The Ultrastructure of Hypertrophied Cardiomyocytes

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Biopsies of seven patients with aortic insufficiency were taken intraoperatively and examined with the electron microscope. In hypertrophied cardiomyocytes excessive Z-band material accumulated subsarcolemmally in existing Z-band zones or occasionally freely in sarcoplasma. Other ultrastructural alterations were: accumulation of glycogen granules, increase in rough endoplasmic reticulum, alterations in mitochondria and nuclei, and rise of apoptotic bodies. A mild increase of fibrillar collagen in heart interstitium was found.

Key words: cardiomyocytes, hypertrophy, biopsy

INTRODUCTION

Mechanical overload is a major factor for cardiac hypertrophy at the pressure or volume overload (1). In response to numerous other pathologic stimuli, the myocardium undergoes hypertrophic changes characterized by an increased myocardial cell size in the absence of cell division and activation of fetal cardiac genes (2). Angiotensin II (Ang II), the effector peptide of the renin–angiotensin system (RAS) regulates the volume and electrolyte homeostasis and is involved in cardiac and vascular growth. When cardiomyocytes are stretched, second messengers such as protein kinase C (PKC), mitogen-activated protein (MAP) kinases and other kinases are activated (1). How mechanical load induces cardiac hypertrophy and concomitant fibrosis remains elusive (3–5). Neither are exhaustively elucidated submicroscopical changes during the process of cardiac hypertrophy (6). It is generally accepted that cardiac hypertrophy is one of the most critical risk factors of heart diseases (7).

The purpose of this study was to evaluate ultrastructural changes in human cardiomyocytes and heart soft connective tissue skeleton in patients that developed cardiac hypertrophy of different degree due to rheumatic deterioration of aortic valves and, as a sequence, impaired camera load and pressure.

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MATERIALS AND METHODS

Biopsies of seven patients with isolated aortic valve rheumatic disease were obtained during open heart surgery intended for aortic valve replacement using cardiopulmonary bypass. Small transmural specimens were taken from the left ventricle before a cross-clamping of the aorta from the beating heart and were fixed by immersion in 4% buffered paraformaldehyde for 24 h at 4 °C, postfixed for 1 h in 2% osmium tetroxide, dehydrated in a graded series of alcohol, treated with propylene oxide and embedded into Epon. For electron microscopic examination, thin sections were cut with an ultratome, mounted on uncoated copper grids, stained with saturated uranyl acetate and lead citrate.

RESULTS

All analyzed left ventricle myocardial biopsies showed a mild increase in myocardial cell diameter and intercellular fibrosis. Cardiomyocytes were considered normal if they did not exceed 15 μ in transfer diameter and 60 μ in length. Histology and electron microscopy disclosed myocardial and myofibrillar hypertrophy of different extent in each pattern. Nevertheless, the majority of cells still had normal cellular arrangement and were connected to adjacent cells by end-to-end and side-to-side junctions.

A increase in intercalated discs and Z-bands and especially in Z-band material located subsarcolemmally in subsarcolemmally elongated masses that ex-

