Structural Remodeling of Cardiac Myocytes in Patients With Ischemic Cardiomyopathy

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Background. Chronic ischemic heart disease may lead to ventricular dilation and congestive heart failure (ischemic cardiomyopathy [ICM]). The changes in cardiac myocyte shape associated with this dilation, however, are not known.

Methods and Results. Left ventricular myocyte dimensions were assessed in cells isolated from explanted human hearts obtained from patients with ICM (n = 6) who were undergoing heart transplantation. Cells were also examined from three nonfailing donor hearts with normal coronary arteries (NCA). Compared with cells from patients with NCA, myocyte length was 40% longer in hearts from patients with ICM (197 ± 8 versus 141 ± 9 μm, p < 0.01), cell width was not significantly different, and cell length/width ratio was 49% greater (11.2 ± 0.9 versus 7.5 ± 0.6, p < 0.01). Sarcomere length was the same in myocytes from both groups. The extent of myocyte lengthening is comparable to the increase in end-diastolic diameter commonly reported in patients with ICM.

Conclusions: These data suggest that increased myocyte length (an intracellular event), instead of myocyte slippage (an extracellular event), is largely responsible for the chamber dilation in ICM. Furthermore, maladaptive remodeling of myocyte shape (e.g., increased myocyte length/width ratio) may contribute to the elevated wall stress (e.g., increased chamber radius/wall thickness) in ICM. (Circulation 1992;86:426–430)

Key Words • myocytes • myocardium, human • heart failure

Chronic ischemic heart disease is the most common cause of death in the United States; it results primarily from coronary atherosclerosis. The ventricular myocardium of patients with this disease characteristically demonstrates localized scarring and diffuse areas of interstitial fibrosis. The term ischemic cardiomyopathy (ICM) is often used when there is significant impairment of left ventricular function caused by atherosclerotic coronary disease. The progression of chronic ischemic heart disease to overt congestive failure and ventricular dilation portends a dismal prognosis. It has been suggested that the anatomic basis for this dilation is caused by: 1) a slight increase in myocyte length, 2) sliding displacement (slippage) of the heart muscle cells in the myocardium with reduction in the number of muscle layers in the wall, and 3) necrosis of cardiac muscle cells caused by coronary insufficiency with subsequent replacement by scar tissue. Although it is clear that myocyte necrosis and myocardial fibrosis occur, the relative contributions of altered myocyte shape and slippage of myocytes to the dilation process have not been resolved.

There is a paucity of information concerning myocyte dimensions in normal and diseased human hearts. Such data are typically limited to cell diameter measurements of autopsy material subjected to various sampling errors and artifacts. In this study, volume, length, and cross-sectional area measurements from cardiac myocytes isolated from patients with and without congestive heart failure are reported for the first time using methods of proven reliability.

Methods

Upon removal of the recipient’s diseased heart, transmural pieces of fresh tissue weighing approximately 25 g were excised rapidly from the left ventricular free wall near the apex and immediately placed into ice-cold cardioplegic solution. The collection site varied slightly as areas with obvious scarring were avoided. A coronary arterial branch was cannulated with polyethylene (P.E. 50) tubing for perfusion with media containing collagenase. If arterial branches were too small, an epicardial vein was cannulated for retrograde perfusion. Similar results were obtained with either arterial or venous perfusion. Distal superficial branches were ligated to obtain better perfusion of penetrating arteries or veins.

The cell isolation protocol, used previously to isolate myocytes from experimental animals, has been de-
were centrifuged and the cell volume and cell diameter=2r). Myocyte diameter was also calculated from cross-sectional area using the formula for a circle (area=πr²; diameter=2r).

Sarcomere length was measured using a Bioquant Meg IV image analysis system. Ten sarcomeres each were measured from 20 different myocytes from each tissue sample. Student's t test was used to compare individual data from the ICM and normal coronary arteries groups.

**Results**

All hearts from patients with ICM had significant coronary artery disease and diffuse and localized areas of myocardial fibrosis. Left ventricular ejection fraction averaged 21% (Table 1). Unsuitable donor hearts with widely patent coronary arteries, normal chamber volumes, and normal ejection fractions served as nonfailing controls.

