Ultrastructure and Mechanical Properties of Chordae Tendineae from a Myxomatous Tricuspid Valve

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SUMMARY

The ultrastructure and mechanical properties of chordae from a surgically removed myxomatous tricuspid valve were examined. Transmission and scanning electron microscopy showed the internal structure of the abnormal chordae to consist of a loose and relatively sparse network of disorganized collagen fibrils, with no well defined collagen bundles present. The abnormal tissue was found to be more extensible than normal and it ruptured at a stress that was only about 6% of the breaking stress for normal chordae. Chordal rupture, a common complication in this disorder, can be attributed to this decrease in chordal strength. At high strains, a decreased elastic modulus was also observed, which may be attributed to tissue changes and an alteration in collagen fibril structure and/or composition.

Additional Indexing Words:
Electron microscopy

MYXOMATOUS degeneration of human heart valves is a frequent incidental finding at necropsy1,2 and can be demonstrated during life (using noninvasive techniques) in over 5% of the population.3,4 Although most individuals experience no problems related to this degenerative process, a small portion develop valvular insufficiency that may require valve replacement.5-8 The tricuspid valve is less commonly affected than the mitral valve, but either or both valves may be involved.9-11 Gross examination usually reveals enlarged, often thickened, floppy valve leaflets.
with a somewhat doughy appearance. Both the valve cusps and the chordae tendineae may be involved and the affected chordae may be elongated as well as thickened. Most studies of the disorder have focused on its incidence and clinical features or on histological studies of the valve tissues. There have been few ultrastructural studies of myxomatous tissue and the mechanical properties of such tissue have not been characterized. This report describes our measurements of the mechanical properties of a chorda tendinea from a surgically removed myxomatous tricuspid valve and relates those measurements to the structural changes shown to be present.

CASE REPORT

A 60-year-old woman of Italian descent presented with a 4 year history of slowly progressive, easy fatiguability, and a recent onset of an irregular heart rhythm. In addition, she had noted an increase in her abdominal girth over the 4 weeks prior to her admission. She was experiencing two pillow orthopnea but no nocturnal dyspnea, ankle swelling or chest pain. She had been taking digitalis and diuretics for 4 years. Family history was non-contributory. On examination, there was jugular venous distension to 9 cm above the sternal angle with prominent "v" waves. The right ventricle was palpable and the apex was located in the sixth interspace, 13 cm to the left of the sternum. A grade III/VI pansystolic murmur, heard loudest along the left sternal border, radiated to the apex and varied with respiration. Along the left sternal border, fourth interspace, a grade I/VI mid diastolic rumble was audible. The liver was pulsatile and extended 4 cm below the right costal margin. There was positive hepatojugular reflux. Percussion of the abdomen revealed the shifting dullness of ascites. Peripheral pulses were all palpable and there was no edema. There was no evidence of arachnodactyly, hypermobile joints, ecchymoses or hyperelastic skin. Criteria for right ventricular enlargement were present on the chest film, electrocardiogram and echocardiogram. The electrocardiogram also showed atrial fibrillation, while the echocardiogram showed paradoxical septal motion but no identifiable valvular abnormalities. Cardiac catheterization and angiography were performed. The right ventricular end-diastolic pressure was 11 mmHg. The right atrial mean pressure was 12 mmHg with a "c-v" wave of 18 mmHg and a rapid "y" descent. Severe tricuspid regurgitation with a dilated right atrium and a dilated, well contracting right ventricle was present. The left ventricle, aortic root, and coronary arteries were normal. A mitral valve prolapse, involving the posterior leaflet postero-medial commissural scallop, was present with trivial mitral
insufficiency. Tricuspid valve replacement was recommended. At surgery, the tricuspid annulus was dilated and the valve leaflets appeared redundant and flimsy. The chordae were intact. The valve was excised and replaced with a porcine xenograft and the patient made a satisfactory postoperative recovery.

**METHODS**

(1) Histology and transmission electron microscopy: Selected portions of the valve and chordae were fixed in 10% formalin and processed routinely for paraffin embedding. Sections were stained with hematoxylin and eosin, Periodic Acid-Schiff, Masson Trichrome, Gomori aldehyde fushsin, and alcian blue-safranin at a pH of 2.5. In addition, 1 mm square cubes of valvular and chordal tissue were immediately fixed in 2% glutaraldehyde and processed routinely for transmission electron microscopy. Thin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss model 9-A electron microscope.