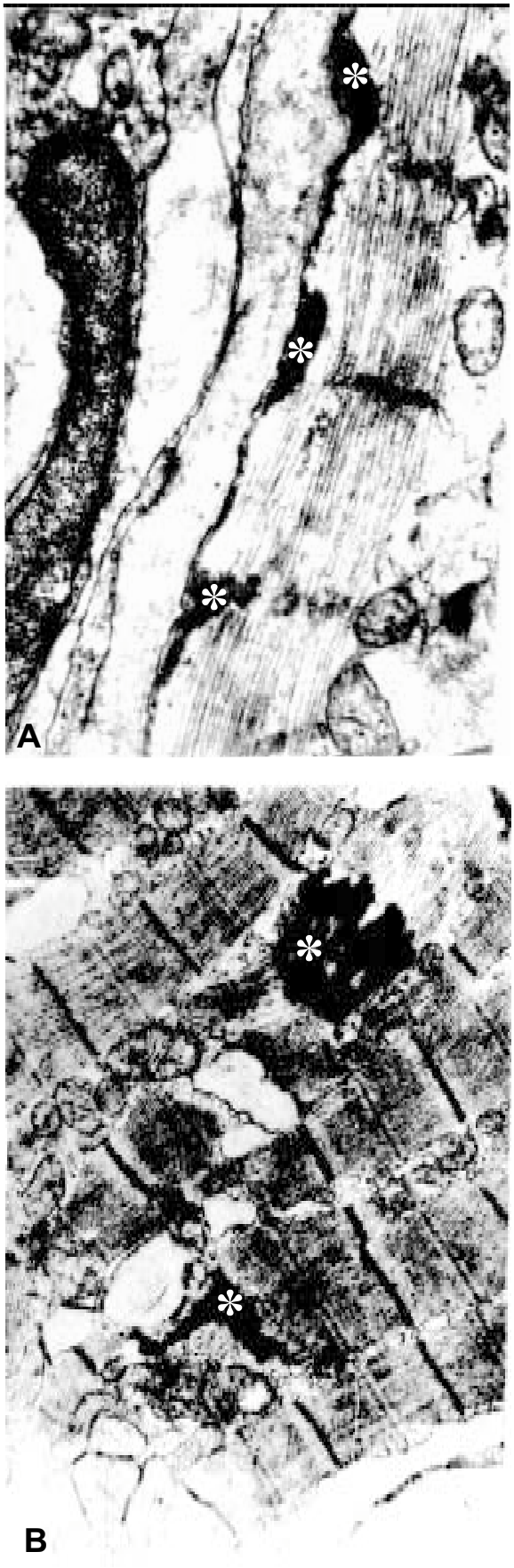

Fig. 1. Excessive Z-band material in hypertrophic cardiomyocytes (asterisc*s). A – x25000, B – x15000

tended over the length of several sarcomeres and in just existing Z-bands or freely in sarcoplasma was found (Fig. 1 A and B). Some Z bands showed focal thickening, splitting and fragmentation with irregular elongated extension of Z-band material into other regions of the myofibrils. Contractile elements adjacent to altered Z bands frequently showed focal disorganization and partial loss of myofilaments. A rough endoplasmic reticulum unusual for cardiomyocytes was present occasionally. A variable degree of degeneration of muscle cells and apoptotic bodies was present (Fig. 2 A and B). Lysosomal residual bodies were not frequent. Some myocytes looked atrophic. In others, accumulations of glycogen granules were present (Fig. 3 A). The mitochondria varied in size and shape and were located mostly between myofibrils and at the nuclear poles, occasionally arranged in aggregates. The T-system of the cardiomyocyte revealed only tiny alterations. Microscopic examination revealed mild continuos or marked (Fig. 3 B) fibrosis in the ventricular myocardium and showed apoptotic cells and bodies in the destroyed myocytes along the border of the mild fibrotic area. The pathological changes were not uniformly distributed throughout the myocardium. Most disturbances were found in the subendocardial zone.

The histological markers of acute myocyte damage were also occasionally present.

Activated lymphocytes or activated macrophages were found occasionally in the endomysium. Mast cells were lying between myocytes and in close contact with blood vessels. The density of cardiac mast cells and the intensity of their degranulation were markedly higher in patients with histological marks of myocarditis. In such patients, increased lymphocytic infiltrates and inflammatory endothelial activation were evident. Some of the cardiomyocytes were more hypertrophic, and the shape of their nuclei was bizarre. Irregular distribution of chromatin, intranuclear mitochondria or tubular nuclear structures was found only occasionally.

A morphological evaluation demonstrated increase in myocardial fibrosis. The collagen weve, struts, and strands were more evident in the subendocardial zone.

DISCUSSION

Cardiac hypertrophy develops to compensate the hemodynamic pressure overload of the myocardium-linked stretching of cardiomyocytes. However, cardiac hypertrophy itself poses a serious risk to patients with heart failure (7, 14). An increased diameter of the cardiomyocyte impairs the diffusion of

