Impairment of the Myocardial Ultrastructure and Changes of the Cytoskeleton in Dilated Cardiomyopathy

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This study was designed to determine the morphological correlate of chronic heart failure. Myocardial tissue from eight patients undergoing transplantation surgery because of end-stage dilated cardiomyopathy was investigated by electron microscopy and immunocytochemistry using monoclonal antibodies against elements of the cytoskeleton: desmin, tubulin, vinculin, and vimentin. The tissue showed hypertrophy, atrophy of myocytes, and an increased amount of fibrosis. Ultrastructural changes consisted of enlargement and varying shape of nuclei, numerous very small mitochondria, proliferation of T tubules, and accumulation of lipid droplets and glycogen. The most obvious ultrastructural alteration was the decrease of myofilaments, ranging from rarefication to complete absence of sarcomeres in cells filled with unspecified cytoplasm. Immunocytochemistry showed that desmin was localized at the Z lines. In diseased myocardium, the amount of desmin was increased, but it was disorderly arranged. Tubulin formed a fine network throughout the myocytes and was significantly increased in cardiomyopathic hearts. Vinculin, a protein closely associated with the cytoskeleton, occurred not only at the sarcolemma and the intercalated disc but also within the myocardial cells. Ultrastructural changes and alterations of the cytoskeleton were severe in about one third of all cells. About one third of all cells showed moderately severe changes, and the remaining cells were normal. Vimentin was present in the interstitial cells and was increased in relation to the increase of fibrosis. We conclude that the increase of fibrosis, the degeneration of hypertrophied myocardial cells, and the alterations of the cytoskeleton are the morphological correlates of reduced myocardial function in chronic heart failure. (Circulation 1991;83:504–514)

Patients with chronic dilated and progressive cardiomyopathy exhibit severe heart failure in the late stage of the disease. These patients show a decreasing left ventricular ejection fraction; they are in New York Heart Association functional stage IV; and despite drug treatment, they will die of congestive heart failure unless treated with transplantation surgery.

Morphological cellular changes in chronic heart failure due to dilated cardiomyopathy have not yet been systematically described. However, several ultrastructural studies on catheter biopsy material removed from human hearts at various stages of this disease have described hypertrophy of myocytes, degeneration of subcellular organelles, and an increased fibrosis.1–6 Catheter biopsies, however, have the disadvantage of providing only very small tissue samples from the subendocardial layer. With the development of transplantation surgery, it is now possible to study large samples of myocardial tissue from explanted hearts of cardiomyopathic recipients in a well-defined stage, namely that of heart failure. Therefore, we investigated the subcellular structure and the cytoskeleton of failing hearts from large samples of tissue from explanted hearts.

The cytoskeleton has been shown to be a well-organized structure that maintains the various subcellular organelles in their normal spatial arrangement and, thus, represents a scaffolding structure within the cells (for review, see Bershadsy and Vaziliev7). The cytoskeleton proper consists of microtubules, actin filaments, and intermediate filaments, such as desmin for muscle cells and vimentin for interstitial cells,8 but many other proteins, such as

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Vinculin and laminin, are closely linked to these structures. Furthermore, cytoskeletal organization has been discussed as one of several factors regulating the synthetic activities of the cell.7 Therefore, it seemed worthwhile to further elucidate the pathophysiology of dilated cardiomyopathy by investigating changes of the cytoskeleton and associated proteins by use of monoclonal antibodies and fluorescence light microscopy. The results of immunocytochemistry were compared with ultrastructural findings obtained by electron microscopy from the same hearts. In addition to the ultrastructural abnormalities in myocardial cells, the results indicate that alterations of the cytoskeleton appear to contribute considerably to the deterioration of contractile function of the myocardium afflicted by dilated cardiomyopathy. In addition, an increased amount of fibrosis may further compromise cardiac function, as indicated by numerous interstitial structures showing labeling for vimentin.