(2) Mechanical properties and scanning microscopy: Three additional chordae were removed carefully. One was reserved for mechanical testing and two were processed for scanning electron microscopy. Normal tricuspid chordae were obtained from 2 autopsy patients who had no clinical record of tricuspid valve disease for comparison. In our previous studies of normal mitral valve tissues, we observed that age and chordal size, if they differ significantly, are important variables to be considered when comparing microscopic architecture and mechanical properties. However no variation was observed for patient sex or body habitus. The controls were both males, ages 60 and 72, and the tricuspid chordae selected for comparison were all of comparable cross-sectional area to the myxomatous chordae. These normal chordae were removed from the valves not more than 12 hours after the patient's death and were kept in 0.01% merthiolate solution at a temperature at 6°C until studies could be performed. This antibacterial solution preserves the mechanical properties of collagen and elastin and was also used for storing the three abnormal specimens until the studies could be carried out. All studies were carried out within 24 hours of specimen removal.

The chordal mechanical properties studied included static testing of the stress-strain behaviour plus a determination of the breaking stress and strain. The mechanical testing was done at room temperature (21 ± 1°C) using an Instron tensile testing machine that applied uniaxial stress. The details of the experimental technique have been described previously.
Stress was applied until rupture occurred. A strain rate of 160% min\(^{-1}\) was used and, the tissues were kept moist throughout the testing period. The stress-strain curves and breaking stress and strain of these specimens was obtained from the tension-time records given by the Instron machine and the initial chordal cross-sectional area. The chorda from the myxomatous valve had a cross-sectional area of 0.019 cm\(^2\) while the chordae from the normal valves had an average cross-sectional area of 0.010 cm\(^2\). Larger size normal chordae were not available.

The techniques for the preparation of the specimens for scanning electron microscopy have also been reported previously.\(^{19}\) The specimens were first washed in saline and fixed for 2 hours in 5% glutaraldehyde in 0.1 M phosphate buffer (pH-7.1). Specimens were stored overnight in a 5.4% sucrose in 0.1 M phosphate buffered solution at a temperature of 6°C. The chordae were then ripped apart gently to expose their internal structure. They were treated for 2 hours in a solution of 1% osmium tetroxide in the same phosphate buffer. After washing twice with demineralized water, the specimens were dehydrated in a series of acetone immersions before being transferred to benzene, then 50% benzene in propylene oxide and finally to 100% propylene oxide. This was followed by treatment with 50% camphene in propylene oxide and then 100% melted camphene. The camphene infiltrated specimens were left overnight to allow the camphene to sublime. The dehydrated specimens were then coated with a layer of gold and examined under a scanning electron microscope.

**Results**

(1) Gross examination: The excised tricuspid valve was received from surgery as three separate tissue fragments. The distal one-half of one fragment showed a moderate degree of gelatinous thickening. The attached chordae tendineae were mostly delicate and thinned and there was no evidence of chordal rupture. The other two fragments of the valve showed mild, focal nodular thickening along the free margins. No thrombi or vegetations were present.

(2) Light microscopy: Sections of the chordae showed accumulations of myxomatous ground substance, with fragmentation and loss of collagen and elastin fibres. This material was alcian blue positive. Similarly, sections of the distal portion of the valve showed a full-thickness accumulation of myxomatous ground substance and fibrosis of both the atrial and ventricular surfaces of the adjacent valve. There was no inflammation, neovascularization or thrombus formation.
Fig. 1. (a) Transmission electron micrograph of chorda tendinea from the abnormal valve showing decreased collagen fibres with the remainder being haphazardly arranged. Increased ground substance with irregular vacuoles and electron-dense granules are also noted. ×18,000. (b) Chorda tendinea from a normal tricuspid valve, obtained at autopsy from a 49 yr old male. Note the closely packed, regularly arranged collagen bundles. ×18,000.

(3) Transmission electron microscopy: Foci of disorganized and fragmented collagen fibres were noted on ultrastructural examination (Fig. 1). The amount of collagen was reduced and a marked increase in ground substance was apparent, with irregular vacuolated spaces and some electron dense material in the matrix. These changes were most prominent within the valve, but they were also present within the chordae examined. The collagen fibres appeared normal and retained a normal periodicity. The
Fig. 2. Scanning electron micrograph showing the internal structure of the myxomatous chordae. Original magnification ×640. Each marker is equal to 10.53 μ. No orderly arrangement of collagen bundles is visible.

Fig. 3. Scanning electron micrograph of the internal structure of a normal tricuspid chorda tendinea. Magnifications and markers are as in Fig. 2. Dense bundles of collagen are oriented longitudinally.

fibroblasts were unremarkable.

(4) Scanning electron microscopy: The internal structure of the myxomatous tricuspid chordae is illustrated in Fig. 2. In contrast to the normal samples, no distinct collagen bundles were observed (Fig. 3). At high magnification, these chordae were found to be comprised of a disorganized loose network of fine collagen fibrils. Although this bore a general
Fig. 4. Stress-strain curves for the normal tricuspid chorda versus the curve for the myxomatous chorda.

