Calcium overload in human giant cell myocarditis

H G Olbrich, G Herrmann, G Vandeplasse, H Michaelis, M Schneider, E Krause, G Kober

Abstract

Myocardial calcium overload was observed in a patient with giant cell myocarditis. The myocardial calcium content estimated by atomic absorption spectrophotometry amounted to 120 mEq/kg dry weight, and the von Kossa stain disclosed multiple foci with patchy calcifications of myocardial fibres. Cytochemical examination of the ultrastructural calcium localisation using the phosphate-pyrosulphonate method showed considerable variation in the subcellular calcium distribution. In normal myocytes calcium precipitates were confined to the inner leaflet of the sarcolemma, T-tubules, intercalated disks, and sporadically to mitochondria. In contrast, extensive calcification of mitochondria and loss of sarcolemmal calcium was evident in necrotic myocytes. A number of grossly normal myocytes also showed an increase of calcium precipitates in slightly swollen mitochondria.

These findings suggest that myocardial calcium overload in this case started in viable myocytes and was not merely a secondary phenomenon occurring after cell death.

Myocardial calcium overload has been reported in metastatic calcification of the myocardium associated with increased serum calcium concentrations such as occur in chronic renal disease,¹ osseous lesions,² or overdoses of vitamin D,³ as well as dystrophic calcification without increased serum calcium concentrations as seen in ischaemic heart muscle^{4,5} and toxic cardiomyopathies.⁶ These also include idiopathic calcification, as seen in the hereditary cardiomyopathy of Syrian hamsters,⁷ and sporadic cases in man.⁸⁻¹⁰ In dystrophic and idiopathic calcification it still is a matter of debate whether calcium overload is a phenomenon secondary to cell death, or is a factor that itself affects viable cells and leads to irreversible cell damage.¹¹ Most of the previous reports dealing with calcification in human hearts are confined to light microscopical and biochemical findings, which probably miss the early stages in the development of calcium overload. This report shows myocardial calcification in giant cell myocarditis. Besides light microscopical and biochemical findings it describes ultrastructural features of calcium overload in this rare myocardial disease.

Case report

A 48 year old man was admitted to hospital for the investigation of rapid progressive heart failure. He had been well until two weeks previously when he was admitted to a local hospital because of fever, dyspnoea, and weakness. Pneumonia in the left lower lobe was diagnosed. He had returned from a visit to Indonesia six weeks earlier. The patient was a frequent traveller for business to tropical regions—namely, South East Asia and South America—and therefore had had continuous annual medical check-ups since 1971. There was no history of cardiac disease except for two episodes of sinoatrial block of unknown cause in 1973 and 1974. The rest of his medical history included a tonsillectomy in 1960, a thyroidectomy in 1970, scarlet fever in 1978, and repeated episodes of colitis since 1980.

On examination, the patient had a cachectic appearance (height 1.86 m, weight 64.2 kg, body surface 1.9 m²). His pulse was 112/minute and the blood pressure 90/70 mm Hg. There were signs of heart failure with an enlarged liver, distended jugular veins, and inspiratory rales at the right lung base. No peripheral oedema was present. Percussion showed that the heart was enlarged; there was a grade 2 systolic murmur and a third heart sound audible at the apex.

The complete blood count was normal except for a mild leucocytosis of 10.1/nl. Apart from a slight to moderate increase in aspartate aminotransferase activity (23 U/l), gamma-glutamyl transpeptidase (103 U/l), alkaline phosphatase (392 U/l) and a slight decrease in serum albumin (30 g/l), the routine laboratory findings, including renal function, blood glucose, and thyroid hormones were within normal limits. Serum calcium concentration and phosphate activity were normal. Cultures from specimens of venous blood, sputum, and urine were negative. The patient was positive for hepatitis B surface antigen. Serological investigations including tests for Q fever and *Trypanosoma cruzi* were negative except for titres of *Candida albicans* (1/640) and herpes zoster (IgA 0.5).

The electrocardiogram showed a first degree atrioventricular block and a complete right bundle branch block. The chest x ray pictures showed pronounced cardiac enlargement (heart volume 1637 ml/1.73 m²; normal <800 ml/1.73 m²), with moderate pleural effusions on both sides, and evidence of a pulmonary infiltrate in the postero-basal segment of the left lower lobe. The echocardiogram showed dilation of all heart chambers with severe global hypokinesia. An ultrasound

Zentrum der Inneren Medizin, Abteilung für Kardiologie, Universität Frankfurt, Federal Republic of Germany

H G Olbrich
H Michaelis
G Kober

Zentrum der Pathologie, Universität Frankfurt
G Herrmann
M Schneider

Department of Life Sciences, Janssen Pharmaceutica, Beerse, Belgium
G Vandeplasse

Zentrum der Chirurgie, Abteilung für Thorax-, Herz- und Gefäßchirurgie, Universität Frankfurt
E Krause

