Localised deposition of amyloid in tears of the rotator cuff

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Age-related localised deposition of amyloid in connective tissue has been found in degenerative articular and periarticular tissue. Biopsies of the supraspinatus tendon of 28 patients undergoing repair of the rotator cuff were analysed histologically for the presence of localised deposition of amyloid. There was a long history of impingement in 20 patients, and eight patients had suffered an acute traumatic tear with no preceding symptoms. Localised deposition of amyloid identified by Congo Red staining was detected in 16 samples (57%). Amyloid was present in 14 (70%) of the degenerative tears, but in only two (25%) of the acute tears. Immunohistochemical staining showed that the amyloid deposits were positive for P component, but negative for κ and λ light chains, prealbumin, and β2 microglobulin.

Critical electrolyte staining revealed highly-sulphated glycosaminoglycans at sites of deposition of amyloid. The presence of localised deposition of amyloid in tears of the rotator cuff is likely to represent irreversible structural changes. These findings support the theory that impingement and tears are due to intrinsic degenerative changes within the tendons of the rotator cuff.

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Both extrinsic impingement and intrinsic degeneration have been investigated in studies on the pathogenesis of tears of the rotator cuff. Recent evidence, including pathological changes in the acromion, has suggested that at this site the problem is predominantly intrinsic.1 Cadaver analysis of supraspinatus tendons has shown an increased incidence of degenerative change in the matrix with age, including accumulation of glycosaminoglycans (GAGs) and fibrocartilaginous transformation.2,5 Biopsies of supraspinatus tendons from patients with chronic tendonitis have also shown a substantial increase in sulphated GAGs.4 It is postulated that these changes, in addition to those in collagen composition, predispose to rupture of the tendon.

The deposition of amyloid is a process in which normally soluble proteins polymerise to form insoluble fibrils. Localised deposition of amyloid has been reported in articular hyaline and fibrocartilage as well as in the joint capsule and in meniscal and ligamentous tissues.5-10 Highly-sulphated GAGs are an integral component of these deposits. It is postulated that accumulation of such GAGs in the extracellular matrix is related, specifically, to the deposition of amyloid, playing an important role in the folding of amyloidogenic proteins into fibrils and protecting the deposits from proteolytic degradation.13,14 Localised deposition of amyloid in osteoarthritic tissues is age-related,15 and is associated with degenerative changes in the tissue matrix. Changes in the composition of GAGs in the matrix, particularly a relative increase in highly-sulphated GAGs, may encourage the deposition of amyloid.12,15

We have examined whether irreversible structural and functional degeneration and failure of the rotator cuff are associated with changes in the matrix. In particular, we have investigated whether localised deposition of amyloid is associated with tears of the rotator cuff and further assessed whether this process is related to qualitative and quantitative changes in the composition of GAGs in the tendon matrix.

Patients and Methods

Tissue was obtained from 14 men and 14 women with a mean age of 64.4 years (52 to 77) in the course of surgery on the rotator cuff. The samples, from the torn edge of the defect in the supraspinatus tendon, were fixed in 10% buffered formalin and routinely processed. Paraffin-embedded sections 5 μm thick were stained with either haematoxylin and eosin or alkaline Congo Red. All samples were analysed by a consultant histopathologist (NAA) who was blinded as to the chronicity of symptoms. Sections of tissue...
containing amyloid were then stained immunohistochemically by an indirect immunoperoxidase technique using monoclonal and polyclonal antibodies (Dako, UK) at a dilution of 1:400, directed against known amyloid proteins (β2M, κ light chain, λ light chain, prealbumin) and monoclonal antibodies directed against undiluted amyloid A protein and undiluted P protein. Negative controls consisted of the omission of the primary antibody and its replacement with a phosphate-buffered saline solution.

The Alcian Blue magnesium chloride critical electrolyte concentration technique was used to indicate the presence and nature of matrix GAGs. It was also used to assess the composition and topographical distribution of GAGs in amyloid-containing tissues. In general, neither unsulphated GAGs nor polycarboxylated GAGs bind Alcian Blue in situ at concentrations higher than 0.2M MgCl₂. By contrast, there is persistent staining of highly-sulphated GAGs, such as heparin sulphate and keratan sulphate, at 0.7M MgCl₂. Paraffin sections of the biopsy specimens were stained for 18 hours in solutions of 0.1% Alcian Blue 8GX in 0.25 acetate buffer (pH 5.8) with MgCl₂ added at concentrations of 0.06M, 0.3M and 0.7M.

The presence of amyloid was correlated with age and the chronicity of symptoms. Patients with a long history of impingement and no preceding trauma were classified as having a chronic tear while those with no preceding symptoms and an acute traumatic episode as having an acute tear.

For statistical analysis we used Student’s t-test for comparison of means and Fisher’s exact test for frequency observations.

Results

Deposition of amyloid in rotator-cuff connective tissue. Localised deposits of amyloid were present in samples obtained from 16 patients (57%). The mean age of the patients with tissue shown to contain amyloid was 67.25 years compared with 60.66 years in those with no amyloid (Student’s t-test, p = 0.02).