Table I. Mechanical Properties of Tricuspid Chordae Tendineae

<table>
<thead>
<tr>
<th></th>
<th>Normals</th>
<th>Myxomatous valves</th>
</tr>
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<tbody>
<tr>
<td>Initial elastic modulus*</td>
<td>$1.73 \times 10^7$ dynes cm$^{-2}$</td>
<td>$2 \times 10^6$ dynes cm$^{-2}$</td>
</tr>
<tr>
<td>Final elastic modulus**</td>
<td>$0.85 \times 10^9$ dynes cm$^{-2}$</td>
<td>$0.60 \times 10^9$ dynes cm$^{-2}$</td>
</tr>
<tr>
<td>Breaking stress</td>
<td>$1.6 \times 10^9$ dynes cm$^{-2}$</td>
<td>$9.3 \times 10^9$ dynes cm$^{-2}$</td>
</tr>
<tr>
<td>Breaking strain</td>
<td>27.3%</td>
<td>25.7%</td>
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* This is given by the slope of the stress-strain curve at its origin.
** This is given by the final slope of the linear portion of the stress-strain curves.

similarity to the collagen network of normal chordae, it was not as well or- ganized and less dense than the normal tissue. The well-organized collagen fibril networks observed in the normal tricuspid chordae were much the same as those demonstrated previously in normal mitral chordae.\(^{19,20}\)

(5) Mechanical properties: The stress-strain curves (i.e. the percent elongation resulting from an applied force) for the myxomatous tricuspid chorda and the curves for comparable normal tricuspid chordae tendineae are illustrated in Fig. 4. Both the normal and abnormal chordae showed the typical response exhibited by biological materials. Initially, the chordae stretched easily. As the length (strain) increased further, the resistance to further elongation also increased. Thus, the slope of the stress-strain curve gradually increased as strain increased. The curves we obtained demonstrated that the myxomatous chorda was considerably more extensible than the normal tricuspid chordae of comparable size and age. The strain rates employed were identical for both normal and abnormal tissue.

The initial and final elastic moduli of both the normal and abnormal chordae are shown in Table I. The final elastic modulus for the myxoma-
tous chorda was a full order of magnitude less than the modulus for the normal tissue. Also, the myxomatous chorda ruptured at a level of stress of only about 6% of that exhibited by normal chordae. However, the breaking strains (i.e. the degree of stretch at rupture) were similar.

**DISCUSSION**

The differences between the mechanical properties of normal and myxomatous tricuspid valve chordae are illustrated in Fig. 4 and Table I. The static stress-strain curves showed myxomatous tissue to be much more extensible than normal. Also, despite the similarities of the breaking strains for the two tissues, there was a major difference between the stresses at which rupture occurred. The myxomatous tissue failed when the applied stress had reached a point that is only about 6% of that withstood by the normal tissue. Thus, the myxomatous tissue stretched more easily than normal and, although it broke after the same amount of elongation, the force applied to reach that length was far less than normal. These features provide an explanation for the clinical observations that chordal elongation is common, allowing prolapse of the valve into the atrium and that chordal rupture can be a frequent complication of the myxomatous process.7),20)

For the normal controls, the final slope of the stress-strain curve (the final modulus) was on the order of $10^9$ dynes/cm². This is consistent with the modulus reported for collagen and is similar to modulus demonstrated for both normal tricuspid and normal mitral valve chordae tendineae.21) However, the final elastic modulus for the myxomatous tricuspid chordae was found to be on the order of $10^8$ dynes/cm². This value is a full order of magnitude less than that of the normal chorda which suggests that the collagen fibrils were not responding to stress in a normal fashion.

Kern and Tucker5) examined myxomatous valve leaflets using transmission electron microscopy and reported that the collagen fibres of the affected valves had a disorderly and degenerate arrangement and that the typical cross banding of the collagen was absent. Although our transmission electron microscopic results showed a disorganized arrangement, no abnormalities in the collagen fibril cross banding were observed. However the mechanical testing showed a decrease in both the final tissue modulus and the breaking stress that could not be totally explained by the disorganization of the fibril bundles. This implies that there may be another, more fundamental change in the tissue. Recently, Hammer et al22) demonstrated a marked alteration in the biochemical composition of mitral valve tissue from a patient with mitral and tricuspid valve prolapse. Their electrophoretic
analysis of the collagen content showed that the mitral leaflet and chordal tissue had normal quantities of type I collagen, but that type III and AB collagen were virtually absent. They felt that these abnormalities provided a molecular basis for the pathological and histological findings. These abnormalities and the presence of increased ground substance may explain the changes we recorded in the final elastic modulus and breaking stress of the myxomatous tricuspid chorda.

To our knowledge, the architecture of myxomatous valve tissue, as observed by scanning electron microscopy, has not been previously reported. Our studies showed a markedly disordered appearance of the collagen bundles instead of the normal lengthwise orientation. In addition, fewer collagen fibrils were present than in normal control preparations and the myxomatous tissue was more easily stretched and ruptured at a lower stress than the normal tissue. These structural and mechanical features of the myxomatous tissue provide an explanation for some of the problems encountered clinically in such patients.

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REFERENCES


