INTRODUCTION

Post-traumatic joint arthrofibrosis is detrimental to the functional capability of patients. Surgery is often required, but is not always successful in restoring motion. Anti-fibrotic therapy has not been widely used for the management of stiff joints, partly because the pathophysiology of joint fibrosis remains poorly understood.

Myofibroblasts are considered central in the genesis of joint fibrosis and have been shown to be increased in the capsule of humans with elbow contractures, as well as rabbit models of arthrofibrosis.\(^1,^2\) α-SMA has rapidly become the most accepted marker of myofibroblasts.\(^3\) However, the timeline of myofibroblast proliferation in joint contractures is largely unknown. The aim of this study was to determine the absolute and relative number of myofibroblasts present in contracting joint capsules at different times.

METHODS

Eighteen skeletally mature New Zealand White female rabbits were divided into 3 experimental groups: Group I (n = 6) underwent 2 weeks of immobilization, Group II (n = 6) underwent 8 weeks of immobilization, and Group III (n = 6) underwent 8 weeks of immobilization plus 16 weeks of remobilization. The right limb was operated on in all animals to create 3-mm defects in the non-cartilaginous portions of the femoral condyles, hyperextend the joint to disrupt the posterior capsule, and immobilize the joint in maximum flexion with a Kirschner-wire for 8 weeks.\(^4\) Five additional rabbits did not undergo any surgery. They were allowed free cage activity and were otherwise treated identically. All animals survived without any complications. All experiments were approved by our Institutional Animal Care and Use Committee.

After sacrifice, specimens were sectioned, fixed, and then incubated. Mouse monoclonal anti-αSMA antibody was applied. Visualization was carried out using anti-mouse labeled HRP polymer. Sections were counterstained with hematoxylin to identify nuclei. Positive and negative controls were assessed, with capsular blood vessels serving as α-SMA positive tissue and bovine serum albumin substitution primary antibody serving as a negative control. Sections were viewed under light microscopy. Identifiers were removed to the investigator performing the counts. Three images of capsule were taken on 6 serial sections of tissue. Images were captured at 400X, and the number of myofibroblasts and total cell number were quantified. Myofibroblasts were identified as cell nuclei associated with α-SMA stain. Blood vessels were manually excluded based on morphology.

Total myofibroblast numbers, total cell count numbers, and percent of myofibroblasts were measured and compared to the non-operative limbs, in addition to the animals receiving no surgery. Direct comparison between experimental groups was done using a two-sample t-test assuming unequal variances (p < 0.05). Data are represented as mean ± standard deviation (SD).

RESULTS

The percent of myofibroblasts was significantly elevated in the operated limbs compared to the control limbs at 2 weeks (20% vs. 7%, respectively; p = 0.014) (Figure 1). There was no difference in the percent of myofibroblasts between the operated and control limbs at 8 or 24 weeks (p = 0.96 and 0.19, respectively). The percent of myofibroblasts dropped from 20% at 2 weeks to 3% at 8 weeks (p < 0.001) and 2% at 24 weeks (p = 0.0001) (Figure 2). The decrease from 8 to 24 weeks was not significant (p = 0.19). There was no statistical or clinical difference between the contralateral limbs and the limbs of animals that received no surgery (p = 0.4).

DISCUSSION

The appearance, and therefore the relevance, of the myofibroblast appears to be an early phenomenon. There was a statistically greater percentage of myofibroblasts at its peak in the contracted limb at 2 weeks, which subsequently decreased over time. Thus, what has not been previously emphasized is that pharmacologic interventions targeted at myofibroblasts will likely require administration during the early stages of fibrosis. In addition, there was no difference in myofibroblast numbers between the contralateral limb and limbs that received no surgery, indicating a lack of systemic effect. This further validates the unproven assumption that the contralateral limb may serve as a valid histologic control.

REFERENCES