INTRODUCTION

Tendinopathy and tears of the rotator cuff are common. However, the molecular and biochemical pathophysiology of these disorders is incompletely understood. It is likely that a set of physical stimuli initiate a cascade of responses that ultimately lead to disruption of matrix synthesis and repair and then inferior material properties and mechanical failure. The aim of this study was to determine which protein based signalling molecules (cytokines) might play important roles in this cascade.

METHODS

In the first part of this study, the expression of 5760 genes was evaluated in a running rat tendinopathy model. In the second part of the study, the expression of the families of cytokines that were upregulated in the running rat model were evaluated in human rotator cuff tendon. 

Rat model: Twelve Sprague-Dawley rats underwent a daily treadmill running regimen to model tendon overuse resulting in degeneration as previously described. After four weeks of running, rats were sacrificed by CO₂ inhalation and both supraspinatus tendons were collected. Twelve non-exercised rats were used as controls. Custom microarrays consisting of 5760 rat oligonucleotides in duplicate were used. All genes were represented by at least two independent targets on each microarray, and the signal from each target was used to calculate an average for each gene.

Human tendon: Following ethics approval, seventeen supraspinatus tendon samples were collected from patients with rotator cuff tears undergoing arthroscopic shoulder surgery. Samples of the subscapularis tendon in which there was no evidence of damage on pre-operative imaging and at the time of surgery were also collected from the same patients. Control samples of subscapularis tendon were collected from ten patients undergoing arthroscopic stabilisation surgery without rotator cuff tears. Tendinopathy was assessed on a five point histological scale in a blinded fashion, where grade 4 = marked degeneration, 3= advanced degeneration, 2= moderate degeneration 1= mild degeneration, 0= normal tendon. Total RNA was isolated from tendon tissue using Trizol Reagent (Life Technologies, Grand Island, NY, USA) as per the manufacturer’s instructions. The expression of genes of interest were the evaluated by semiquantitative RT-PCR and immunohistochemistry in the human tendon samples. Positive and negative control specimens were included, in addition to the surgical specimens for each individual antibody staining technique. Differences among experimental groups were assessed by Student’s t-test with the level of statistical significance set at p<0.01.

RESULTS

Rat model: There were significant changes to the gene expression of the following cytokines in the supraspinatus tendons subjected to running: upregulation of IL-6 (1.2 fold), IL-11 (3.6 fold) and IL18 receptor (1.75 fold), downregulation of IL-2 (3.34 fold).

Human tendon: All torn human supraspinatus tendon showed Grade 4 histological changes consistent with marked tendinopathy with mucoid change and frank chondroid metaplasia. Matched subscapularis tendon showed Grade 2-3 appearances of moderate-advanced degenerative change with mucoid ground substance and fibrinoide degeneration of the collagen. All control samples showed Grade 1 fibroteninous tissue with large distinct collagen fibres.

The mRNA for interleukins 6, 15 and 18, TNF-α and MIF were detected in all samples of torn supraspinatus tendon. IL-15 and MIF were expressed in all matched subscapularis tendon samples while 16/17 expressed IL-18, 14/17 IL-6 and 13/17 TNF-α. IL-12, IL-11 and IL-2 were not detected in any tendon samples (but were detected in other tissues). The expression levels of IL-18, IL-15, IL-6 and MIF was significantly higher in the torn edges of supraspinatus when compared to matched subscapularis tendon and normal control tendon (p<0.001). TNF-α mRNA expression was found to be significantly elevated (p<0.001) in subscapularis tendon compared to torn supraspinatus samples.

DISCUSSION

Previous studies of tendinopathy have suggested a role for stress activated protein kinase activation and apoptosis in tendon degeneration. The cytokines overexpressed in this study have been shown to promote the intensive production of reactive O₂ metabolites and are potent agonists of protein kinases. Our finding of significantly increased levels of IL18,15,6, MIF and TNF-α support the hypothesis that these molecules when expressed during the degenerate and healing phases of tendon injury result in the subsequent production of reactive O₂ species and protein kinases causing apoptosis and tendon damage or failure of the normal reparative process. Our finding of marked tendinopathy in matched subscapularis tendon may also provide a useful human tendinopathy model.

REFERENCES

4 Das NS Eur Heart J 2006; 27:1385
5 Goedert M. EMBO J. 1999 Jun 16;18(12):3563-71

** Douglas Hanly Moir Pathology, Sydney, Australia
# University of Glasgow, Glasgow, Scotland, UK

53rd Annual Meeting of the Orthopaedic Research Society
Paper No: 0363