INFLAMMATION OF INJURED PATELLAR TENDONS AFFECTS COLLAGEN FIBRIL DIAMETERS

Alaseirlis, D.A., Li, Y., Cilli, F., Fu, F.H., *Wang, JH-C.

* MechoBiohnology Laboratory, Departments of Orthopaedic Surgery and Bioengineering, University of Pittsburgh
210 Lothrop St., BST, E1647, Pittsburgh, PA 15213, 412-648-9102, FAX: 412-648-8548
wanghc@pitt.edu

INTRODUCTION

Connective tissue wound healing begins immediately after injury (Nerlich, 2003) and continues for months or even years (Monaco and Lawrence, 2003). The first phase of this healing process is inflammation, which overlaps with the proliferation phase. The inflammation phase is noted by inflammatory cell recruitment, the release of inflammatory factors (e.g., TNF-a), vascular ingrowth, and increased tissue construction. The inflammatory phase is necessary for wound healing; but, as an in vivo study has shown that skin wound healing in TNF-receptor deficient mice (Rp55 deficient mice) was accelerated compared to wild type mice, which suggests that inflammation caused by TNF-β at the wound site negatively affects wound healing by reducing collagen accumulation as well as angiogenesis (Mori et al., 2002). Therefore, we hypothesized that decreasing the early inflammatory response to injury would improve the quality of the healing tendon. To test this hypothesis, injuries were created by transecting the right patellar tendons of C3H/HeJ and C3H/HeN mice, and collagen fibrils of injured and non-injured tendons from both mice were examined at 7 days post-injury.

MATERIALS AND METHODS

Two strains of mice were used: three C3H/HeJ mice, and three C3H/HeN mice. C3H/HeJ mice have a genetic deficiency in the production of TNF by macrophages and other cytokines in response to endotoxin and C3H/HeN mice have no genetic deficiency. The right patellar tendon of both mouse strains was transversely transected, whereas the left patellar tendon was left intact for control. After 7 days, both right and left patellar tendons were harvested, and tendon samples were prepared for examination with transmission electron microscopy (TEM).

A software program (NIH Image J) was then used to analyze the TEM images, with a negatively stained catalase crystal used as a standard to estimate collagen fibril diameters. A total of 261 to 552 collagen fibrils were used to construct frequency distributions of collagen fibril diameters. A total of 261 to 552 collagen fibrils were used to construct frequency distributions of collagen fibril diameters. For statistical analysis, the Kolmogorov-Smirnov test was used to compare collagen fibril distributions between C3H/HeJ and C3H/HeN mice for both injured and non-injured tendons. An unpaired student t-test was also used to compare the means of collagen fibril diameter from the C3H/HeJ and C3H/HeN mice. A difference was considered to be significant if the p value was less than 0.05.

RESULTS

It was found that the collagen fibrils of the healing tendons in the C3H/HeJ mice were consistently larger than those of the C3H/HeN mice (Fig. 1A&B). In addition, the space between collagen fibrils of healing tendons in C3H/HeJ mice was more uniform than in C3H/HeN mice. The collagen fibrils of non-injured tendons, however, had a similar size and organization (Fig. 1C, D).

Furthermore, the collagen fibril diameter of injured tendons in C3H/HeJ mice was 46.2 ± 10.7 compared to 29.4 ± 7.2 (nm) in C3H/HeN mice, where the difference was significantly different (p < 0.01). The collagen fibril diameters of non-injured tendons in C3H/HeJ and C3H/HeN mice were 100.8 ± 57.8 compared to 95.6 ± 43.2 (nm), respectively (Fig. 2). This difference in collagen fibril diameters was not significantly different (p = 0.13).

Fig. 2 The comparison of collagen fibril diameters from injured and non-injured tendons of C3H/HeJ and C3H/HeN mice.

DISCUSSION

Using genetically deficient mice, C3H/HeJ, we found that reducing the inflammatory response increased the diameter of collagen fibrils and improved the organization of collagen fibrils of healing patellar tendons compared to control mice, C3H/HeN. Previous studies have shown that inflammatory cytokines influence the healing quality of injured tissues. For example, in TNF-Rp55 deficient mice that had reduced leukocyte infiltration, skin wound healing was enhanced (Mori et al., 2002). Also, using knocked-out mice, it was found that TNF-α affects connective tissue breakdown by promoting acute smoke induced inflammation (Churg et al., 2002). With genetically defective mice, C3H/HeJ, inflammatory cytokines at early healing times impair healing and delay improved tensile strength of skin wounds. Thus, this study was consistent with previous studies in that injured tissue inflammation reduces the quality of the healing tissue.

The findings of this study are important, because the organization and collagen fibril diameter of the healing tissue remain inferior to those of normal tissue (Scott and Hughes, 1986) after a period of weeks or several months and because collagen fibril diameter is associated with the mechanical strength of the healing tissue (Birch et al., 2003). Therefore, to enhance the quality of injured tendons in the early stages of healing, it may be desirable to reduce tissue inflammation of injured tendons with anti-inflammatory drugs. Future studies should evaluate the effect of decreased inflammation on these properties at a later healing time-point and determine the effect of decreased inflammation on the mechanical properties (e.g., stiffness, ultimate tensile strength) of the healing tendon.

REFERENCES

Birch et al., 2003; Churg et al., 2002; Monaco and Lawrence, 2003; Mori et al., 2002; Nerlich, 2003; Scott and Hughes, 1986

ACKNOWLEDGEMENT

This study was supported in part by the Arthritis Investigator Award, Whitaker Biomedical Engineering Grant, and NIH grant AR049921 (JHW). We would like to thank Joseph P. Suhani in the Department of Biological Sciences at Carnegie Mellon University for his assistance in electron microscopy.

Fig. 1 TEM photographs showing collagen fibrils of injured and non-injured patellar tendons of C3H/HeJ and C3H/HeN mice. A. Injured tendon from a C3H/HeJ mouse; B. Injured tendon from C3H/HeN mice; C. Non-injured tendon from a C3H/HeJ mouse; and D. Non-injured tendon from a C3H/HeN mouse.