Correspondence to:
Dr Hans-Georg Olbrich,
Abteilung für Kardiologie,
Zentrum der Inneren
Medizin, Klinikum der J W
Goethe-Universität,
Theodor-Stern-Kai 7,
D-6000 Frankfurt/M 70,
Federal Republic of
Germany

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scan of the abdomen showed no abnormalities other than distension of the inferior vena cava, congestion of the liver, and moderate ascites. Use of cardiac catheters disclosed a severely dilated cardiomyopathy with a left ventricular ejection fraction of 21%, moderate mitral and tricuspid regurgitation, and normal coronary arteries. A biopsy specimen of the left ventricular endomyocardium disclosed evidence of an acute interstitial myocarditis with severe fibrosis.

Because of rapid progressive deterioration, a heart transplantation was required. Despite high doses of catecholamines, heart failure remained intractable. A left ventricular assist bypass was applied under general endotracheal anesthesia to maintain an adequate pressure. The following day a donor heart was available and orthotopic heart transplantation was performed, but the patient died with right ventricular failure of the donor organ, most probably due to an acute increase in pulmonary vascular resistance because of the development of shock lungs. The weight of the explanted heart was 563 g; both ventricles were severely dilated.

At necropsy the anastomoses of the implanted heart were normal. Gross inspection of the inner organs showed acute congestion of the lungs, liver, and kidneys, and a necrotising pancreatitis.

Methods

Tissue specimens were fixed in formaldehyde and processed for routine light microscopical examination using the haematoxylin and eosin and the von Kossa stains. For immunohistochemistry, paraffin wax embedded tissue specimens from the explanted heart were examined by the avidin-biotin-peroxidase complex method. The tested primary antibodies are listed in the table. For cytochemical localisation of calcium left ventricular biopsy specimens were processed using the phosphate-pyroxantimonate method.¹² Duplicate unfixed portions of the left ventricle were processed for the determination of the myocardial calcium content using atomic absorption spectrophotometry.

Results

Neither giant cells nor granulomatous inflammation were identified in the extramyocardial tissues. The histological examination of the explanted heart disclosed widespread myocardial fibrosis as well as huge patchy inflammatory infiltrates comprising neutrophils, lymphocytes, some immunoblasts and plasma cells, many macrophages and numerous multinucleated giant cells. These findings were more prominent in the septum. Inflammatory cells were often adjacent to degenerated muscle cells (fig 1a). Severely degenerated myocytes were often surrounded by a garland of activated macrophages with a tendency for confluence. Giant cells sometimes contained clods of an homogenous eosin material, apparently remnants of myocytes, and their nuclei showed a striking resemblance to those of activated macrophages. Immunohistochemical characterisation of the giant cells is shown in the table. Based on these findings, acute lymphocytic myocarditis with giant cells was diagnosed. The von Kossa stain disclosed multiple foci with patchy calcifications of myocardial fibres (fig 1b). Giant cells rarely seemed to be affected by calcification. Calcified foci were scattered irregularly throughout both ventricles and the septum, predominantly sited in areas of dense inflammatory infiltrates. The calcium content of the left ventricular myocardium, as determined by atomic absorption spectrophotometry, averaged 120 mEq of total calcium/kg dry weight.

The ultrastructural localisation of calcium with the phosphate-pyroxantimonate method in the explanted heart showed a considerable variation in the subcellular calcium distribution. In most of the morphologically healthy looking myocytes calcium deposits were regularly distributed as 20 nm particles associated with the sarcolemma, T-tubules, and intercalated disks. Only a few calcium deposits were found in the mitochondria. In several cells, even though no gross morphological abnormalities were evident, many single calcium deposits had accumulated in slightly swollen mitochondria (fig 2a). In contrast, severely injured myocytes, showing signs of irreversible cell damage, displayed an excess

Immunohistochemical markers of giant cells shown by avidin-biotin-peroxidase complex method

Markers/antibody	Clone/source	Reactions		
		Macrophages	Giant cells	Cardiomyocytes
Muscle actin*	HHF35	(-)	(-)	(+++)
Desmin†	D33	(-)	(-)	(+++)
Granulocyte associated antigen†	C3D-1	(-)	(-)	(-)
Leucocyte common antigen†	2B11 + PD7/26	(-)	(-)	(-)
Lysozyme†	rabbit	(+)	(+)	(-)
Macrophage†	KP1	(+++)	(++)	(-)
Macrophage†	MAC387	(+++)	(+)	(-)
Mycobacteria†	rabbit	(-)	(-)	(-)
Myeloperoxidase†	rabbit	(+++)	(+)	(-)
Myoglobin†	rabbit	(++)	(++)	(+++)
Myosin‡	rabbit	(+)	(+)	(+++)
Neutrophil elastase†	NP57	(-)	(-)	(-)
Alpha-1-antichymotrypsin†	rabbit	(+++)	(++)	(-)
Alpha-1-antitrypsin†	rabbit	(+)	(+)	(-)
S-100 Protein†	rabbit	(-)	(-)	(-)
Vimentin§	VIM3B4	(+++)	(++)	(-)

Staining intensity: (+) = weakly positive, (++) = moderately positive, (+++) = strongly positive. Sources of reagents: *Enzo Biochemicals, New York; †Dakopatts, Copenhagen; ‡Merck, Darmstadt; §Progen, Heidelberg.

