EXPRESSION OF TGF-\(\beta_1\) AND \(\alpha\)-SMOOTH MUSCLE ACTIN POSITIVE CELL IN INJURED MEDIAL COLLATERAL (MCL) AND ANTERIOR CRUCIATE (ACL) LIGAMENTS

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Introduction

Ligaments are one of the most frequently injured tissues of the musculoskeletal system. The extra-articular MCL undergoes a classic healing response which results in inferior biomechanical properties, with the modulus of healing 20-50% of controls (4, 5). The intra-articular ACL has even poorer healing with a healing response that is “functionally insufficient”. It has been shown that \(\alpha\)-smooth muscle actin (SMA) positive cells are myofibroblasts (1) and contribute substantially to the contractile period of the healing and might allow recovery of original ligament length and in situ strain (3). It also has been reported that the expression of SMA by the myofibroblast is regulated by transforming growth factor \(\beta\) (TGF-\(\beta\)) (2). The objectives of this study were to (1) describe the spatial-temporal expression of the myofibroblast in the entire injured MCL and ACL, and (2) to look at the expression of TGF-\(\beta_1\) and its receptor I and II in the injured ligamentous tissue.

Material and Methods

A “mop-end” tear model (5) was created in the left MCL of 21 adult New Zealand white rabbits. The antero-medial bundle of the right ACL was transected in mid-substance, thus creating a partial ACL tear. Non-injured contralateral ligaments were used as controls. Three animals each were sacrificed at 3, 7, 14, and 21 days, and 4, 6 and 12 weeks post-injury. Ligaments were retrieved entirely, fixed and sliced in three micrometer-thick sections. Ligament healing was assessed histologically with routine hematoxilin and eosin staining. Expression of \(\alpha\)-smooth muscle actin (SMA) positive cells or myofibroblasts was revealed by immunohistochemistry using a mouse anti-human \(\alpha\)-smooth muscle actin monoclonal antibody. Expression of TGF-\(\beta_1\) was revealed by immunohistochemistry using a rabbit anti-human TGF-\(\beta_1\) polyclonal antibody, and expression of TGF-\(\beta_1\) receptor I and II by rabbit anti-human TGF-\(\beta_1\) receptor I and rabbit anti-human TGF-\(\beta_1\) receptor II polyclonal antibodies respectively. The spatial-temporal expression of \(\alpha\)-smooth muscle actin and TGF-\(\beta_1\) receptor I was quantified by image analysis (Zeiss KS 400 3.0).

Results

Histological analysis of healing of the MCL revealed an inflammatory phase (Day 3-7), a proliferation phase (Day 7-3 weeks), and a maturation phase (3 – 12 weeks). Analysis of the ACL showed an inflammatory phase (Day 3-2 weeks) with increased vascularisation of the injured region of the ligament and a limited proliferation phase (2 - 6 weeks). The healing process failed to restore the integrity of the injured tissue. The \(\alpha\)-smooth muscle actin positive cells were seen as early as the third day post-injury in the MCL. Their numbers increased up to the third week before decreasing progressively toward the 12th week (Table 1A). These cells were first located at the periphery of the ligament and migrated toward the center of the lesion (Figure 1A). A few \(\alpha\)-smooth muscle actin positive cells were found in the ACL. These were mostly seen in the lesion from day 7 to 2 weeks. The TGF-\(\beta_1\) and TGF-\(\beta_1\) receptor I and II were noted in the injured MCL at day 3 and increased up to 7 days. At this time, the TGF-\(\beta_1\) expression was located within inflammatory cells (Figure 1B). At 2 weeks, the expression of TGF-\(\beta_1\) was low, followed by a new peak at 3 weeks, and then a progressive decrease from the 4th to the 12th week. At three weeks, the expression of TGF-\(\beta_1\) was mainly noted within the proliferating fibroblasts. In the ACL, the expression of TGF-\(\beta_1\) was also noted from the day 3 (Figure 1C), with a decrease at 2 weeks, a new peak at 3 weeks, and then a rapid decrease from to 4 to 12 weeks post-injury.

Discussion

The histological healing found in this study confirms previous reports. However, this study shows that myofibroblasts appear in the lesion as early as the third day post-trauma in a centripetal migration. This corresponds to the presence of the first wave of TGF-\(\beta_1\) in the injured MCL and ACL. Interestingly, the density of myofibroblasts was higher in the MCL. This may partially explain the difference in the healing response which results in inferior biomechanical properties, with the modulus of healing 20-50% of controls (4, 5). The intra-articular ACL has even poorer healing with a healing response that is “functionally insufficient”. It has been shown that \(\alpha\)-smooth muscle actin (SMA) positive cells are myofibroblasts (1) and contribute substantially to the contractile period of the healing and might allow recovery of original ligament length and in situ strain (3).

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Table 1: Expression of SMA and TGF-\(\beta_1\) RI in MCL (A,B) and ACL (C, D).

Figure 1: A. \(\alpha\)-smooth actin positive cells migrating from the periphery to the center of an MCL lesion at 7 days post-injury. B. TGF-\(\beta_1\) expression in an MCL injury at 7 days post-injury. C. TGF-\(\beta_1\) expression in an ACL injury at 7 days post-injury.