Morphology and Relationship to Extensibility Curves of Human Mitral Valve Chordae Tendineae

Koon O. Lim, Ph.D., and D.R. Boughner, M.D., Ph.D.

SUMMARY Human mitral valve chordae tendineae in which elastic response curves are nonlinear have also been found to exhibit extensibility that increases with chordal size and decreases with chordal age. We used selective enzymatic digestion and scanning and transmission electron microscopy to explain these observations. Removal of the outer elastin sheath by enzymatic digestion did not significantly affect the elastic response of this tissue. Scanning electron microscopy revealed that the collagen fibers in the central core of young chordae exhibited a very wavy pattern but the pattern in adult specimens was relatively straight. The increased waviness accounted for the greater extensibility of the young specimens. The collagen fibers from young and old chordae consisted of a network of collagen fibrils that became more collapsed when the tissue was fixed under tension. This network arrangement of the fibrils explains the nonlinearity in the elastic response of the tissue. Transmission electron micrographs showed that the density of collagen fibrils decreased as chordal size increased. The number of fibrils per $10^{-5}$ cm$^2$ of the central core decreased from 182.4 (SE = 1.3) to 131.3 (SE = 1.6) as average chordal cross-sectional area increased from 0.0016 cm$^2$ to 0.0268 cm$^2$. This difference in fibril density provides an explanation for the greater extensibility shown by the thicker chordae. The collagen fibril diameters ranged from 516 Å to 552 Å.

HUMAN MITRAL VALVE chordae tendineae, like tissues such as skin, rat tail tendons, and arteries, exhibit a nonlinear quasi-static elastic response. In rat tail tendons, which are mostly collagen, the initial segment of the nonlinear stress-strain curve was attributed to a straightening of the wavy collagen fiber bundles. It was suggested that in skin the behavior was due to an initial stretching of the outer elastin sheath by enzymatic digestion did not significantly affect the elastic response of this tissue. Scanning electron microscopy revealed that the collagen fibers in the central core of young chordae exhibited a very wavy pattern but the pattern in adult specimens was relatively straight. The increased waviness accounted for the greater extensibility of the young specimens. The collagen fibers from young and old chordae consisted of a network of collagen fibrils that became more collapsed when the tissue was fixed under tension. This network arrangement of the fibrils explains the nonlinearity in the elastic response of the tissue. Transmission electron micrographs showed that the density of collagen fibrils decreased as chordal size increased. The number of fibrils per $10^{-5}$ cm$^2$ of the central core decreased from 182.4 (SE = 1.3) to 131.3 (SE = 1.6) as average chordal cross-sectional area increased from 0.0016 cm$^2$ to 0.0268 cm$^2$. This difference in fibril density provides an explanation for the greater extensibility shown by the thicker chordae. The collagen fibril diameters ranged from 516 Å to 552 Å.

Method

Ninety chordae from 19 mitral valves were studied. The chordae were selected from deceased patients 18–79 years of age (10 male, 9 female), who had had no clinical record of mitral valve disease and whose valves appeared normal at autopsy. The valves were removed as soon as possible and not later than 12 hours after death. They were kept, if necessary, in 0.01% thimerosal (Merthiolate), 0.9% saline, at a temperature of 6°C until tests could be performed.

SELECTIVE ENZYMATIC DIGESTION

To determine the role of each of the two major components, elastin and collagen, in the elastic response of chordae tendineae, the technique of selective enzymatic digestion was used to remove the outer elastin sheath. Thirty-seven chordae from nine mitral valves (five male and four female subjects, 45–76 years of age) were divided into two groups with chordal cross-sectional areas of 0.001–0.003 cm$^2$ and 0.004–0.006 cm$^2$, and assigned randomly to act as controls or test specimens. The test specimens were digested in a solution of 0.1 M NH$_4$HCO$_3$ buffer containing 3 mg of α-chymotrypsin (Sigma, C4129 type II) per 2 ml of buffer, 2.5 mg of trypsin (Sigma, T8253 type II) per 2 ml of buffer, and 20 μl of elastase ( Worthington ES34E747) per 100 ml of buffer, at a temperature of 37°C for 10 hours with occasional agitation. The pH of the buffer solution was 7.8. The control specimens were similarly incubated in the buffer solution of 0.1 M NH$_4$HCO$_3$ without the enzymes. This digestion procedure removed both the amorphous and fibrillar components of the elastin. After the digestion and incubation periods test specimens and controls were removed, washed out, and kept in 0.01% Merthiolate, 0.9% saline, solution at 6°C until further testing.

