MYOFIBER BRANCHING IN IDIOPATHIC CARDIOMYOPATHY UNDER THE SCANNING ELECTRON MICROSCOPE

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Myofiber changes in idiopathic cardiomyopathy were examined by the scanning electron microscope in order to elucidate the three-dimensional architecture. Muscle specimens were sampled from the ventricular walls of 13 autopsy cases (7 with the congestive type, 3 with the hypertrophic type and 3 controls), and observed according to the Evan's method. In addition, the same samples were investigated histopathologically using the tribasic staining originated by Kurotaki, and the muscle cell diameters were analyzed with a pendigitizer computer system. In congestive cardiomyopathy, the hypertrophic myofibers branched more frequently and varied more in thickness than in the controls. The complex myofiber architecture seemed to be the result of the compensation for muscle weakness. On the other hand, hypertrophic cardiomyopathy revealed three-dimensional myofiber disarray, which was fundamentally constituted from a ring formation of the myofiber branches, but only at the ventricular septal wall. The structure appeared only to promote muscle stiffness.

Concerning morphological changes of myofibers in idiopathic cardiomyopathy, many distinguished investigators have already reported their observations. They have indicated that the congestive type shows severely damaged myofibers with interstitial fibrosis and the hypertrophic type reveals characteristic alteration, that is, so-called myofiber disarray. However, these myofiber changes were examined by only two-dimensional techniques, that is, by both light microscope and transmission electron microscope or by either one. Thus, the present study aims to observe the myofiber changes in idiopathic cardiomyopathy under the scanning electron microscope, and to elucidate their three-dimensional architectures.

MATERIALS AND METHODS

Thirteen autopsied hearts were examined: 7 of the congestive type, 3 of the hypertrophic type and 3 normal heart as the controls. In age and heart weight, there were no significant differences between the 2 types of cardiomyopathies (congestive type: age, 44.0 ± 11.5 years, heart-weight, 620 ± 62g; hypertrophic type: 35.3 ± 14.3 years, 607 ± 47g and the controls: 28.3 ± 0.9 years, 238 ± 8g). These hearts were previously fixed with 10% formaldehyde for the usual pathological examinations.

Two groups of muscle samples were obtained from 5 walls at the middle level of the ventricle (the left anterior, the posterior and the septal

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Fig. 1. Myofibers in a normal heart.

wall, and in addition the right septal and the anterior wall). Those specimens were investigated using 3 methods. Firstly, the samples were observed under the scanning electron microscope according to Evan's methods. The specimens, which were sliced into rectangular prisms of 5 mm in length, 3 mm in width and 1 mm in height, were shaken in 8N-hydrochloride at 60°C for about 60 min and then immersed into 100 units/ml collagenase (type II) at 37°C for about 2 hours. They were then postfixed with 2% tetraoxide osmium, dehydrated by acetone, treated with the critical point drying method and finally observed by the scanning electron microscope (Hitachi, HSF-2). Secondly, the other samples, which were chopped into 1 mm cubes, were postfixed with 2% tetraoxide osmium, dehydrated by ethanol and embedded into epoxy resin according to conventional methods for the transmission electron microscope. Two kinds of semithin sections, one presenting longitudinal views of cardiac myofibers and the other transverse aspects, were cut from blocks of each ventricular wall. After dyeing with tribasic staining originated by Kurotaki, they were used for histopathological observation. Thirdly, the myofiber diameters were measured using only the semithin sections presenting transverse aspects of the fibers by a pendigitizer computer system (Ohsawa, Oscon). Out of the 200 myofiber diameters, a median value of the cardiac muscle cell was calculated by measuring diameters at the site where nuclei were present in order to compare muscle cell size.

RESULTS

Figure 1 represents a picture of the myofibers under the scanning electron microscope at the left ventricular anterior wall of a normal heart. The myofibers show almost uniform thickness of about 15 micrometer, and are orderly arranged in the direction of the long fiber axis. In some places, the myofibers branch into one half or one third thinner fibers than the original. There were no fundamental differences in myofiber architecture among the ventricular walls of the controls.

A representative case of congestive cardiomyopathy is shown in Fig. 2. These pictures were taken at the left ventricular anterior wall,
Fig. 2. Myofiber branchings in a patient with congestive cardiomyopathy.

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Fig. 3. Myofiber disarrays in a patient with hypertrophic cardiomyopathy.

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Fig. 4. A comparison in muscle cell diameter between congestive cardiomyopathies and hypertrophic cardiomyopathies. The shadowed portion indicates a normal range. Abbreviations: LV = left ventricle, RV = right ventricle, Ant.W. = anterior wall, Post.W. = posterior wall, Sep.W. = septal wall, n = number

where the present case revealed the most damaged muscle among the 5 walls of the histopathological study. In Fig. 2-A, the hypertrophied myofibers (about 26 micrometer on the average) became disarranged in the long axis direction, and frequently branched into many fibers of varying thicknesses, i.e., from very large to very small ones. Here and there, these fiber branches seemed to connect with each other. By means of this complex interaction of the branches, the tissue appeared to just retain muscle tension. Many capillaries ran through the complex myofiber architecture. In the center of Fig. 2-B, a fiber branch with a free-end edge, and another tapering like a thread can be seen. Probably, this myofiber will immediately drop out from the tissue. In some places, residual collagen fibers were found.

Figure 3 also illustrates a representative case of hypertrophic cardiomyopathy. These pictures were taken at the ventricular septal wall, where myofiber disarray was frequently seen in the optical studies. Resulting from much myofiber disarrangement, the tissues lost muscle direction and the fiber long axes were thrown into confusion (Fig. 3-A). However, upon careful observation of the figure, many ring formations which are composed of myofiber branches as shown in Fig. 3-B can be seen. The cardinal structure of the myofiber disarray appears to be a ring shape formed by fiber branches. In the present study, such characteristic features could not be detected at the other hyperthickened walls.

