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Are cardiomyocytes able to generate pre-amyloid peptides?

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Abstract

We performed ultrastructural testing of a cardiac biopsy taken from a heart with amyloidosis in which transthyretin mutation and light chain A amyloidosis were excluded. Cardiomyocytes of the affected heart showed accumulation of endosomal-like structures in which soluble amyloid oligomeric conformation was deposited. Intracellular accumulation of β-amyloid as well as phosphorylated tau protein seen in the immunohistochemical study suggest that the heart tissue may generate an amyloidogenic peptide leading to cardiomyocyte destruction and heart dysfunction.

Key words: cardiomyocytes, amyloidosis, β-amyloid, phosphorylated tau protein.

Introduction

Recent studies suggest that soluble oligomeric species which are intermediates in the fibril formation process in amyloid diseases may play an important role in the amyloid pathogenesis. Amyloid oligomer, a precursor of fully aggregated forms of amyloid, represents a generic conformation and it has been suggested that toxic β-aggregation processes may be characterized by a common mechanism [5,17]. Novel insights into the structure of amyloid oligomers were made possible with the development of anti-amyloid oligomer conformation-dependent antibody A11 [10]. The fact that various oligomers are A11-immunopositive suggests that amyloid oligomers share a common structure and implies that various protein-misfolding diseases may have a common pathogenic mechanism [5,17]. There is some evidence that amyloid beta (Aβ) oligomers are in fact more toxic to cells than mature fibrils [20]. Cytoplasmic pre-amyloid oligomers were observed within cardiomyocytes in desmin-related cardiomyopathy [13,18,19]. Pattison and co-workers [15] reported an animal model of cardiomyocyte amyloid oligomer expression leading to heart failure and confirmed that intracellular amyloid oligomer accumulation can contribute to cardiomyocyte death and heart failure. In the present study, we determined the preferential effect of specific Aβ aggregation in cardiomyocytes affected with restrictive cardiomyopathy (RCM).

Material and methods

An endomyocardial biopsy was performed in the right ventricle of a patient with diagnosis of idiopatic RCM. The suspected transthyretin and light chain A amyloidosis was eliminated on the basis of genetic analysis. For electron microscopy the specimen was

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fixed in 1% osmium tetroxide in the same buffer. Following that it was dehydrated and embedded in Spurr resin. The sections double stained with uranyl acetate and lead citrate were examined with a JEM II electron microscope. For indirect immunofluorescence examination cryostat sections were stained using monoclonal antisera against anti-β amyloid (NCL-β), anti-phosphorylated tau protein (NCL-Tau 2) and anti-transthyretin (DAKOA0002 Rabbit a-hu Prealbumin) as was previously presented [4,16].

Results

Ultrastructural analysis of the ventricular septal myocardium revealed an abnormal accumulation of

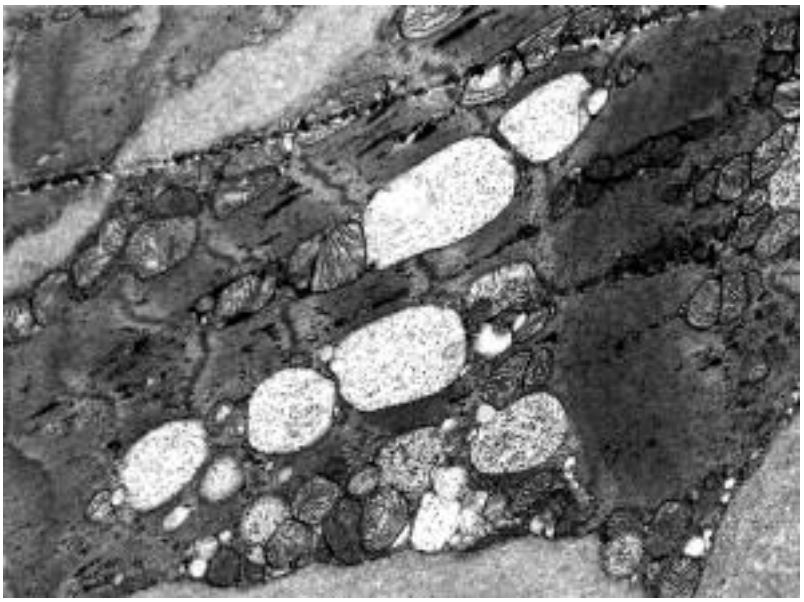

Fig. 1. Intracellular endosomal-like vacuoles distributed through cytoplasm of myocyte. × 21 000.