Morphological changes in left ventricular myocyte structure are shown in Table 2. Myocyte volume was similar in left ventricular tissue samples from patients with normal coronary arteries and those with ICM. In contrast, the shape of cardiac myocytes of patients with ICM was significantly different from cells obtained from nonfailing human myocardium. Although cell width was similar in both groups, myocyte length was significantly longer (p<0.01) and myocyte length/width ratio was substantially larger (p<0.01) in patients with ICM. Sarcomere length was similar in myocytes from both groups. Representative myocytes from a nonfailing human heart and from a patient with ICM are shown in Figure 1.

**Discussion**

The availability of fresh myocardial tissue from explanted hearts obtained from patients undergoing heart transplantation makes it possible to collect isolated myocytes from human hearts. In our experiments, myocyte length and myocyte length/width ratio were significantly larger in hearts from patients with ICM. Based on the “control” data and other observations in experimental animals, it is unlikely that this difference in myocyte length/width ratio is related to causes other than congestive heart failure and the associated ventric-
ular dilation. For example, length/width ratio of isolated myocytes is within a range of 7–9.5 in normal hearts from other mammalian species (rats, hamsters, guinea pigs, cats, ferrets). Additionally, data from adult rats and cats indicate that there are no sex-related differences in myocyte length/width ratio. Consequently, the differences in sex between controls and patients with ICM should not affect myocyte length/width ratio comparisons. Finally, data from rats indicate that length/width ratio is preserved in ventricular myocytes during aging, during the period of normal myocyte growth from weaning to adulthood, and during severe volume-overload–induced hypertrophy. This variable may be slightly reduced in compensated hypertrophy because of pressure overload in which a selective increase in myocyte diameter and no change in cell length occurs.

Sarcomere length was similar in myocytes from non-failing human hearts and those from patients with ICM. Therefore, the longer cell length, observed in myocytes from patients with ICM, appears to be caused by the addition of new sarcomeres. The possibility that myocyte length measurements were overestimated because of cells attached end-to-end was discounted after analysis of vinculin (concentrated at the intercalated disk) immunolabeling in some samples (data not shown). The increased length of cardiac myocytes from patients with ICM was not associated with abnormal sarcomeres, as labeling of actin with rhodamine-phalloidin demonstrated a normal registration of the banding pattern (Figure 1B).

The mechanism by which cardiac myocytes regulate the growth of contractile units in series (increased myocyte length) and parallel (increased myocyte cross-sectional area) is not well understood. Grossman et al have suggested that increased afterload (e.g., increased systolic pressure, increased systolic wall stress) leads to an increase in wall thickness and myocyte cross-sectional area with little change in chamber volume or myocyte length. Increased preload (increased diastolic pressure, volume overload, increased diastolic wall stress), however, leads to ventricular dilation and an increase in cell length (series addition of contractile units). Those authors also suggested that wall thickness and myocyte diameter increase during volume overloading as a result of the elevated wall stress associated with chamber dilation. Recent data on myocyte remodeling in pressure- and volume-overload cardiac hypertrophy in experimental animals strongly support this hypothesis.

Hearts from patients with ICM are typically dilated, hypertrophied, and have disproportionately thin walls, suggesting inadequate myocardial thickness for the degree of dilation. Therefore, it appears that the changes in myocyte length and myocyte length/width ratio observed in hearts from patients with ICM mirror the gross anatomic changes in ventricular circumference and wall thickness, respectively. Furthermore, the increase in myocyte length alone can account for a 40% increase in chamber circumference and ventricular chamber diameter. It is probable that end-diastolic sarcomere length is also increased as end-diastolic wall stress is markedly elevated in these patients. Consequently, it appears that a substantial increase in cell length coupled with a small increase in sarcomere length can easily account for the increase in chamber size that is typically observed in patients with ICM. Therefore, it appears that slippage of myocytes may not be a necessary component of the ventricular dilation associated with this disease.