**Methods**

Hearts removed from eight patients at the time of transplantation surgery were investigated. All patients had chronic cardiac failure due to end-stage dilated cardiomyopathy, for which they finally underwent cardiac transplantation (for clinical data, see Table 1).

At the time of transplantation surgery, the diseased hearts were removed from each patient's chest, and 10×5×5-mm samples were removed from the left ventricular wall, were immediately quick-frozen in liquid nitrogen, and were then stored at −70°C. About 20 samples were taken from each heart from different regions of the left ventricle. All samples removed were investigated by microscopy to obtain a representative overview of the morphology of the entire left ventricle. In addition, small samples were taken from the same areas for electron microscopy.

**Control Tissue and Isolated Cells**

Cardiac tissue and isolated cells were used for controls. Because fresh human donor hearts were unavailable, left ventricular myocardium from normal pigs was prepared in an identical manner and served as control tissue.

Isolated myocytes were prepared from normal rat hearts by using the collagenase perfusion procedure described by Piper et al.9 Freshly prepared myocytes were centrifuged onto gelatin-covered coverslips and were used for further procedures.

Human fibroblasts 3T3 were grown on coverslips in culture and were used 5–7 days after seeding, which is a time of intense mitotic activity.

**Electron Microscopy**

From the explanted human hearts, small samples were taken from different areas from the left ventricle, immediately fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4, 400 mosm) at 4°C, rinsed, postfixed in 1.5% OsO₄ in veronal acetate buffer, rinsed, dehydrated in a graded series of ethanol, incubated in a mixture of propylene oxide and epon, and embedded in epon. Semithin sections of a 1-μm thickness were prepared, stained with Toluidine blue, and viewed in the light microscope. Thin sections (60 nm) were prepared, stained with uranyl acetate and lead citrate, and viewed in an EM 300 or CM 10 electron microscope (Philips, Eindhoven, The Netherlands). The typical appearances of the tissue and all abnormal ultrastructural features were recorded by photography. The findings from electron microscopy were then compared with those from immunocytochemistry.

**Immunofluorescence**

Cryostat sections 3–4 μm thick were used for all immunocytochemical reactions in human or porcine tissue. Reactions were carried out on coverslips in freshly isolated myocytes and on cultured cells. Nuclei were counterstained during 30 seconds with 0.002% propidium iodide (modified from Jones and Kniss).10

To establish the optimal method of treatment of the frozen sections and to exclude possible artifacts, all reactions with various monoclonal antibodies were carried out with different fixatives: 4% paraformaldehyde, acetone, 90% ethanol, or 100% methanol, and the optimal fixative was chosen according to the quality of the staining pattern. For each antibody, the optimal dilution was also tested. Immunostaining was considered optimal when the fluorescence was distinctly localized at certain structures, for example, cross-striation for desmin localization at Z lines, when the background was dark, and the fluorescence was intense. Treatment with Triton X was carried out in the earlier, but omitted in the

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<th>Table 1: Clinical Data in Patients With Chronic Cardiac Failure Due to Dilated Cardiomyopathy</th>
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All patients were treated with digitalis, angiotensin converting enzyme inhibitors, and diuretics.

NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; PAP, pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure.
later, experiments because no clear improvement of the localization pattern was observed. The following recipes are the final results of previous testing series.

Desmin

Frozen tissue sections of human myocardium were placed on gelatin-coated slides and treated with acetone at −20°C for 10 minutes. Subsequent processing was performed at room temperature in a moist chamber. A mouse monoclonal antibody against desmin diluted 1:4 in phosphate-buffered solution (PBS) (Boehringer Mannheim, Mannheim, FRG) was applied for 60 minutes. Several rinses with PBS were followed by incubation in biotinylated goat antimouse immunoglobulin G (IgG) 1:20 (Amersham International, Great Britain) for 60 minutes. After being rinsed several times with PBS, the sections were incubated for 30 minutes with streptavidin fluorescein isothiocyanate (FITC) at a dilution of 1:20. Rinsing with PBS was followed by mounting the tissue sections with Mowiol (Hoechst, Frankfurt/M., FRG) and coverslips.

