Overload Damage Results in Early Inflammation in Tendon

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Introduction: The role of inflammation in tendon injury is uncertain and is a topic of current interest [1]. Tendon injury is likely the result of damage accumulation during cyclic loading over an extended period of time. However, cellular factors and the role of inflammation in the processes leading to the initiation of tendinopathy are not easily investigated in human subjects, because the affected tendons are normally only assessed after advanced tendinopathic changes have occurred. Therefore, in vitro studies of the inflammatory response to induced tendon overload damage can serve as a valuable source of information on the early stages of tendinopathy. The role of the interfascicular matrix (IFM) in the initiation and propagation of tendinopathy remains obscure. The interfascicular matrix is the highly cellular region between collagen-rich fascicles (see Figure 1), that contains a variety of non-collagenous proteins (elastin, fibromodulin, proteoglycans) and minor collagens [2].
The interfascicular matrix is thought to create sliding planes that facilitate tendon extension and recoil. However, the exact role of this region in tendon remodelling and damage or injury initiation remains uncertain.

**Methods:** Fascicle bundles were dissected from three bovine extensor tendons (n = 4 per tendon). Samples were divided into three groups:
1. statically loaded to 2% strain for 24hrs;
2. cyclically loaded for 300 cycles (5 minutes) from 2-12% strain (1Hz) followed by 2% static strain for 24hrs;
3. cyclically loaded for 1800 cycles (30 minutes) from 2-12% strain (1 Hz) followed by 2% static strain for 24hrs.

Tests were performed in custom-made loading chambers, allowing loading of multiple samples, with samples maintained in sterile DMEM at 37 degrees. After testing, samples were snap frozen in optimal cutting temperature (OCT) embedding medium cooled in hexane on dry ice and stored at -80 degrees before 20 μm cryo-sections were cut from each sample, resulting in 108 sections for immunostaining. Dual immunostaining for the following marker pairs was performed:
- inflammatory markers - cyclooxygenase (COX-2) and interleukin (IL-6);
- matrix degradation markers: matrix metalloproteinases MMP-1 and MMP-3; and MMP-13 and the collagen degradation marker C1,2C.

Cell nuclei were stained with DAPI (blue) to distinguish the highly cellular interfascicular matrix regions from the less cellular fascicular regions containing elongated tenocytes. Sections were imaged with a confocal microscope (Leica TCS SP2). Images were thresholded with a single level threshold (for a given stain) and overlaid to allow a qualitative assessment of the extent and intensity of immunostaining within IFM and fascicles.

**Results:** Cyclic loading of samples resulted in visible matrix damage, with disrupted collagen fibres and fibre kinks (white arrows in Figure 2 and 3). Damage was especially localised to the interfascicular matrix, with less damage being identified within the collagen-rich fascicles. Inflammatory markers were only expressed in the cyclically loaded samples, and were more highly expressed in the samples loaded for 1800 than 300 cycles. COX-2 staining appeared stronger than IL-6 staining and was localised predominantly in the vicinity of the interfascicular matrix, see Figure 2.
Matrix degradation markers MMP-1 and C1,2C were present in statically strained control samples (Figure 3a and d) as well as the loaded samples (Figure 3b, c, e, f). MMP-1 and C1,2C were co-localised in many areas. These stains were again more localised to the interfascicular matrix, but were also expressed within fascicles and associated with tenocytes, especially in the loaded samples.

Little MMP-3 or MMP-13 was evident in control sections. However, in loaded samples, some sections showed slight increase in staining of these enzymes, again localised mainly to interfascicular matrix regions (not shown).
**Discussion:** This study suggests that inflammatory markers may be expressed rapidly and early after tendon overload exercise (within 24h following 5 or 30 minutes of overload exercise), indicating that inflammation may be an early occurrence in tendinopathy. Interestingly, both inflammation and damage-induced matrix remodelling seem to be concentrated in or in vicinity of the interfascicular matrix. This may be due to increased transfer of strain through the fascicles into the softer interfascicular matrix, leading to different loading conditions in the interfascicular matrix, with a greater shear component to facilitate fascicles sliding within tendon. However, the greater response of interfascicular matrix to loading could also be explained by its high cellularity, with more cells producing the target enzymes. Possible different roles of interfascicular matrix cells and tenocytes in the initiation, progression and resolution of inflammation and the consequences for tendinopathy are yet to be elucidated.

**Significance:** This study contributes to the knowledge of the early occurrence of inflammation in tendons, and highlights the role of the interfascicular tendon matrix in the processes of inflammation and collagenous matrix remodelling resulting from overload exercise.

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