se/Zn deficiency, already reported in previous studies,[8–20] could be explained by the low antioxidant reserve of the heart, averaging 1% of that expressed by the liver, and the compulsory activity of the heart compared with the intermittent and voluntary activity of the skeletal muscles.

Structural changes in selenium/zinc-deficient cardiomyopathy

Histology, immunohistochemistry, and electron microscopy showed no evidence of myocarditis, or of specific endomyocardial lesions.

Table 2 Mean serum (ng/g) and myocardial trace element concentration (ng/g) in patients with malabsorption-related dilated cardiomyopathy (group A), idiopathic dilated cardiomyopathy (group B), and non-dilated cardiomyopathy controls (group C) as determined by neutron activation analysis

Element (μg/L)	Group A ($n = 8$)		Group B ($n = 5$)		Group C ($n = 6$)	
	Serum	Myocardium	Serum	Myocardium	Serum	Myocardium
Ag	0.1 ± 0.1	43.1 ± 21.4	0.1 ± 0.2	38.1 ± 10.7	0.2 ± 0.3	42.9 ± 11.7
Au	0.07 ± 0.06	16.9 ± 10.2	0.01 ± 0.01	21.3 ± 4.7	0.01 ± 0.01	14.9 ± 10.8
Co	0.4 ± 0.2	9.9 ± 5.7	0.4 ± 0.3	45.0 ± 23.0	1.3 ± 0.2	30.4 ± 20.6
Cr	0.01 ± 0.01	251.7 ± 159.4	0.01 ± 0.01	197.9 ± 126.2	0.01 ± 0.01	177.2 ± 87.5
Cs	1.4 ± 0.9	10.0 ± 2.0	0.01 ± 0.01	14.1 ± 3.2	0.01 ± 0.01	11.7 ± 2.0
Fe	0.01 ± 0.01	26 041.0 ± 19 944.9	0.01 ± 0.01	58 969.4 ± 21 009.9	0.01 ± 0.01	26 268.3 ± 10 606.9
Hg	0.5 ± 0.6	26.3 ± 12.3	0.7 ± 0.3	153.0 ± 79.8	0.6 ± 0.2	21.3 ± 7.4
La	0.3 ± 0.1	3.8 ± 1.6	0.2 ± 0.1	2.5 ± 0.7	0.2 ± 0.09	2.4 ± 0.9
Rb	195.2 ± 44.7	40 63.5 ± 1966.3	456.9 ± 78.9	65 25.0 ± 3365.5	523.0 ± 152.1	31 50.0 ± 1266.5
Sb	0.01 ± 0.01	6.1 ± 2.9	0.01 ± 0.01	10.1 ± 5.0	0.01 ± 0.01	4.5 ± 2.1
Sc	0.01 ± 0.01	3.0 ± 1.1	0.01 ± 0.01	2.2 ± 1.3	0.01 ± 0.01	3.5 ± 1.3

Table 3 Patients with malabsorption: comparison between baseline and follow-up characteristics of the Se/Zn-treated (group A1) and untreated (group A2) cohort

Variables[a]	Group A1 ($n = 10$)		*P*-value	Group A2 ($n = 8$)		*P*-value
	Before Se/Zn	After Se/Zn		Before Se/Zn	After Se/Zn	
Age, years	56.3 ± 5.8		–		58.7 ± 6.7	–
Sex						
Male	6 (67%)		–		5 (55%)	–
Female	3 (33%)		–		4 (45%)	–
Time from intervention, years	13.1 ± 2.5		–		12.0 ± 2.9	–
NYHA functional class	3.1 ± 0.7	2.0 ± 0.5	<0.001	2.7 ± 1.2	2.7 ± 1.2	1.000
ECG findings						
Heart rate	100.8 ± 7.6	81.3 ± 5.1	<0.001	102.6 ± 10.8	101.9 ± 7.9	0.778
Repolarization abnormalities	9 (90%)	2 (20%)	0.005	7 (88%)	7 (88%)	1.000
Echocardiography						
LVEDD, mm	67.2 ± 3.4	59.9 ± 3.8	<0.001	67.0 ± 6.6	67.4 ± 6.7	0.447
EF, %	27.6 ± 7.0	41.7 ± 8.2	<0.001	27.7 ± 5.1	27.8 ± 4.4	0.886
Morphometry						
Total fibrosis, %	18.7 ± 7.6	18.3 ± 6.3	0.807	17.3 ± 6.1		–
Cardiomyocyte diameter, μm	32.3 ± 4.3	29.9 ± 4.5	<0.001	31.3 ± 4.5		–
Myocardial Se content (ng Se/g tissue)	172.3 ± 37.5	368.9 ± 50.9	<0.001	171.8. ± 33.0		–
Serum Se content, μg/L	71.7 ± 6.0	132.9 ± 12.9	<0.001	72.2. ± 5.3	72.8 ± 10.4	0.815
Myocardial Zn content (ng Zn/g tissue)	11 640.0 ± 4752.0	18 087.2 ± 4752.0	<0.001	11 638.7 ± 1810.8		0.397
Serum Zn content, μg/L	748.2 ± 103.5	1081.9 ± 198.1	0.002	745.8 ± 145.5	759.2 ± 151.5	0.150
GPX activity (nmol/NADPH/mg protein)	6.1 ± 1.9	10.9 ± 2.3	<0.001	6.3 ± 1.8		–
LC3-II (arbitrary densitometric units)	126.1 ± 18.2	51.2 ± 13.1	<0.001	133.8 ± 28.5		0.741