Control tissue sections for the specificity of the immunocytochemical reactions were developed using the protocol described above, except for omission of the first antibody. These controls have been used in human and porcine tissue and in myocytes and fibroblasts for all the different antibodies tested. Normal porcine myocardium and isolated rat myocytes served as control tissue and were treated in the same way, both for a specific antigen–antibody reaction and for controls.

Tubulin

Cryostat sections of human myocardium underwent fixation with freshly prepared 4% paraformaldehyde for 10 minutes at room temperature. Rinsing with PBS was followed by incubation with a 1:1 mixture of monoclonal antibodies against α- and β-tubulin (Biomakor, Rehovot, Israel) for 60 minutes. Dilution of the antibodies was 1:500 for α-tubulin and 1:200 for β-tubulin. The sections were frequently rinsed with PBS and were then incubated with biotinylated sheep antimouse IgG (Amersham International) for 60 minutes. After rinsing with PBS, streptavidin labeled with FITC was applied for 30 minutes.

For localization of tubulin in normal myocardium, cryostat sections of porcine heart tissue and isolated myocytes from rat heart centrifuged onto coverslips were used. Positive controls were human fibroblasts in culture.

Vinculin

Cryostat sections of human myocardium were fixed in 4% paraformaldehyde at room temperature, frequently washed in PBS, and then incubated with a mouse monoclonal antibody against vinculin (Biomakor) at a dilution of 1:50 for 60 minutes at room temperature. Frequent rinsing with PBS was followed by incubation with sheep antimouse biotinylated IgG (Amersham International) for 60 minutes, rinsing with PBS, and incubation with FITC-labeled streptavidin.

For localization of vinculin in normal tissue, porcine myocardium was treated in an identical manner.

Vimentin

Frozen tissue sections were treated with acetone for 10 minutes at −20°C. A mouse monoclonal antibody against vimentin diluted 1:3 (Boehringer Mannheim) was applied for 60 minutes. After rinsing in PBS, incubation with biotinylated goat antimouse IgG 1:20 (Amersham International) was carried out. Rinsing in PBS was followed by application of streptavidin FITC for 30 minutes.

Microscopy

The sections were viewed with an Olympus Vanox T microscope at magnifications of 25×40 or 25×50 and photographed on HP5 IIford film or on Agfa professional film for color slides (Agfa-Chrome 100).

Results

Ultrastructural Changes

The cells in hypertrophic myocardium were wider and longer than cells in normal myocardium, sometimes three to four times their normal size, which is 12×45 μm (own measurements). The nuclei of these cells were enlarged, exhibiting clumping of the chro-
matin and inclusion of cytoplasmic areas (Figure 1). Frequently, the mitochondria occurred in large clusters in cytoplasm free of myofibrils, and they varied in size and shape from very small to very large (Figure 2). The lack of myofibrils in many cellular areas, in the center and in the periphery of a myocyte, was a very obvious ultrastructural feature (Figures 1–3).

Next to hypertrophied cells, other cells were atrophic and contained only remnants of myofilaments (Figure 4).

Other changes were the occurrence of prominent dilated parts of the T tubular system (Figure 5),

**Figure 2.** Electron photomicrograph showing clusters of mitochondria (mit) of various shape and size occurring in areas free of myofilaments. T, prominent T tubules. Bar, 10 μm.

**Figure 3.** Electron photomicrograph showing lack of contractile material in a cell exhibiting large areas of cytoplasm (cyt) and numerous small mitochondria (mit). Myo, remnants of myofibrils; N, nucleus; L, lipofuscin. Bar, 10 μm.

**Figure 4.** Electron photomicrograph showing two atrophic cells separated by an intercalated disc (ID). Cell on the right contains only few myofibrils but numerous lipid depositions (Li). Cell on the left exhibits myofibril-free cytoplasm but also a few myofilaments. Cells are surrounded by fibrotic tissue (F). Bar, 10 μm.