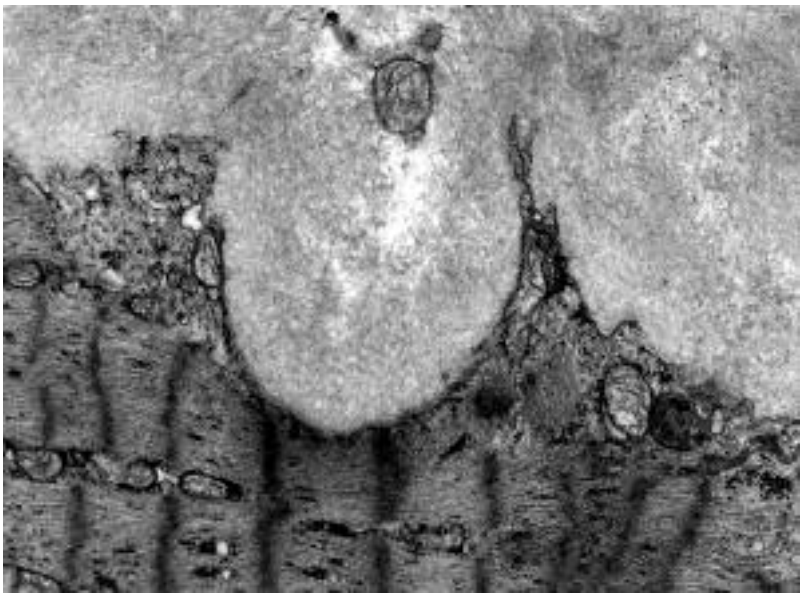

Fig. 2. Diffuse tightly packed extracellular deposit. × 30 000.

Fig. 3. Large centrally located endosomal-like vacuole. × 23 000.

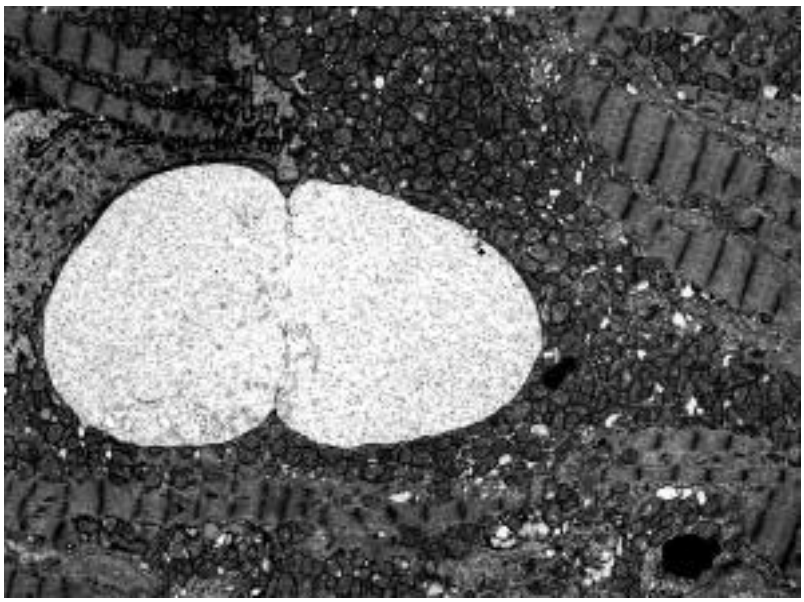

Fig. 4. Phenomenon of endosomal fusion. × 9000.