Figure 1 Left ventricular myocardium from patient with giant cell myocarditis. (A) Area showing fibrosis, inflammatory cell infiltrate, and multinucleated giant cells (arrows). (Haematoxylin and eosin.) (B) Large area of myocardial calcification and dense inflammatory cell infiltrate. (Von Kossa stain.)

Figure 2 Localisation of calcium in the left ventricular myocardium from patient with giant cell myocarditis. (A) Calcium deposits are bound to the sarcolemma and T-tubules (long arrows). Mitochondria are slightly swollen and contain a considerable amount of calcium deposits (short arrow) but very few osmiophilic matrix granules (arrowhead). (B) Extensive calcium accumulation in swollen mitochondria of a necrotic myocardial cell.

sive accumulation of clustered calcium deposits in the mitochondria; the sarcolemma was devoid of precipitate (fig 2b).

Discussion

The term giant cell myocarditis covers a variety of rare and still poorly understood diseases. In contrast to systemic diseases being associated with the occurrence of giant cells in the myocardium, such as tuberculosis and sarcoidosis, giant cell myocarditis is confined to the myocardium.^{13 14} The diagnosis in the reported case was based on the failure to find evidence of inflammatory or granulomatous changes with giant cells in extramyocardial tissues. The aetiology and pathogenesis of giant cell myocarditis are still unknown. Viral infections and abnormal immunological phenomena have been regarded as possible causes for the disease.¹⁵ Neither isolation of nor serological evidence for an infectious organism except for a positive titre for *Candida* could be obtained in the present case. There was no evidence for *Candida* infection in the myocardium itself, but it cannot be ruled out that an abnormal immune response secondary to *Candida* infection led to the fatal disease.

The nature of the giant cells in giant cell myocarditis has been controversial and is still a matter of conjecture. They are considered to be rather a heterogeneous group of cells of both a macrophage and a myogenic origin.^{14 16-20} The giant cells observed in the present case were morphologically quite uniform. The histological and immunocytochemical findings, including the positive stain for KP1, a recently described monoclonal antibody for macrophages,²¹ suggest that these cells merely originated from macrophages. This agrees with the recent immunohistochemical investigations on giant cells carried out by Theaker *et al.*²²

A pronounced myocardial calcification was associated with giant cell myocarditis. Compared with normal data from animals as well as from humans,^{10 23} the total myocardial calcium content in our patient was very high. As the serum calcium concentration was normal this myocardial calcium overload might be regarded as either idiopathic or dystrophic.

In the present case the ultrastructural calcium distribution was localised cytochemically in a human myocarditis. In morphologically healthy myocytes the phosphate-pyroxanthionate method disclosed a calcium distribution which corresponds to that found in other

mammalian species.²⁴⁻²⁷ According to these reports, the calcium precipitate is predominantly confined to the inner leaflet of the sarcolemma, the T-tubule system, and the intercalated disks. In contrast, only a little precipitate is found in the mitochondria. The sarcolemmal bound calcium precipitate represents an exchangeable calcium pool,^{28,29} which might be related to the calcium binding of acidic phospholipids of the membrane bilayer.^{30,31} In myolytic cells we observed an extensive intramitochondrial calcium accumulation up to complete mitochondrial calcification; the plasma membrane was devoid of calcium precipitate. This might be regarded as the expression of a final intracellular calcium overload in irreversibly injured myocytes.³² Between normal myocardium and those severely damaged calcified myocytes we found a considerable variation in the mitochondrial calcium deposition. The observation of an intramitochondrial calcium accumulation in cells which otherwise morphologically appeared to be quite normal, might reflect an early stage of change in calcium homeostasis, resulting in an increased intracellular calcium concentration. We think it worth while to emphasise this as it suggests that myocardial calcium overload in this case started in viable myocytes and was not merely a secondary phenomenon occurring in irreversibly damaged cells.

The reason for myocardial calcium overload in the present case is unknown. Possible pathogenetic mechanisms include an increase in endogenous catecholamines in the final stage of heart failure combined with the contribution from intensive treatment with exogenous catecholamines.^{33,34} The focal occurrence of calcified myocytes showed by the von Kossa stain could also reflect microvascular disturbances, probably due to the inflammatory process or to increased cardiac sympathetic activity.³⁵ Furthermore, a direct toxic action—for example, an immune response leading to membrane damage with consecutive intracellular calcium gain—cannot be excluded.³⁶

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