**Figure 5.** Electron photomicrograph showing dilated and numerous T tubules (T). Bar, 5 μm.
multiple intercalated discs, and accumulation of glycogen and fat droplets. Lipofuscin, myelin figures, and vacuoles were numerous.

The interstitial space was widened and contained macrophages, fibroblasts, fibrocytes, collagen fibers, and many particles of cellular debris (Figure 6). The capillaries, arterioles, and venules showed a rather normal appearance except for a thickened basement membrane. It should be mentioned that not all tissue blocks showed these changes, reflecting the focal localization of structural changes in diseased myocardium. About one third of all cells showed severe alterations, one third of the cells were normal, and the rest of the cells showed moderately severe changes.

**Immunofluorescence**

*Desmin.* In normal porcine myocardium and in rat myocytes, immunofluorescence against the intermediate filament desmin was localized at the Z lines. In isolated rat myocytes, because of the exact localization of desmin at each Z line (Figure 7), it was even possible to count the number of sarcomeres. Isolated myocytes showed an increased fluorescence at the cellular ends, indicating labeling of the intercalated discs separated by the isolation procedure. In tissue sections from porcine myocardium, the intercalated discs exhibited an irregular labeling of desmin, but all Z lines were regularly stained, resulting in a distinct and regular pattern of cross-striations.

In diseased human myocardium, desmin was localized in some areas of the myocardial cells at the Z lines, identical to that in rat myocytes or normal pig myocardium. In many cellular areas, however, desmin was irregularly distributed within the cell. It was not only present at the Z lines, generally in increased amounts, but it also occurred in the A bands and I bands of the sarcomeres and within cytoplasm devoid of myofilaments (Figure 8, top panel). Frequently, regions intensely stained for desmin were observed (Figure 8, middle and bottom panels). This distribution pattern corresponded to the irregular arrangement of sarcomeres, in particular to streaming of Z line material and to the occurrence of large cytoplasmic regions lacking myofilaments as observed in the electron microscope. This lack of contractile material occurring in areas of accumulation of desmin filaments was confirmed by immunocytochemical double staining for desmin and actin with an antibin monoclonal antibody (HHF35, Enzo Biochem, New York). Smooth muscle cells of blood vessels showed labeling with desmin, but endothelial cells did not show labeling.

*Tubulin.* In normal myocardium, that is, either in isolated rat myocytes or in porcine cardiac tissue, immunofluorescence against tubulin was evident as fine structural elements, most probably microtubules, around the nucleus and, to a lesser degree, throughout the entire cell (Figure 9).

In diseased human myocardium, tubulin was present in an increased amount, mostly bound to structural components of the cell or as diffuse immunofluorescence (Figure 10).

It is noteworthy that there was a relative paucity of labeling for tubulin in normal and diseased myocardium; that is, not all myocardial cells were positive. To test the specificity of the antibody reaction, cultured proliferating 3T3 fibroblasts were used. These exhibited a ubiquitous staining for tubulin-containing filaments as the classic cytoskeletal elements (Figure 11). Therefore, the inhomogeneous labeling for tu-
FIGURE 8. Photomicrographs showing localization of desmin in diseased human myocardium. All photomicrographs show an irregular sarcomeric structure and an increase in fluorescence intensity, that is, an increased amount of desmin. Nuclei are stained red. Dark areas are interstitium. Top panel: Moderate changes of desmin localization (arrows). Middle panel: More obvious disturbance of desmin localization (arrow). Bottom panel: Severe desmin disarrangement and accumulation (arrow).

FIGURE 9. Photomicrograph showing that localization of tubulin is sparse in normal porcine myocardium. Nuclei are stained red.

FIGURE 10. Photomicrograph showing increased amount of tubulin in diseased human myocardium.