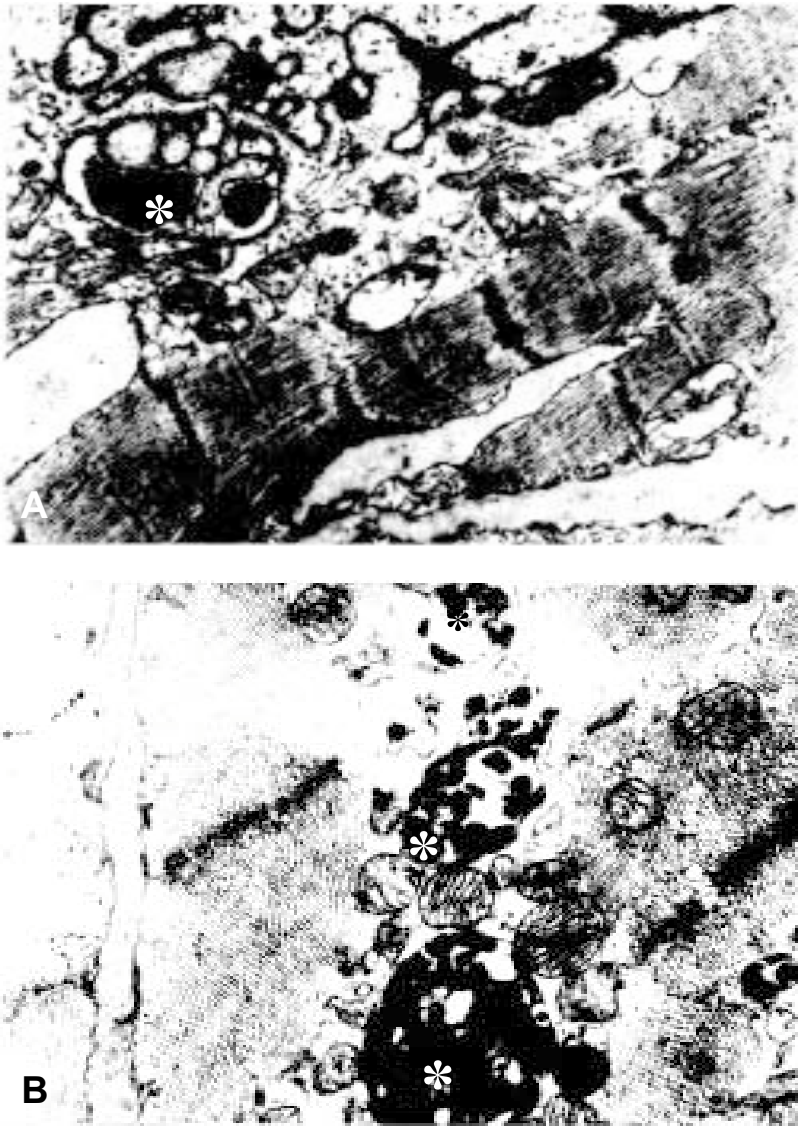

Fig. 2. Hypertrophic cardiomyocytes. A – apoptotic body (asterisc*), x20000. B – residual bodies (asteriscs), x30000

plasma components from a blood vessel to the center of the myocyte, destroys the demand–supply ratio of nutrients and metabolites, and leads to myocyte dystrophy and death.

The response of the myocardium to an increased stress load is not stereotyped. Myosin isoforms gradually change during the development of hypertrophy, whereas collagen density changes only during the chronic phase of hypertrophy (8). Our recent investigation showed a tiny collagen network consolidation in the earliest phase of heart damage (9). Cardiac hypertrophy can regress if properly treated, but the myocardium of the post-hypertrophic heart no longer has the same composition as it had before hypertrophy (8).

Loss of myocytes is an important mechanism in the development of cardiac failure of either ischemic or nonischemic origin. However, whether programmed cell death (apoptosis) is imperatively implicated in terminal stages of cardiomyocyte is not clear (10, 11). Our study showed sporadic myocityc apoptotic bodies.

Z-band changes are indicative of abortive rather than normally proceeding sarcomerogenesis. Various forms of Z-band material deposits reflect an imbalance between the rates of the synthesis of Z-band material and other myofibrillar components.

In mammalian myocardium, relaxation is mainly triggered by the reuptake of calcium from the cytosol to the lumen of the sarcoplasmic reticulum. Structural deteriorations in this organelle may lead to cell's overtiring and necrosis (12).

The renin–-angiotensin system is considered as a system in which circulating Ang II is delivered to a target cell and tissue. O Ang II evokes an increase in protein synthesis, MAP kinase activity both in myocytes and in heart fibroblasts. Autocrine and paracrine pathways of Ang II are known to promote *de novo* protein synthesis (1). The problem is that these newly introduced ways of synthesis may initiate a new step in the pathogenesis.

Endomyocardial fibrosis may be also a sequence of nonmanifested vasculitis involving the endocardium or the myocardium, or both. Myocardial fibrosis alters the myocardial structure and function. It is characterized by pathological accumulation of collagens, predominantly of types I and III. The cardiac soft skeleton is composed of nonmyocyte cells and a structural protein network which play a dominant role in governing the heart structure.

Separation of adjacent cardiomyocytes impairs not only their electric coupling, but also the tension exerted upon a given cell by the contraction of adjacent cells and microcirculation (13). This can also cause muscle cells to undergo degeneration and atrophy. Separation appears to start with dilatation of the unspecialized regions of intercalated discs and side-to-side junctions.