Amyloid tissue was identified by histological examination of collagenous connective tissue of the rotator cuff stained with Congo Red and apple green birefringence under polarised light (Fig. 1a). The presence of amyloid P protein in the deposits was confirmed by immunohistochemical analysis. Staining for other amyloid proteins was negative. In most cases, deposits of amyloid were relatively small and well-defined but in two cases, those in the dense connective tissue of the cuff were relatively large. They were present in areas of degeneration of collagenous tissue throughout the biopsy samples and in some cases, beneath the bursal synovial lining. The deposits of amyloid in tissues of the cuff were in areas where the matrix of the connective tissue showed the presence of highly-sulphated GAGs as identified by staining with Alcian Blue at 0.7M (Fig. 1b).

Deposition of amyloid in acute and chronic tears. The 20 patients with degenerative tears and eight with acute tears had mean ages of 65.1 and 62.8 years, respectively (p = 0.48). Amyloid was present in 14 (70%) of the samples from patients with degenerative tears and in two (25%) of those with acute tears. Amyloid was therefore more frequently seen in patients with a chronic history compared with those with an acute traumatic episode (Fisher’s exact test, p = 0.044). No correlation was observed between repairability or size of the tear and the presence of amyloid.

Discussion

Systemic deposition of amyloid has been reported in bursae around the shoulder in patients undergoing renal dialysis, but to our knowledge localised deposition has not been previously described in tears of the supraspinatus tendon.
In all cases in our study, the amyloid was positive only for the P protein component and negative for other protein components associated with systemic amyloid disease. Deposits of amyloid were found in areas of degeneration of the connective tissue matrix of the cuff in which there was a concentration of highly-sulphated GAGs, suggesting that tears may be associated with intrinsic degeneration. When present, amyloid was found throughout the biopsy sample. Biopsies of seemingly normal tendon were not taken; the incidence of deposition of amyloid in these areas is not known.

Virchow is credited with having first described amyloidosis. Deposits of amyloid are known to have three major components: the amyloid fibril protein, the amyloid P component and GAGs. Once deposited in tissues, amyloid is resistant to proteolytic degradation. This explains its persistence and continuing accumulation, and leads ultimately to the structural and functional failure of the affected tissue. A common feature of all the known amyloid proteins is that their precursors in polypeptide chains condense to form amyloid fibrils. All deposits have a fibrillar ultrastructure forming extensive antiparallel β pleated sheets. The amyloid P component is a consistent feature of all types of amyloid, irrespective of the nature of the protein. It is identical to the P component in circulating serum and has some structural homology with acute-phase proteins such as C-reactive protein. It has ligands which bind specifically for GAGs and amyloid protein fibrils, although its precise role in the amyloidogenic pathway and deposition of amyloid has not been clarified. Its presence is an important immunohistochemical marker for the deposition of amyloid. GAGs are an integral part of all types of amyloid and are thought to be important in the amyloidogenic pathway, accelerating the formation of fibrils and protecting against proteolytic degradation.

The cause of tears of the rotator cuff still remains unclear and controversy continues about which factors initiate the process of tearing. Extrinsic impingement and intrinsic tendon degeneration have both been identified in studies investigating the pathogenesis of tears. Proponents of the extrinsic theory have suggested that changes in the coracocromial arch and the shape of the acromion are associated with disease of the cuff. Although these studies indicate a strong association between the presence of tears of the cuff and the shape of the acromion, subsequent evidence by Ozaki et al has suggested that these changes are secondary to degeneration of the cuff.

The evidence for intrinsic degeneration comes from studies as early as 1934 by Codman who identified that the pattern of failure of the cuff was distinctive, with a rim rent. Most frequently, the tear begins on the deep surface of the tendon and extends outwards to involve the full thickness. The vascular anatomy of the cuff has been extensively reviewed with early studies suggesting an avascular critical zone. More recent evidence, including laser Doppler studies, has cast doubt on this theory.

Many of the histological studies have been made on cadaver specimens. Riley et al have investigated the composition of collagen and the changes in GAGs in the extracellular matrix in specimens obtained from patients undergoing surgery for chronic impingement. They found changes in the composition of collagen with increased amounts of type III in supraspinatus tendons as well as marked changes in the content of GAGs with increases in sulphated GAGs; both of these having been implicated in the deposition of amyloid.

Localised deposition of amyloid has been reported in a number of tissues including articular, hyaline and fibrocartilage, as well as in meniscal and ligamentous tissue. It has been found in joint tissues in osteoarthritis and rheumatoid arthritis. Its deposition in these conditions is most likely to be age-related because of changes in GAGs in the matrix, rather than specifically disease-related.

Deposition of this type of amyloid is also related to the composition of GAGs in the matrix, although the protein component has yet to be identified.

In our study, the localised deposition of amyloid also appears to be age-related and, although all our patients were over the age of 53 years, there was a positive correlation between deposition of amyloid and age. The role of deposition of amyloid in the pathogenesis of tears of the rotator cuff is not known. We observed that deposition was more commonly seen in those patients with chronic symptoms of impingement and deficiency of the cuff than in those with acute tears. The presence of amyloid deposits may thus be related to changes in the content of GAGs which may be associated with the structural and functional failure of the cuff. These findings suggest that degeneration of the intrinsic tissue in this group of elderly patients is likely to contribute to tears of the rotator cuff.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References


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