DEGENERATION OR REMODELLING : MATRIX METALLOPROTEINASES (MMPS) IN THE TORN ROTATOR CUFF

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Introduction : In the shoulder, the rotator cuff plays a central role in joint function and tears of this tissue give rise to pain and disability. The overall incidence of cuff tears increases with age, individuals over 80years having a 51% incidence of a tear. The aetiology of rotator cuff tears remains unclear. Current hypotheses implicate direct abrasion of the cuff tendons. However, clinical experience indicates that many patients do not have structural abnormalities, which can directly damage the cuff and many cuff tears seem to start on the joint side. The extent of debridement of the torn rotator cuff is limited by the need to reattach the tendon, and successful repair is only achieved in 30% patients. After surgery, pain relief and patient satisfaction are acceptable but return of strength and clinical outcome measures are inconsistent.

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes capable of degrading almost all the components of the extracellular matrix. Roles for MMPs have been determined in a wide range of physiological processes including implantation and placentation, embryogenesis, tissue remodelling and wound healing, and linked to a variety of pathological processes including conditions with excessive matrix destruction such as rheumatoid arthritis, osteoarthritis, periodontitis and multiple sclerosis.

Objective : To relate the expression of a wide range of MMPs with well characterised histological changes in the torn cuff. To determine if there is any difference in activation of gelatinase MMPs (MMP2 & MMP9) between the resected and the torn tissue edges.

Methods : Rotator cuff tissue was obtained from ten patients (age 40-80y) undergoing surgical repair. The resected edge nearest the muscle insertion was identified by a suture. The size of tear was 1-4.5cm, time from presentation to surgery was 1 month (acute) to between 0.5-4y (chronic). Fresh tissue was collected onto crushed ice, and taken to the pathology department and divided for both formalin fixation and tissue culture. Immunohistochemical staining with monoclonal antibodies to a range of MMPs, endothelial, macrophage and fibroblast markers was performed (Table 1). Visualisation used a standard DAB chromagen technique (Envision, Dako Ltd.). Collagen structure was examined under polarised light. (Ethical approval 99/066).

Table 1.	**Source**	**Antigen retrieval**
Collagenase 1 (MMP-1)	TCS Biologicals Ltd	NA
Collagenase 2 (MMP-8)	TCS Biologicals Ltd	NA
Collagenase-3 (MMP-13)	Neomarkers	NA
Gelatinase A (MMP2)	Neomarkers	pressure cooker
Gelatinase B (MMP9)	Oncogene Ltd	pressure cooker
Stromelysin-1 (MMP3)	Neomarkers	NA
Stromelysin-2 (MMP10)	Neomarkers	NA
Stromelysin-3 (MMP11)	Neomarkers	NA
MMP 14	Chemicon Ltd	NA
TIMP1	Chemicon Ltd	trypsin
TIMP2	Chemicon Ltd	NA
CD31 (endothelial)	Dako	trypsin
CD34 (endothelial)	Dako	trypsin
CD68 (macrophage)	Dako	pressure cooker
Mac386 (macrophage)	Dako	trypsin
Vimentin	Dako	NA
Ki-67 (mib-1)	Dako	pressure cooker
SMA	Dako	NA

Specimens for tissue culture were diced, washed and cultured in DMEM for 2-5days at 37ºC, 5%CO_2 in a humidified incubator. The supernatant was analysed for zymogen and active MMP2 and MMP9 by gelatin zymography.

Comparison was made within each specimen, of differences between the torn and resected edge, for cell type, MMP distribution and gelatinase MMP activation.

Results : Acute - There was an infiltrate of macrophages, little collagen degeneration, the fibroblasts were MMP1 positive and MMP2 negative. Endothelial cells were MMP1 and MMP2 positive.
Chronic – resected edge - Mature collagen, plump fibroblasts and proliferating endothelial cells were identified adjacent to the resection edge. The fibroblasts were again MMP1 positive and MMP2 negative. Endothelial cells were MMP1 and MMP2 positive.
Chronic - torn edge - Areas of degenerate collagen were seen by polarised light. Within this matrix were rounded cells with foamy cytoplasm, vimentin positive and CD68 negative. These fibroblasts were intensely positive for MMP1 and MMP2, and positive for MMP-3, -10, -11, -13 and -14. Little evidence of proliferation was detected.
Zymography : Tissue culture supernatant demonstrated active and latent MMP2 production in all cases. The proportion of active MMP2 to latent MMP2 was 44% at the torn edge and 11% at the resected edge. Latent MMP9 was detected occasionally.

Discussion : Successful repair of the torn rotator cuff will be constrained by both the strength and cellular activity of the remaining tissue. Repair requires deposition of new collagen and remodelling of that collagen along the axis of tensile forces. Thus an adequate vascular supply supporting enzymatically active fibroblasts is necessary. The prolonged interval between trauma and surgical repair, with intervening disuse immobility, remedial physiotherapy and phamaceutical support make it difficult to disentangle the factors contributing to either primary tendon degeneration or spontaneous repair and remodelling.

Macrophage infiltration was evident in the acute tear but very sparse in all other cases, suggesting any acute inflammatory reaction had ceased in the chronic cases. Areas of collagen degeneration were readily identified in the debrided tissue. However, clear evidence of cellular activity typical of wound repair was also visible, including fibroblast and endothelial cell proliferation. The most striking finding was the association between areas of poor collagen structure with rounded fibroblasts demonstrating high enzymatic activity. These fibroblasts stained intensely for both MMP1 and MMP2 and a wide range of other matrix metalloproteinases. The increase in the activation percentage of MMP2 by the torn tissue might be related to the coincidental production of MMP1 and MMP2 by these fibroblasts. While little proliferation was noted, the cells were undertaking extensive enzyme synthesis, suggesting adequate nutritional support.

Whether all this MMP activity is evidence of a causative degenerative mechanism or active remodelling is unclear. More work needs to be done to clarify the balance between matrix degeneration and remodelling in the rotator cuff, particularly in tissues in which the loading has changed as a result of pain, disuse or a tear.

Summary :

- Fibroblast shape differed between areas of mature and of degenerate collagen
- Fibroblasts within the torn edge demonstrated positivity to a wide range of MMPs
- MMP content differed between fibroblasts within areas of mature or degenerate collagen, especially MMP2
- The percentage of MMP2 activated was greater by tissue cultured from degenerate areas than tissue from the resected edge
- MMPs play a role in the extensive matrix alterations ongoing in the torn rotator cuff, however, whether this is degeneration or remodelling is unclear.

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