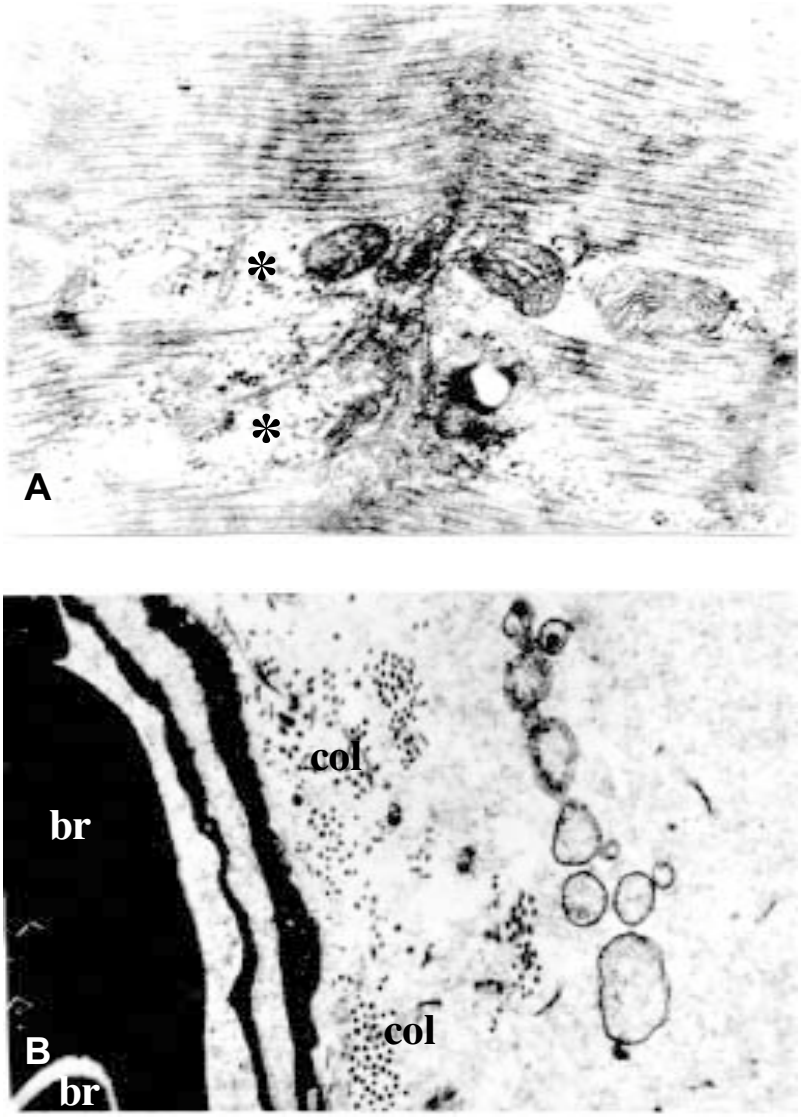

Fig. 3. Hypertrophic myocardium. A – accumulation of glycogen granules in myocyte (asteriscs), x40000. B – perivascular fibrosis (bv – blood vessel, col – collagen fibres), x10000

Myocardial remodeling is a central feature in the progression of myocardial failure secondary to cardiomyocyte hypertrophy (15, 16). Proper treatment can prevent the catastrophe.

CONCLUSIONS

Hyperthrophy of ventricular myocytes in rheumatic diseases is accompanied by mild fibrotic changes in the endomysium.

Most cellular changes of mild cardiac hyperthrophy-associated pathology are reversible.

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HIPERTROFUOTŲ KARDIOMIOCITŲ ULTRASTRUKTŪRA

S a n t r a u k a

Elektroniniu mikroskopu buvo ištirtos septynių ligonių sergančių aortos vožtuvo reumatine yda, kairiojo skilvelio biopsijos, paimtos atliekant dirbtinio aortos vožtuvo implantacijos operaciją. Nustatyta, kad hipertrofuotuose kardiomiocituose perteklinė Z linijų medžiaga kaupiasi po sarkolema, Z diskų zonose, rečiau – laisvai sarkoplazmoje. Kiti ultrastruktūriniai hipertrofuotų miocitų pokyčiai: susikaupusios glikogeno granulės, susidaręs grūdėtasis endoplazminis tinklas, pažeidimai mitochondrijose ir branduoliuose, taip pat apoptoziniai kūneliai. Šioks toks skaidulinio kolageno pagausėjimas buvo aptiktas kairiojo skilvelio intersticijume, dažniau – subendokarde. Dauguma šių pokyčių klasifikuojami kaip grįžtamieji.