FIGURE 11. Photomicrograph showing cultured 3T3 fibroblast with regular staining for tubulin. It was used as positive control for the tubulin-staining method. Nucleus is stained orange.
bulin in myocardial cells was assumed not to be due to technical artifacts but to the rarity of tubulin-containing structures, which is well known from ultrastructural studies.11,12

The relatively increased amount of tubulin labeling in diseased myocardium corresponds with an elevated number of microtubules as observed by electron microscopy.

Vinculin. In normal myocardium, immunofluorescence against vinculin was found as a fine line at the intercalated discs and, to a lesser amount, at the lateral border of the myocytes, that is, the sarcolemma (Figure 12). Immunostaining in the inner part of the myocardial cells was occasionally observed as small dots, most probably corresponding to the invaginations of the sarcolemma at the level of the T tubules. Occasional staining of Z lines, mostly at the periphery of the cell, was observed.

In diseased human myocardium, the immunostaining against vinculin was much more pronounced and was present in much greater amounts. Vinculin labeling appeared as wide irregular lines at the intercalated discs, was very pronounced at the sarcolemma, and also occurred as punctate staining or as rather wide irregular lines or dots within the myocardial cells (Figure 13). This localization corresponds with the ultrastructural appearance of the intercalated disc, that is, the multiplicity, the increased width, and the tortuous course in human end-stage dilated cardiomyopathy. The increased number of localization points of vinculin within the myocardial cells may be due, at least in part, to the presence of multiple, enlarged T tubules, that is, more numerous invaginations of the sarcolemma. Vinculin was also found in endothelial and smooth muscle cells of blood vessels.

Vimentin. In normal and diseased myocardium, vimentin-positive structures were found only in the interstitial space. Labeling was confined to the cellular elements, that is, fibrocytes, fibroblasts, endothelial cells, and smooth muscle cells of blood vessels. The extracellular matrix was not stained by vimentin but is readily labeled with fibronectin (unpublished results from our laboratory).

In normal porcine myocardium, labeling for vimentin was distinct, but the amount of positive cells was rather small and corresponded to the small amount of connective tissue in normal hearts. In diseased human myocardium, large areas in each tissue section were filled with vimentin-positive cells that reflect the increased amount of interstitial tissue, that is, fibrosis, in cardiomyopathic hearts (Figure 14).

The mode of labeling of connective tissue cells was similar in normal and diseased myocardium.

Discussion

The purpose of this study was to define the morphological correlates of cardiac failure in end-stage dilated cardiomyopathy. The data presented indicate that the degeneration of almost all cellular organelles, especially the lack of contractile material, in the hypertrophied cardiomyocytes represents one important component of the altered morphology. In addition, disproportionate increases of cytoskeletal proteins such as desmin, tubulin, and vinculin were present. Many myocardial cells lacking myofilaments, but containing an abnormal amount of cytoskeletal elements, will probably survive but certainly do not contribute significantly to the contractile function of the heart.

Critique of Methodology

Proper controls are usually a great problem when studying diseased human tissue because normal human hearts are scarcely available for transplantation, and even more difficult to obtain for research work. However, because the normal ultrastructure of car-
diac muscle is well known, recognizing pathological alterations is relatively easy. In the case of controls for immunocytochemical findings, normal tissues from different animal species were found to show identical patterns of labeling so that identification of pathological changes was possible. The changes, as described here, were interpreted as a true augmentation of the amount of the protein labeled because the staining pattern was different and the intensity was increased. Therefore, redistribution, rather than augmentation, was excluded as the cause for the observed changes.

In this study, an attempt was made to combine morphological results obtained by two different techniques, that is, electron microscopy and fluorescence microscopy. The great advantage lies in the fact that these two techniques complement each other; that is, structural components not visible with electron microscopy can be detected by light microscopy and vice versa. With the electron microscope, alterations of all cellular organelles and sarcomeric structures can be detected, but desmin and vimentin filaments, vinculin, and tubulin cannot be observed in a representative manner. Microtubuli, that is, the structures containing tubulin, are occasionally visible in the electron microscope, but this depends on the direction of sectioning and on the random chance that these structures, 25 nm in diameter, were occurring at the level of sectioning. At the light microscopic level, however, the fine structure of mitochondria or nuclei cannot be defined, whereas the identification of cytoskeletal proteins is possible when the localization of specific monoclonal antibodies is investigated by fluorescence microscopy. Therefore, the combination of different morphological techniques is believed to provide a better insight into the pathology of cardiac muscle.