Muscle cell diameter analysis is summarized in Fig. 4. The upper column shows the data of the congestive type and the lower is of the hypertrophic type. The vertical line represents the muscle cell diameter, that is, the median value, and the abscissa shows the site of the ventricular wall. The shadowed portions indicate a normal range, which was obtained from the controls. In the congestive type, all median values of the muscle cell diameters were larger than in the normal at each ventricular wall. At points where the muscle appeared to be severely damaged as seen by the optical investigation, the complex myofiber branching was easily found under the scanning electron microscope. On the other hand, in the hypertrophic type, the median always showed larger value than in the normal case at the ventricular septal wall, where such three-dimensional myofiber disarray was found. Meanwhile, at the other ventricular walls, the medians differed from wall to wall, and from case to case, i.e., normal value in a case and large ones in the others at each wall.

DISCUSSION

In the present study, diagnosis of cardiomyopathy was made according to the definition proposed by the Research Group of the Intractable Disease of the Welfare Ministry of Japan. Coronary artery disease, myocarditis, alcoholic cardiomyopathy, peripartum cardiomyopathy and endomyocardial diseases had been excluded in the usual clinical and pathological examinations. In this paper, the hypertrophic types could not be classified into subtypes, i.e., the obstructive and the nonobstructive types, because of the very small number of the autopsied hearts. They consisted of one case with an obstruction of the left ventricular outflow tract and 2 without obstruction. Mysteriously, one of them was combined with valvular pulmonary stenosis. Though these cases contained 2 kinds of subtypes of cardiomyopathy, as mentioned above, the difference in the cases seem to be no hindrance in making this study. This is because, as Maron, et al? have already clarified, not much difference exists at the ventricular septal wall in myocardial abnormalities between the obstructive and the nonobstructive types. And then, controls were justified with clinical laboratory data and pathological findings.

Up to the present time, no researcher has been able to elucidate the three-dimensional structure of myofibers existing in the tissue of idiopathic cardiomyopathy except a pioneering trial.11
Pathological changes of the myofibers have been observed by conventional light microscopical examination. In 1978, Uehara, et al. succeeded in examining some cardiac muscle cells isolated from formalin-fixed autopsy hearts under the scanning electron microscope. However, this approach did not clarify the three-dimensional structure of the myofibers, which are organized by connections from cell to cell at the intercalated disc. Meanwhile, Evan, et al. proposed a new technique to examine the surface structure of cells existing in tissue by a hydrochloride-collagenase method. I applied this method to cardiac muscle and the three-dimensional structure was clearly revealed under the scanning electron microscope. However, this technique could not demonstrate an accurate picture of the intercalated disc in autopsied human myocardiun.

Many previous investigators have reported that congestive cardiomyopathy shows some pathological findings such as hypertrophic cardiac muscle cells, variety in myofiber thickness, some degenerative cellular changes (in the myofibril, the mitochondria, the nucleus and the sarcoplasm) and interstitial fibrosis. These results, however, were obtained from conventional histopathological research using both the optical microscope and the transmission electron microscope or either, that is, the two-dimensional technique. Evan's method has provided some important information about the three-dimensional structures of the myofibers in congestive cardiomyopathy. In a wall which shows severely damaged muscle, some of the hypertrophic myofibers branched into thinner fibers which varied in thickness from the original and which seemed to connect to each other forming a very complex networks (Fig. 2-A). Tapering or free-ending of the myofibers could be seen at site of the most damage (Fig. 2-B). This myofiber branchings seemed to be the reasonable result of muscle tension which occurred in the damaged atrophic myofibers, because the tension may be more easily communicated to other hypertrophic myofibers through some of the fiber branches than in the normal heart (Fig. 1). In congestive cardiomyopathy, the cardiac muscle cells always hypertrophied at all the walls (Fig. 4). The muscles with cell hypertrophy showed a common tendency: higher frequency in myofiber branching and more variety in myofiber thickness than in the normal heart. This tendency is thought to be closely related to a compensation mechanism in tissue level for muscle weakness.

On the other hand, in hypertrophic cardiomyopathy, the three-dimensional architecture of the so-called myofiber disarray which was seen by the conventional pathological observations, was beyond imagination. The individual myofibers lost their long axes as muscle tissues, and the disarrayed fibers seemed to be entangled with each other (Fig. 3-A). In fine structures, the myofiber disarray consisted basically of a ring formation of the myofiber branches (Fig. 3-B). The ring fibers appeared to be joined together to form a link, and finally forming the disarray, which may increase muscle stiffness.

The specificity of the disarray in hypertrophic cardiomyopathy is controversial. But, in the present study, the structures could not be found in any of the ventricular walls except in the septal wall in hypertrophic cardiomyopathy. The cardiac muscle cell diameters in the septal wall of this type were larger than in the normal wall. Moreover, the disarray was not seen in either volume overload or pressure overload hearts in our other study. These results do not give support to the fact that the disarray is specific for hypertrophic cardiomyopathy, because the sensitivity in detecting the structure seems to be closely correlated to the grade of the section thickness in the conventional pathological study. Concerning these problems, further studies are necessary.

Consequently, the following conclusions were obtained from the present study:

1) In congestive cardiomyopathy, the hypertrophic myofibers branched frequently and varied much in thickness. The complex myofiber architecture, made up by interconnection of fiber branches, seemed to occur in order to compensate for muscle weakness at the tissue level.

2) In hypertrophic cardiomyopathy, the so-called myofiber disarray, which was based on a ring formation of the myofiber branches, exists only at the ventricular septal wall. The structure appeared to increase muscle stiffness.

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