two molecularly different structures. The appearance of intracellular endosomal-like vacuoles that were irregularly distributed through the cytoplasm was the most unexpected finding observed in the numerous cardiomyocytes (Fig. 1). The diffuse tightly packed extracellular deposits closely adjacent to the surface of myocytes as well as small capillary walls were the second abnormality observed in the affected tissue (Fig. 2). Intracellular endosomal-like vacuoles were round, oval or elongated, indicating variable diameter ranging from 0.5 to 6 nm. Those compartments larger than 2 nm in diameter were frequently located centrally (Fig. 3). Some of these showed features of endosomal fusion (Fig. 4). Other, smaller vacuoles were located close to the plasmalemma (Fig. 5). In longitudinally oriented cardiomyocytes endosomal-like

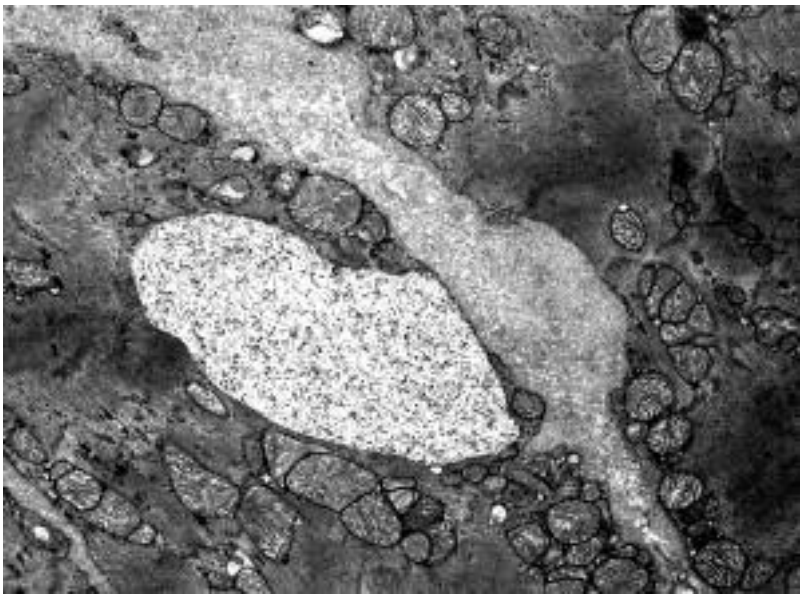

Fig. 5. Small vacuole located close to the plasmalemma. × 18 000.

structures extended along two or three sarcomeres (Fig. 6A). All these compartments contained very small osmophilic molecules that potentially had an amyloid oligomeric conformation (Fig. 6B). Extracellular deposits were manifested by the appearance of very short, small, delicate, unbranched, needle-like fibrils shaperendly distributed (Fig. 2). They were different in structure from those molecules seen in endosomal-like structures. The appearance of two different aberrant deposits in the investigated cardiac tissue prompted us to examine whether intra- and extracellularly located deposits correlated with some kind of protein activity. To reveal the nature of vacuolar contents we used two antibodies against Aβ and phosphorylated tau proteins. Figure 7 demonstrates that Aβ positive deposits were located within cardiomyocytes. The same results were obtained using antibodies against phosphorylated tau protein (Fig. 8). Activity of transthyretin antibodies was limited to the extracellular space, forming in some places delicate, intensely staining roads (Fig. 9).

Discussion

Amyloidosis is well characterized in many tissues including the heart, where it manifests with the formation and accumulation of extracellular proteinaceous fibrils [9]. Although Aβ was initially identified in extracellular amyloid plaques, growing evidence indicates that Aβ is also generated intracellularly [7]. Recently it has been proposed that cytoplasmic soluble oligomeric forms of amyloidogenic proteins or pre-amyloid oligomers are responsible for various diseases [12]. The mechanism of soluble Aβ oligomer formation remains unclear. Glabe [5,6] suggested that multiple Aβ oligomer forms were produced via different pathways, indicating the complexity of the oligomer formation mechanism. Aβ oligomers of different size and shape have been reported, accounting for their biological and structural diversity. Recent data suggest that Aβ oligomer accumulation may also play an important role in cardiac diseases. Pre-amyloid oligomer (PAO) aggregates were observed within cardiomyocytes in desmin-related myopathy [13,18,19]. In addition, Pattison *et al*. [15] presented a mode of cardiomyocyte specific PAO expression leading to heart failure. In our report, two distinct ultrastructural abnormalities seen in the affected cardiac tissue have been presented. The first is the appearance of endosomal-like structures of varying size located intracellularly within cardiac myocytes. They contained widespread deposits potentially having an amyloid oligomeric conformation. These structures, peripherally located in close contact with the plasma membrane, may create an impression that they want to exhaust an abnormal content. The most intriguing finding was the intense staining of endosomal bodies with beta amyloid and phosphorylated tau protein antibodies. Such a phe-

