Gene Expression and Protein Analysis in Ruptured Human Achilles Tendons. A Comparison between Ruptured and Healthy Area
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Introduction: Matrix metalloproteases (MMPs) are involved in remodelling of the extracellular matrix (ECM) of tendons, and the various MMPs can be either up or down-regulated in tendinopathy (1). A balance between MMPs and tissue inhibitors of metalloproteases (TIMPs) is required to maintain ECM homeostasis (2). Their precise role in tendinopathy is still unclear, and it is conceivable that MMPs play a role in tendinopathies (3). The hypothesis of this study was that the metabolism of these molecules is altered in patients with Achilles tendon rupture.

Materials and Methods: We studied the extracellular matrix of 19 ruptured human Achilles tendons, comparing the tissue composition of specimens harvested from area close to the rupture with specimens harvested from an apparently healthy area in the same tendon. We compared the gene expression and the protein localization of collagen type I, decorin and versican including enzymes involved in their metabolism as matrix metalloproteases (MMP-2 and -9) and tissue inhibitor of metalloproteinase (TIMP-1 and -2) using a Real Time PCR, zymography and FACE analysis. Descriptive statistics were calculated. The significance of means analysis was carried out using $t$-student paired test. Alpha of 0.05 was applied.

Results: The gene expression of proteoglycans core protein, collagen type I, MMPs and TIMPs was more represented in the area close to the tendon rupture

Panel A: gene expression pattern of tendon specimens harvested close and far from the rupture area. Panel B: the same genes reported in Panel A are compared with the expression pattern of the Col I A1 gene. The bars represent a mean of three different experiments on 19 different patients. R: gene expression of the ruptured area. H: gene expression of the healthy area. (p<0.05). The expression of MMPs was confirmed by zymography analysis, showing a marked increase of gelatinolytic activity in area close to the tendon rupture

Zymography of the extracts from human tendons. The ladder indicates the molecular weight of the protein standards. H healthy area, R rupture area. The activity bands are clear band on the blue background. The Bands 72 kDa and 66 kDa represent the zimogen and activated MMP-2 respectively. The bands with molecular weight above 100 kDa correspond the the MMP-2 lipocalin complexes. Zymography was performed on 19 different samples, and the results showed the same experimental pattern (A, 13th sample; B, 16th sample). (p<0.05). The chemical composition of tendon changed showing that in the healthy area the carbohydrate content is higher than the ruptured area

Examples of FACE analysis of the glycosaminoglycan disaccharides obtained from the human tendons: H healthy area, R rupture area. A shows the non-sulphated glycosaminoglycans. B shows the sulphated glycosaminoglycans disaccharides (13th sample). (p<0.05).

Discussion: In the ruptured area, the tenocytes tried to restore the normal proteoglycan pattern increasing the core protein synthesis but without the normal glycosaminoglycan production. Our data support the hypothesis that, in human tendons, the tissue in the area of rupture undergoes marked rearrangement at molecular levels, and support the role of MMPs in the tendon pathology.