In frozen sections, the problem of technical artifacts caused by either fixation or treatment with Triton X was tested thoroughly and was excluded as an influence because control tissue did not exhibit any of the irregular labeling pattern observed in diseased human myocardium, not all cells in human myocardium showed these changes, and the typical alterations in human myocardium were observed independent of the mode of fixation or treatment and also at the same location in serial sections from the same block. The intensity of the fluorescing pattern, however, varied in a dependent manner with the mode of fixation and was optimized individually for each antibody used.

Because we investigated several large samples of explanted myocardium instead of biopsy material, we believe that our data are representative of heart failure and explain, on a morphological basis, the structural equivalent of chronic cardiac insufficiency.

A great advantage of studying large samples of myocardium, as in the present study, lies in the fact that the heterogeneity of tissue alterations becomes obvious. The severity of the ultrastructural and of the immunocytochemical changes varied from cell to cell. About one third of all cells were estimated to be severely altered; one third were normal; and one third had alterations of medium severity. Presently, a semiquantitative study is being performed to determine these numbers more accurately.

Ultrastructural Findings

Degenerative changes included those of the mitochondria, the T tubular system, the sarcoplasmic reticulum, and nuclear material, as well as the occurrence of large amounts of lipofuscin. Nuclear changes included enlargement of nuclei and the presence of multiple nucleoli and may, therefore, be indicative of an increased synthetic activity.

The most prominent ultrastructural alteration was the loss of contractile elements so that large cellular areas were often filled with nonspecified cytoplasm containing only mitochondria, ribosomes, and glycogen. Preliminary quantitative ultrastructural results obtained in four patients showed that the content of myofilaments is reduced from 58% in normal hearts to only 32% in failing hearts.

On the basis of morphometry, a similar, but variable, reduction of contractile material has been re-
ported for dilated cardiomyopathy in human patients,\textsuperscript{2-4,6} and Pagani et al.\textsuperscript{14} using biochemical analysis, recently showed a decrease of myofibrillar protein in explanted human hearts with cardiomyopathy. Loss of myofilaments due to exhaustion of cellular adaptational processes\textsuperscript{15} can easily be imagined to contribute significantly to the reduction of cardiac function. In contrast to the reduction of contractile elements, however, were the increased levels of desmin, tubulin, and vinculin in the myocardium of failing hearts.

**Desmin**

Desmin belongs to the group of intermediate filaments,\textsuperscript{16-18} which are 7–11 nm thick and occur exclusively in muscle cells.\textsuperscript{19} Desmin filaments contribute to the structural organization of striated, including cardiac, muscle\textsuperscript{16,19,20} and have been implicated in normal muscle growth\textsuperscript{21,22} (for review, see Osborn\textsuperscript{23}). Desmin in cardiac muscle is localized at the Z lines, in the interfibrillar space between adjacent Z bands,\textsuperscript{24} and close to the intercalated disc but not immediately adhering to it, as are vinculin and α-actinin.\textsuperscript{24} Craig and Pardo\textsuperscript{25} showed localization of vinculin, spectrin, and γ-actin with desmin at the myofibrillar attachment sites at the sarcolemma. This localization described in the literature was confirmed by our study in cells from rat and pig hearts and in a smaller number of myocytes in human hearts.