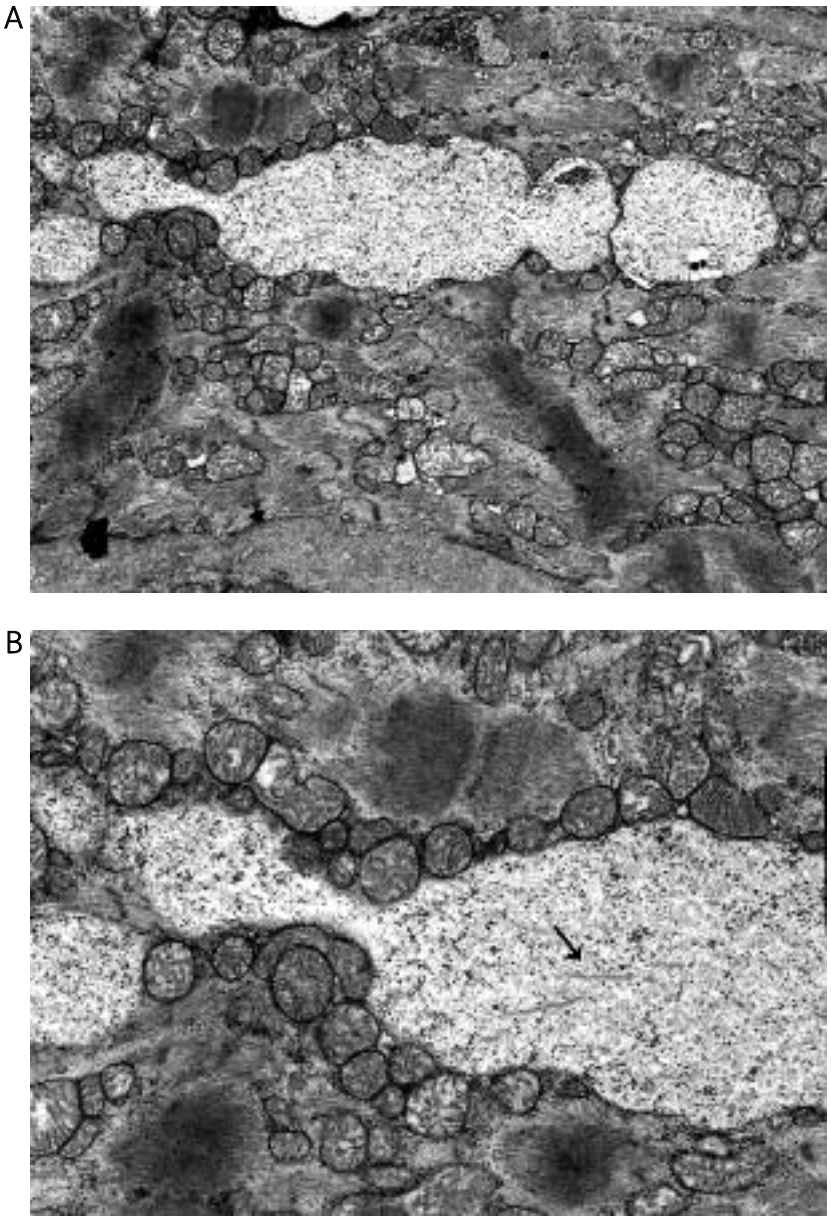

Fig. 6. A) Longitudinally oriented endosomal-like complex extended along sarcomeres. × 15 000. **B)** Higher magnification shows the tendency to filament formation (arrow). × 30 000.