Based on the law of Laplace, wall stress is directly proportional to ventricular pressure and chamber radius and inversely proportional to wall thickness. Patients with congestive failure caused by ICM typically have normal systolic pressures, elevated end-diastolic pres-

<table>
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Student's t test was used to compare data from each group.
*p<0.01.
sure, increased chamber radius, and normal or reduced wall thickness. Consequently, both diastolic and systolic wall stress are increased. The relatively small myocyte diameter in cells from patients with ICM indicates an inappropriate response to the increase in systolic wall stress. Although the underlying reason for this maladaptation is not clear at this time, anatomic restrictions of the microvascular bed may play an important role. If myocyte cross-sectional area were to increase in ICM, capillaries would be pushed farther apart, thereby leading to an increase in diffusion distance. Such a change would exacerbate the problem of tissue hypoxia characteristic of this disease. Conversely, series addition of contractile units (e.g., increased cell length) may not adversely affect diffusion distance if such a change were associated with a parallel increase in capillary length. An increase in cell length without a corresponding increase in myocyte diameter, however, would lead to a further increase in diastolic wall stress (which is believed to be the signal for sarcomeregenesis). The underlying mechanism by which cardiac myocyte shape is adversely altered in patients with ischemic cardiomyopathy is not clear.

Some data from an animal model suggest parallel observations to those reported here for humans. In a recent study, structural remodeling of ventricular myocytes was examined 1 month after producing transmural left ventricular infarction in rats. Changes in the shape of surviving left ventricular myocytes were similar to those noted in the hearts of patients with ICM; cell length increased significantly, but myocyte cross-sectional area was not changed. It is possible that a common mechanism leads to ventricular dilation and congestive failure from cardiac diseases of various etiologies. Because there appears to be inadequate regulation of myocyte diameter (cross-sectional area), examination of the molecular mechanisms that regulate myocyte shape during the progression to cardiac dilation and failure may provide important insight into this disease process.

**Limitations of Study**

Obtaining viable myocardial tissue from normal humans is a recognized problem in studies of this type. Although it is realized that having only three individuals in the control group represents a statistical problem, we felt very fortunate to have collected cell size data from these unsuitable donor hearts. These hearts should be adequate controls for the ICM group as they had widely patent coronary arteries, normal chamber volume, and normal ejection fraction (e.g., comparison of nonfailing nondilated hearts with failing dilated hearts). It is likely, however, that myocyte length/width ratio of two of the unsuitable donor hearts (numbers 2523 and 2524) is slightly below normal, as both hearts appeared to have a mild degree of concentric hypertrophy caused by hypertension (e.g., normal chamber volume and increased wall thickness). Heart number 2617 is an excellent control because the patient had no history of hypertension or other diseases before her death.

Fur-
If data from the ICM group are compared to similar data from adult male rats collected in another study, cell length is 39% greater (p<0.01), and there is a trend for cell width to be smaller (6%, NS), and cell length/width ratio is 49% larger (p<0.01). Therefore, precisely the same conclusions are reached whether one compares cell size data from the ICM group with similar data from normal rats or with the unsuitable human donors.

The relative contribution of fibrosis to ventricular remodeling was not investigated in this study. Before myocyte slippage can be eliminated as a contributing factor to ventricular dilation, it must be demonstrated that lengthening of myocytes can account for all of the dilation. In future experiments, it should be possible to resolve this issue if myocyte size data are supplemented with histological data (e.g., percentage of myocardium composed of myocytes, percentage of fibrosis) collected from the same hearts.

The reliability of the methods used in this study to determine myocyte dimensions is well documented. Recognized sources of error common with tissue-sectioning methods for measuring cellular dimensions have been eliminated (e.g., tissue shrinkage from processing; overestimation of cross-sectional area due to oblique sectioning angle; inability to recognize maximum cell boundaries, which often fall outside the plane of section; compression of myocyte profiles from tissue sectioning; and differences in cross-sectional area due to variation in contractile state). Because there are no equivalent data concerning cardiac myocyte size in humans, it is difficult to compare our results with other published data. It is encouraging to note, however, that cellular dimensions of cardiac myocytes from humans are similar to those from experimental animals. Furthermore, recent data from animals have established a strong foundation for our understanding of the results submitted here.

Summary

Marked differences in cardiac myocyte dimensions were observed in hearts from patients with ICM compared with nonfailing controls. Myocytes from patients with ICM were significantly longer and had a larger myocyte length/width ratio than cells obtained from controls. Consequently, it appears that changes in myocyte dimensions mirror the respective gross anatomic changes in wall thickness and chamber volume that are typical of ICM. Although this work does not address the primary insult associated with this disease, our observations provide new insight into the possible mechanism by which chronic ischemic heart disease progresses to ICM and overt congestive failure.

Acknowledgments

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References

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