An increased amount of disorderly arranged desmin material was a common finding in diseased human hearts and may correspond to similar observations in experimental hypertrophy in rat hearts\textsuperscript{26,27} and in hypertrophied human hearts.\textsuperscript{28}

Porte et al.\textsuperscript{29} in 1980 and Stocek et al.\textsuperscript{30} in 1981 described an unusual, possibly familial, cardiomyopathy in three brothers. This disease was characterized by progressive deterioration of cardiac function, significant degenerative ultrastructural alterations, and storage of material positive for desmin by immunolabeling. Significant similarities exist between the above reports\textsuperscript{29,30} and our own findings. The disarrangement of desmin filaments, that is, the lack of regular cross-striations, may be the consequence of disorganization of the contractile material. The decrease in sarcomeres in diseased myocytes seems to be compensated for by an accumulation of intermediate filaments, thereby maintaining the stability of the cells.

**Microtubules**

Microtubules were observed in normal hearts as a fine latticelike network mainly in the perinuclear regions but also throughout the entire cell.\textsuperscript{31} This is in agreement with earlier studies on microtubules in cardiac muscle by Goldstein and Entman\textsuperscript{11} and others.\textsuperscript{12,26,27,32} Interestingly, Cartwright and Goldstein,\textsuperscript{12} in a quantitative ultrastructural study, found that the number of microtubules increased from birth to a maximum of 9 days and decreased thereafter to a steady state. Increased tubulin labeling was observed by Samuel et al.\textsuperscript{26} in hypertrophied myocardium of the rat; however, they found increased labeling of microtubules only at the onset of hypertrophy and not at later stages. On the other hand, our study, which is concerned only with very late stage hypertrophy, shows increased tubulin labeling. During the phase of compensated hypertrophy, microtubule numbers may be normal and may increase only during the phases of onset and of decompensation of hypertrophy. Not all myocytes were similarly labeled for microtubules in our study, and this confirms identical results by Watkins et al.\textsuperscript{27}

Microtubules have been reported to interfere with the processes of protein synthesis.\textsuperscript{33,34} It has also been hypothesized that the cellular scaffold directs the deposition of the newly synthesized myofilament bundles\textsuperscript{12} and that, in a more general way, the cytoskeleton has a regulatory role in cellular synthetic processes.\textsuperscript{7} These, however, are apparently severely disturbed in chronic heart failure as evidenced by the lack of contractile material and the numerous other degenerative ultrastructural changes described here.

**Vinculin**

Vinculin is a protein that was first purified independently by Geiger\textsuperscript{35} and Feramisco and Burridge.\textsuperscript{36} It has been demonstrated, by immunocytochemical methods, to be present in a variety of cells, including fibroblasts.\textsuperscript{35,37} In cultured cells, it occurs at the focal adhesion plaques anchoring these cultured cells to the petri dish, and in tissue, these plaques make cell-to-cell contact.\textsuperscript{37} Pardo et al.\textsuperscript{38,39} showed recently that in cardiac muscle vinculin connects actin filaments to either the sarcolemma or the intercalated disc; they described the localization of vinculin at the sarcolemma as part of an attachment system of myofibrils to the plasma membrane; and they described the presence of vinculin in longitudinal sections along the Z line and in transverse sections within tubular invaginations, most probably the T tubular system. These findings are in agreement with our findings in normal tissue, showing that vinculin immunofluorescence was regularly present at the level of the intercalated disc and at the lateral sarcolemma. However, the findings by Pardo et al with regard to the localization of vinculin at the level of the Z lines and at tubular invaginations contrast somewhat with our results. We rarely found these to be present in the T tubular system or at the Z line.

In diseased human myocardium, an increased amount of vinculin was found at the intercalated discs and the sarcolemma as well as within the myocardial cells. The spotlike appearance of vinculin fluorescence within cells may reflect the presence of a dilated, proliferative T tubular system, a common phenomenon in dilated cardiomyopathy. However, the more diffuse distribution seems to represent localization within the cytoplasm.

Although alterations of the intercalated discs are a common ultrastructural finding in cardiomyopathy\textsuperscript{40}
and generally in hypertrophy, this fails to explain the increased amount of vinculin. The enlargement of the nuclei and nucleoli of the myocardial cells may indicate that an increased synthetic activity occurs in diseased myocardium. Vinculin may be produced in increased amounts, or it may not be degraded, leading to increased storage. The lack of contractile material may reduce the stability of the cell by disturbing the cytoskeletal structure, and this may be partially compensated for by increased vinculin production. The same mechanical explanation may also be true for the accumulation of desmin and tubulin.