ECG, electrocardiogram; EF, ejection fraction; GPX, glutathione peroxidase; LVEDD, left ventricular end-diastolic diameter; NYHA, New York Heart Association.
[a]Continuous variables are presented as mean ± SD. Categorical variables are presented as number and percentages.

Hypertrophy with remarkable degeneration of myocardiocytes, presenting myofibrillolysis and enhanced cell apoptosis and autophagy, were the main morphological abnormalities and were associated with cell necrosis as suggested by extensive areas of replacement fibrosis. In particular, the presence of autophagosomes was a pronounced and widespread phenomenon that was absent

in control samples and focally detectable in patients with idiopathic dilated cardiomyopathy of similar severity. Cell autophagy was associated with elevation of LC3-II, a protein that is generated by autophagosome membranes and is, therefore, considered a reliable marker.[14] In accordance with the ultrastructural features, LC3-II was much less expressed in both idiopathic dilated cardiomyopathy and control biopsies.

Summarizing, the observed structural changes, although not strictly specific, are consistent with oxidative damage of cell membranes as well as reduction of GPX activity. These changes, together with a reduction of myocardial GPX activity, provide new insights into the pathogenetic mechanism of Se- and Zn-deficient cardiomyopathy.

In addition, the presence of extensive areas of myocardial fibrosis suggests an additional oxidative stress-mediated cell necrosis and may explain the incomplete recovery of cardiac function registered in our study as in other studies.[9]

Response to selenium/zinc infusion

Group A1 receiving a Se/Zn infusion showed a clear improvement of LV dimension and function compared with group A2 who failed to improve when receiving supportive therapy alone. Clinical improvement in group A1 was paralleled by normalization of myocardial Se/Zn and GPX activity, with striking amelioration of cardiomyocyte degenerative changes including a remarkable reduction of cell autophagy and myofibrillolysis.

Practical implications

Due to the close correlation observed between myocardial and serum trace element levels, the identification of Se and Ze deficiency can be achieved through analysis of the peripheral blood and evaluation of GPX activity in the red blood cells. Appropriate blood checking and supplementation with Se and Zn may prevent the establishment of a progressive cardiomyopathy in patients with intestinal malabsorption.

Finally Se/ Zn-deficient cardiomyopathy should be included in the American Heart Association (AHA) definition and classification of cardiomyopathies[22] as a potentially reversible myocardial disease, and American College of Cardiology (ACC)/AHA guidelines on endomyocardial biopsy[23] should consider malabsorption cardiomyopathy as a possible indication for a biopsy study.

Limitation of the study

Despite the fact that the effects of Se/Zn infusion on malabsorption cardiomyopathy appear clinically and histologically appreciable, some study limitations need to be mentioned. In particular: (i) the retrospective nature of the study design; (ii) the low number of patients enrolled with obvious consequences on statistical significance; and (iii) the type of control group that does not represent a healthy control population.

It could be argued that myocardial Se and Zn deficiency might derive from expansion of myocardial fibrous tissue which eventually become poorer in terms of trace element content. Indeed myocardial volume composition was similar in the cohort of idiopathic and malabsorption-associated cardiomyopathy, suggesting cardiomyocyte deprivation of Se and Zn in the malabsorption group.

Conclusions

An Se-/Zn-deficient cardiomyopathy may occur in patients with chronic intestinal malabsorption. It is characterized by decline of myocardial antioxidant reserve with oxidative damage of the cell membrane and enhanced cell death.

Se and Zn infusion may improve and probably prevent the malabsorption-associated cardiomyopathy. A prospective randomized placebo-controlled trial is desirable to confirm these preliminary results.

Supplementary material

Supplementary material is available at *European Journal of Heart Failure* online.

Conflict of interest: none declared.

Authors contributions: the corresponding author had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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