To examine the effect of removal of the elastin on the mechanical response, the treated specimens and the controls were subjected to uniaxial tensile stress. A floor model Instron tensile testing machine (model TT-C) was used for this purpose. The details of this procedure have been...
described elsewhere. Strain rates of 0.13 cm min⁻¹ and 1.27 cm min⁻¹ were used and the chordae all were stretched at an initial length of 0.8 cm. The amount of force exerted on the specimens and the corresponding stretch were recorded continuously and simultaneously on a chart recorder.

SCANNING ELECTRON MICROSCOPIC STUDIES

Scanning electron microscopy was used to examine the internal structure of the chordae tendineae and, in particular, the microarchitectural arrangement of the collagen fibers in the central core. Forty chordae from 10 mitral valves (subjects' ages, 18-79 years; five male, five female) were examined. The technique used in preparing the specimens for study was essentially that of Watters and Buck. Briefly, the following steps were performed. The specimens were first fixed in 5% phosphate-buffered glutaraldehyde (pH 7.1) and then gently separated to expose the internal structure. The chordae were further treated with 1% phosphate-buffered osmium tetroxide solution. Dehydration was achieved by a series of changes in solutions of alcohol and acetone. Finally, the specimens were coated with gold and examined under a scanning electron microscope. (Hitachi HHS-2R). In addition, 12 chordae from four mitral valves, fixed under tension, were also studied.

TRANSMISSION ELECTRON MICROSCOPIC STUDIES

This part of the investigation was used to determine the density and size distribution of the collagen fibrils in the different-sized chordae. Transverse sections of the middle portion of 14 chordae from four mitral valves were studied. The specimens were prepared by standard procedures of fixation, dehydration, sectioning, and staining for electron microscope examination. A Zeiss electron microscope (EM95S) was used and micrographs were taken. From these electron micrographs the density per unit of transverse sectional area and the size distribution of the collagen fibrils were determined with a particle size analyzer (Zeiss TGZ3).

Results

SELECTIVE ENZYMATIC DIGESTION

A comparison of the histological sections of normal chordae and those treated by enzymes showed that the outer elastin sheaths of the treated specimens were completely removed.

Figures 1 and 2 show the elastic response of chordae tendineae after treatment with a solution containing elastase, trypsin, and α-chymotrypsin in 0.1 M NH₄HCO₃ buffer at a pH of 7.8. The elastic response of controls is also shown. Figure 1 shows the behavior of chordae with cross-sectional areas of 0.001-0.003 cm² when strained at 0.13 cm min⁻¹, and Figure 2 shows the results for chordae with cross-sectional areas of 0.004-0.006 cm² and strained at 1.27 cm min⁻¹. The stress-strain curves in both the figures indicate that the response to mechanical stress of both the test specimens, the elastin sheaths of which had been removed, and the controls were similar. This implied that the more readily extensible elastin layer cannot contribute to the nonlinear elastic behavior of chordae tendineae, hence the mechanical properties of this tissue are governed primarily by the behavior of the inner collagen core.

SCANNING ELECTRON MICROSCOPIC STUDIES

The internal structure of the collagen fibers in adult chordae tendineae as revealed by scanning electron microscopy is shown in Figure 3A and B. We found that the collagen fibers (Fig. 3A) were aligned parallel to the axis of the specimens. This suggested that the collagen fibers are primarily oriented in the direction of the stresses applied to the chordae.