Vimentin

Vimentin also belongs to the family of intermediate filaments that are major constituents of most tissues and are thought to fulfill an organizing function in the cytoplasm. Vimentin occurs in mesenchymal tissue, fibroblasts, fibrocytes, and endothelial cells and thus represents the cellular elements of the extracellular space but not the matrix or the collagen fibrils. The amount of fibrosis, therefore, is even larger than indicated by the presence of vimentin-positive structures. Nevertheless, this staining procedure shows that there is a considerable degree of fibrosis in the diseased myocardium that will further contribute to the reduction of cardiac function.

Functional Significance of the Cytoskeleton

Intermediate filaments were proposed to be mechanical integrators of cellular space; that is, they play a major structural role as scaffold within the cell. This view is confirmed by the present study showing that desmin filaments do indeed connect all Z discs, thereby preventing the sarcomeres from slipping during contraction. It is evident that microtubules and vinculin further contribute to this mechanical function so that, because of the cytoskeleton, all subcellular organelles are kept in place within the cells. The contractile elements are not only fixed by desmin filaments at the Z band level, but they also are connected to the intercalated discs and to the sarcolemma by intermediate filaments as well as by vinculin. The nucleus, on the other hand, is held in its central position in the cell mainly by the presence of microtubules and also by intermediate filaments. Under physiological conditions, all mitochondria are regularly aligned between the sarcomeres so that the cardiac cell appears to be a very well-organized system. The increase in elements of the cytoskeleton in human myocardium with dilated cardiomyopathy may, in part, represent a compensation for the loss of cellular stability due to the loss of contractile material.

It has been shown, however, that the cytoskeleton is not only involved in the maintenance of the cellular structure but also that intermediate filaments in association with microtubules play a role in intracellular particle movement, and that intermediate filaments are closely associated with endosomes and, therefore, are involved in receptor-mediated endocytosis. Microtubules are the major components in spindle formation during mitosis, and they interfere with protein synthesis. Intermediate filaments have been shown to preferentially bind to DNA and to have a high affinity for histones, thereby influencing nuclear morphology and function. According to Traub, an increase in intermediate filaments may indicate an increased turnover of proteins similar to myocardial cells undergoing hypertrophy. Intermediate filaments may be activated by a high Ca2+ concentration that, in turn, stimulates the Ca2+-activated proteinases. Therefore, in severely disturbed cardiomyopathic hearts, the inability to control calcium homeostasis may directly influence the formation of intermediate filament proteins, thereby promoting a response of the nucleic acid and protein-synthesizing machinery of the muscle cell.

In conclusion, severe morphological alterations are observed in failing human hearts. These alterations include increased size of myocytes that exhibit degeneration of subcellular organelles and lack of contractile material and an increased amount of cytoskeletal elements. The amount of interstitial tissue was also increased. We hypothesize that these hypertrophied myocytes degenerate at late stages of dilated cardiomyopathy and that they lose their contractile material because this material is not essential for cell survival, by which cellular contractility is reduced. To compensate for the loss of contractile material and the reduction in cellular stability, the different elements of the cytoskeleton and vinculin are increased. The increased amount of microtubules and intermediate filaments may also have a direct stimulatory influence on nucleic acid and cell protein synthesis. The myocardial cells then contain mostly unspecified cytoplasm and a dense cytoskeletal network that enhances the incapability for contraction. These cells are encased by fibrotic material that further inhibits the contractile function and, by its composition of inflexible cells and fibers, increases the stiffness of the ventricular wall. These morphological changes are considered to be the structural correlates of the reduced function in chronic cardiac failure.

References


KEY WORDS: cardiomyopathy • heart failure • ultrastructure • cytoskeleton
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