DIFFERENCES IN COLLAGEN GENE EXPRESSION IN MALE AND FEMALE ACL INJURED ATHLETES

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INTRODUCTION:

Anterior cruciate ligament (ACL) injuries occur two to eight times more frequently in women than men. The explanation for this difference has focused on either extrinsic or intrinsic factors. Many studies have evaluated gender differences at the gross anatomical level. To date, however, little is known as to whether any gender differences exist below the gross level. For example, it is unclear if gender differences exist in the ligament structure composition at molecular levels. This information can be critical not only because the properties of ligaments are determined by their molecular components, but also because cell functions are regulated at this level. The goal of this study was to verify if there is a gender difference between male and female athletes in the gene expressions of types I and III collagens in ACL fibroblasts using reverse transcript-polymerase chain reaction (RT-PCR).

METHODS:

ACLs were harvested from 17 male and 17 female athletes with acute ACL tears (33.5 ± 13.3 days for females and 16.4 ± 7.8 days for males from injury to surgery) undergoing ACL reconstruction. The age for the female patients was 24.2 ± 12.7 years and 18.0 ± 5.3 years for the males. In order to preserve their pre-injury properties, all samples were taken from an area of the ACL far from the rupture ends during surgery. Additionally, each sample to be used for further RT-PCR study also underwent histological analysis to ensure no post-injury effects existed. Samples were fixed in 10% buffered formalin and embedded in paraffin. Five micron-thick sections were cut and followed by hematoxylin and eosin (H & E) staining. Ten micron-thick serial sections were cut for RNA extraction. The target areas with well-organized collagen fibers and fibroblasts, but no inflammatory cells or blood vessels, were cut macroscopically with a fine needle referring to the microscopic observation of the morphology of the H & E stained sections [1].

The isolated tissues were digested with proteinase K overnight and total RNA from the fibroblasts was extracted with TRIzol reagent according to manufacturer's instruction. One-step RT-PCR analyses were performed using specific primers for collagen I (α1), collagen III, and ß-actin (as internal control). PCR cycle numbers were carefully selected within the linear zone of each gene. PCR products were eletrophoresed in 4% agarose gels and densitometric analyses were performed using the image analysis software AlphaEase™ (Alpha Innotech Corp. San Leandro, CA). The resultant data were expressed as a ratio to the internal control value. Student T-test was employed to analyze the difference between male and female samples.

RESULTS:

Although our samples were selected from non-injury sites of torn ACLs using visual observation, our histology studies showed focal inflammatory infiltrates. However, the filtrations were not uniform, and well-organized collagen fibers and fibroblasts were still preserved (Fig.1). Only the areas with well-organized collagen fibers and fibroblasts were used for further RT-PCR study.

The relative expression of collagen I (normalized to internal control) was 1.57 ± 0.58 in males and 1.14 ± 0.75 in females. The relative expression of collagen III was 0.21 ± 0.18 in males and 0.34 ± 0.26 in females. There was a significant difference between males and females in collagen I ($P<0.05$), but not in collagen III ($P>0.05$) (Fig.2).

DISCUSSION:

Female athletes are two to eight times more likely to suffer a knee or ankle ligament injury than male athletes. Our finding that female athletes have lower gene expression of collagen I—the most abundant extracellular matrix molecule in ACL—inferred that there may be a gender difference between male and female molecular compositions in ACL. This difference may be responsible for the higher ACL injury incidence in female athletes. Since to our knowledge, this is the first time gender differences of ACL have been noted at the molecular level, some issues needed to be addressed to ensure our results were reliable.

The purity of cells was critical for this study. Previous literature has shown that inflammatory cells are absent in the ruptured end of injured ACL, especially within three weeks after injury [2]. In our histology study, we confirmed the absence of infiltrated inflammatory cells in our samples taken from non-injury sites of torn ACL. Blood vessel endothelium cells are also common in ACL. Those non-fibroblast cells, though they produce little collagen, contribute significantly to β-actin and total RNA. Our preliminary study showed that the inclusion of those cells caused major errors in the RT-PCR results. However, the non-uniform distributions of inflammatory cells and blood vessels, as well as the local preservation of well-organized collagen fibers and fibroblasts shown in our histology study, made it possible to obtain pure fibroblasts using selective dissections in each sample referring to the H & E stained sections.

Previous studies showed that ruptured ends of torn ACL underwent repair and remodeling from about eight weeks after rupture [2]. Animal studies also showed that ACLs of immobilized rabbits underwent degeneration from about six weeks after immobilization [3]. In order to avoid the effects of degeneration or remodeling, several measures were adopted. First, we strictly chose patients with acute injury within two months. Second, the ACL samples were harvested far from the torn ends during surgeries. Third, histology analysis was done to assure that only the areas without obvious degeneration or remodeling were used.

As the most abundant extracellular matrix molecules, the transcription, translation, post-translation modification, fibril formation and cross-linking of collagen types I and III are very complicated in ACL. Based on our findings in this study, we are currently working on the collagen protein and structure studies. Those results, together with our current findings, will yield a clearer picture of the gender difference at the molecular level in ACL.

In summary, the gene expressions of collagen types I and III of fibroblasts from ACLs of 17 male and 17 female athletes with acute ACL tears were studied. Female athletes were found to have a significantly lower gene expression of collagen I.

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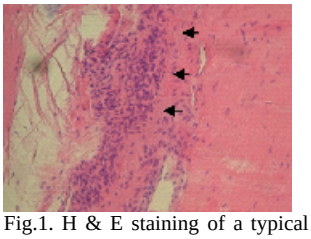

Fig.1. H & E staining of a typical injured ACL sample. Inflammatory infiltrates, mainly lymphocytes and plasma cells were found in some areas (arrows). However, organized collagen fibers and fibroblasts were also preserved (right portion).

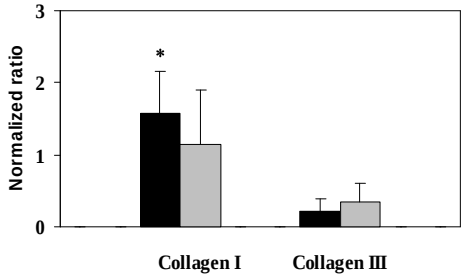

Fig.2. There is a significant difference between male (black) and female (grey) in gene expression of collagen I, but not in collagen III.