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the chordae and were rather straight, with only a few specimens showing a very slight wavy pattern. This was in contrast to the very wavy patterns observed by Rigby et al. in rat tail tendons.

Figure 3B shows the enlargement of one of these fibers. It was found that at the microstructural level the collagen fiber was made up of a network of collagen fibrils, aligned in a direction generally parallel to the chordal long axis. This network arrangement was also observed at this high magnification for the collagen fibers in chordae from a young (18-year-old) male. However, at a lower magnification the young specimens exhibited a much more wavy pattern, as compared to their older counterparts, shown in Figure 4.

If we characterize this wavy pattern by a waviness index (WI) defined as $WI = \frac{\text{ratio of length of wavy fiber to straight length between end points of fiber}}{10}$, WI would have...
a value of 1.0 for a straight fiber such as those from older chordae. For the 18-year-old specimens WI was found to be 1.072 (SE = 0.002), implying that the wavy fibers were 7.2% (SE = 0.4%) longer than a straight fiber with similar fiber end points. Table 1 summarizes the observations with the scanning electron microscope on chordae that were not fixed under tension.

Figure 5 shows the effect of fixing the adult chordae under tension. We observed here that the network of fibrils was more collapsed in many regions along the specimen.

TRANSMISSION ELECTRON MICROSCOPIC STUDIES

Figure 6A and B are transmission electron micrographs of transverse sections of the chordae. The collagen fibrils in the scanning electron micrographs appeared as circular dots in these pictures. Figure 6A shows the central core of the
Table 1  Observations with Scanning Electron Microscope of Chordae Tendineae

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>n</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>F</td>
<td>4</td>
<td>All four specimens have straight collagen fibers</td>
</tr>
<tr>
<td>2</td>
<td>79</td>
<td>M</td>
<td>6</td>
<td>Four specimens have straight fibers and two have fibers that are slightly wavy</td>
</tr>
<tr>
<td>3</td>
<td>68</td>
<td>F</td>
<td>7</td>
<td>Four specimens have straight fibers and three have fibers that are slightly wavy</td>
</tr>
<tr>
<td>4</td>
<td>70</td>
<td>F</td>
<td>7</td>
<td>Six specimens have straight fibers and one has slightly wavy fibers</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>F</td>
<td>1</td>
<td>Straight collagen fibers</td>
</tr>
<tr>
<td>6</td>
<td>65</td>
<td>M</td>
<td>1</td>
<td>Straight collagen fibers</td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>M</td>
<td>2</td>
<td>Both specimens have collagen fibers that are very wavy</td>
</tr>
</tbody>
</table>

n = number of chordae examined.

typical small chordae and Figure 6B that of a much larger specimen. These micrographs show that the density of collagen fibrils was greater for the smaller chordae. The fibril densities of the central core for three different chordal size groups are listed in Table 2, and the average fibril diameters are also presented. An analysis of variance showed that the fibril density decreased significantly as chordal size increased ($P < 0.01$). The average fibril sizes between the first two groups of chordae with smaller diameter were not significantly different (t-test, $P > 0.01$), but the diameter in the third group was found to be smaller. This smaller average fibril size in the large chordae would further decrease the force-supporting areas in them. The distribution of fibril size for any group of chordae was found to be approximately normal.

Discussion

The enzymatic digestion studies showed that the removal of the outer sheath of elastin from human mitral valve chordae tendineae did not significantly affect the elastic response of this tissue under mechanical stress. Thus, the initial segment of the nonlinear stress-strain curve cannot be attributed to the presence of the more extensible elastin. This is in contrast to observations in arteries. For that tissue it had been shown that the initial segment of the nonlinear stress-strain curve was due to the extension of the elastin, and the final slope of the curve was attributed to the collagen response. Our findings therefore indicate that although both arteries and chordae contain essentially the same two structural components the reason for their elastic response cannot be explained by the same theory. This points to the importance of the differences in the architectural arrangement of the structural components of these two tissues in determining their elastic properties. For arteries, there is a network of elastin lamellae interlaced with interlamellar collagen fibers. Such an arrangement makes the arterial wall behave as a two-phase material. In the chordae, however, there is an outer sheath of loosely meshed collagen and elastin fibers, with the latter predominating, and the large central core is of dense collagen bundles with scattered elastin fibers.