nomenon was never previously observed in cardiomyocytes and may suggest that affected cardiomyocytes generate preamyloid peptides. Recently Cataldo *et al*. [1,2] reported the appearance of enlarged endosomes that coincided with an initial stage of soluble Aβ peptides and amyloid deposition. Diffuse extracellular tightly packed deposits that closely surrounded cardiomyocytes and filled the extracellular space were the second ultrastructural abnormality seen in the investigated heart. The extracellular deposit was quite different from that observed within endosomal structures. The very short needle-like fibrils closely adhered to the cardiomyocyte surface and masked the structure of the basement membrane. The extracellular matrix was negative for Aβ as well as phosphorylated tau protein. In some places, transthyretin activity was

observed as very small active points. All these findings indicate that the two types of differently located deposits show great differences in their molecular structure and immunological activity. Morphological specificity limited to the cardiac tissue allows us to speculate that generated soluble Aβ amyloid oligomeric conformation transported to the extracellular space undergoes transformation into a more mature form of preamyloid. Over the last few years, cell biology studies have supported the idea that Aβ amyloid is accumulated intracellularly from the endoplasmic reticulum to the trans-Golgi network and packed into postsecretory vesicles and the endosomal-lysosomal system [8]. Endogenous Aβ deposits were demonstrated within cultured primary neurons and within neurons of mutated mice [7]. All these data may confirm our observations presented in this report.

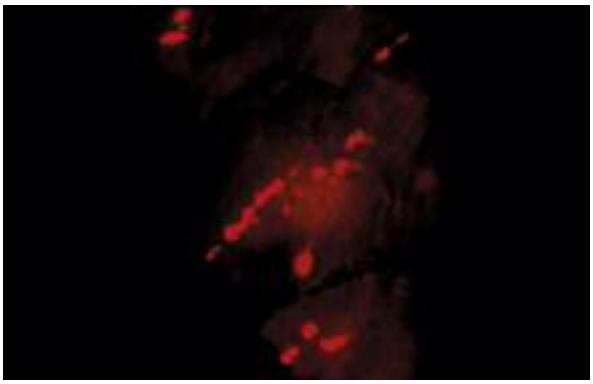

Fig. 7. Aβ positive deposits located within endosomal-like structures. × 480.

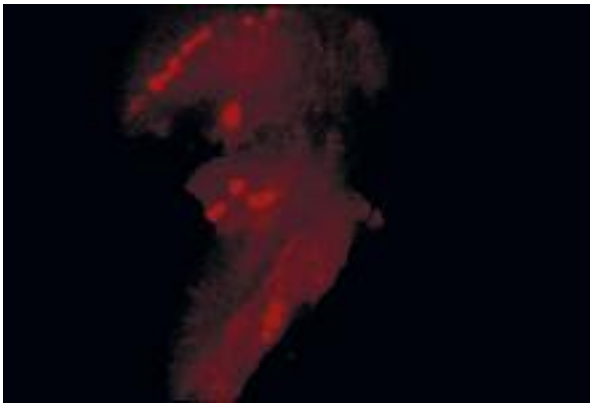

Fig. 8. Phosphorylated tau proteins decorate endosomal-like structure. × 480.

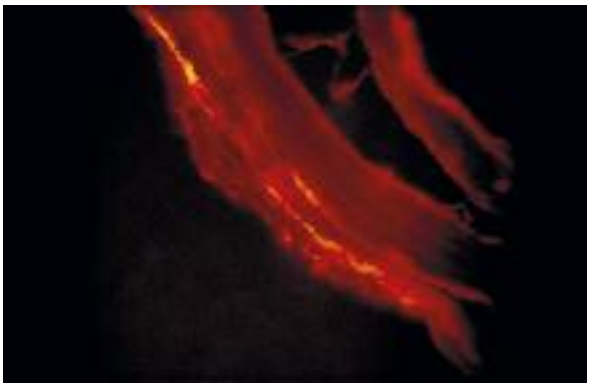

Fig. 9. Transthyretin positive deposits slightly decorate extracellular spaces. × 480.

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