Our studies with the scanning electron microscope showed that, at the microstructural level, the collagen fibers were in fact a network of fibrils. For such a system, when a force is
applied, the initial response is to straighten any wavy pattern that may be present. This is followed by the stretching and collapsing of the fibrillar network, as shown in Figure 5, which illustrates the structure for a chordal specimen fixed under tension. On further stretching, a force greater than that applied initially is needed to strain the tissue as this now will involve the straining of the bonds and bond angles within the fibrils. This response will, therefore, give rise to the observed nonlinear stress-strain curves.

The observation that the collagen fibers in the younger chordae were much more wavy than the fibers in older specimens explains the greater extensibility exhibited by these younger chordae. The waviness index found for the 18-year-old chordae showed that these specimens would extend by a further 7.2% before the fibers were straightened. In our earlier study we observed that the transitional strain (i.e., the strain level when the elastic curve becomes linear) occurs at 8.0% for the mature chordae and at 15% for a 17-year-old specimen. The difference of 7.0% between these two levels of strain would correspond to the straightening of the more wavy pattern in the young specimen. This value compares favorably with the waviness index of 1.072 found for the 18-year-old specimens.

Fibril size and distribution could not be determined accurately by scanning electron microscopy as this involved the coating of the specimen with gold; hence, transverse sections of the chordae were studied by transmission electron microscopy. The transmission electron micrographs (Fig. 6) showed the presence of the fibrils in the central core of the chordae, thus confirming the observations made from the scanning pictures. Results summarized in Table 2 show the fibrils in the smaller chordae to be more closely packed than those in the larger specimens, thus implying that a greater force per unit of area would be necessary to stretch the more dense network of fibrils in thin specimens. As both thick and thin specimens were prepared and handled in a similar manner and random areas of the sections were examined, this observation of differences in fibril density cannot be an artifact. The possibility that the fibers might taper can also be ruled out as only middle portions of the chordal length were examined and compared. Thus, the observation that thicker specimens have lesser fibril density explains the greater distensibility exhibited by the larger chordae. The average size of the collagen fibrils observed in this investigation was also found to be comparable to values reported for collagen fibrils.12

In conclusion, our study has provided an explanation for the greater stiffness observed in adult human mitral valve chordae and the greater extensibility exhibited by the larger chordae. We also showed that the nonlinearity of the elastic response was due to the architectural arrangement of the collagen fibrils of this tissue.

Acknowledgments

We are grateful to the Department of Anatomy for the use of the transmission electron microscope, and to St. Joseph’s Hospital, London, and the Department of Pathology, University Hospital, for the autopsy specimens. We also thank Dr. A.C. Wallace, University Hospital, for the use of the transmission electron microscope.

References


<p>| Table 2 | Fibril Density in Different Chordal Sizes |
|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Chordal cross-sectional area (cm²)</th>
<th>Average diameter (in Å) of collagen fibrils (± SE)</th>
<th>Number of collagen fibrils per 10⁴ cm² (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0013 - 0.0019</td>
<td>545 ± 2</td>
<td>182.4 ± 1.3</td>
</tr>
<tr>
<td>0.0072 - 0.0074</td>
<td>552 ± 3</td>
<td>163.6 ± 1.7</td>
</tr>
<tr>
<td>0.0260 - 0.0280</td>
<td>516 ± 2</td>
<td>131.2 ± 1.6</td>
</tr>
</tbody>
</table>
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Circ Res. 1976;39:580-585
doi: 10.1161/01.RES.39.4.580

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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