Expression TGFβ Receptors and Collagen III/VI in the Subsynovial Connective Tissue in a Rabbit Model of Carpal Tunnel Syndrome

Sun, Y-L; Moriya, T; Zhao, C; An, K-N; Pamadio, P C
Biomechanics Laboratory, Division of Orthopedic Research, Mayo Clinic, Rochester, MN
pamadio@mayo.edu

INTRODUCTION

The subsynovial connective tissue (SSCT) in the carpal tunnel has been implicated in the development of carpal tunnel syndrome (CTS)1. There is a significant increase in TGFβ-R1 expression in the fibroblasts of CTS patients compared with unaffected individuals2, and collagen III is significantly more abundant in the patients than in the controls. In addition, collagen VI is abundant in the SSCT of both CTS patients and unaffected individuals. Recently a rabbit model has been described in which injury of the SSCT leads to median neuropathy, similar to what is seen in human CTS. We wished to see if the SSCT response in this model was similar to that seen in clinical cases of CTS. Our hypothesis was that the expression of TGFβ-R1 and collagen III would be similar when comparing the rabbit animal model with human CTS, and that the expression in normal rabbits and humans would also be similar to each other.

METHODS

Surgical Procedure: Eighteen female New Zealand white rabbits were used for this study. Two types of surgical intervention were applied in 9 rabbits. The first surgical intervention (sham intervention) was achieved by making 2 volar incisions at the proximal and distal ends of the carpal tunnel of one randomly selected forepaw, exposing the flexor digitorum superficialis (FDS) tendon of the third digit. The second surgical intervention (SSCT shear injury) was performed on the middle digit of the contralateral forepaw. Two similar volar incisions were made and the third digit muscle-tendon junction was cut. The cut distal end of the FDS was then advanced 5 mm distally by traction at the distal incision, and a loop was sutured in the tendon to maintain this position of the tendon (Fig. 1). After recovery, the rabbits were allowed 30 minutes of exercise outside their cage twice a week until sacrifice. 12 weeks after surgery. Nine other rabbits were used as a normal control group.

Immunohistochemistry of TGFβ Receptors and Collagen III/VI:

The tissues of the carpal tunnel in each paw were fixed with 10% neutral buffered formalin, dehydrated, paraffin embedded, and sectioned at 5 μm thickness. The sections were deparaffinized with xylene, rehydrated in graded ethanol, and then incubated in 3% H2O2 at room temperature for 10 minutes. The sections were blocked with 1.5% normal horse serum for 1 hour and incubated with anti-TGFβ-R1 (Santa Cruz sc-398-G, Santa Cruz, CA), TGFβ-RII (Santa Cruz sc-17792), TGFβ-RIII (Santa Cruz sc-6199), collagen III (Acirs AF5810, clone III-S3, Hiddendenhausen, Germany), collagen VI (Acirs AF6210) at 4°C overnight. After extensive washing with PBS, the sections were incubated with the biotinylated secondary antibody and Vectastain Elite ABC reagent (Vector Laboratories, Burlingame, CA). Visualization was achieved with AEC substrate (Vector Laboratories). The slides were counterstained with hematoxylin QS (Vector Laboratories).

Analysis of TGFβ receptors was performed by measuring the percentage of fibroblasts expressing TGFβ receptors. The intensity of TGFβ-R1 expression in the control group was 16.0±7.7% (Fig. 3). Surgical interventions significantly increased the percentage of TGFβ-R1 expression in the fibroblasts of SSCT. The percentages of TGFβ-R1 expression in the sham and shear injury groups were 41.1±27.6% and 49.7±25.7%, respectively. No statistical significance was achieved between the two types of surgical intervention. The expression of TGFβ-R2 and TGFβ-R3 in the fibroblasts of the SSCT of the rabbits with or without surgical intervention was also investigated in this study. As with TGFβ-R1, surgical interventions significantly increased the percentage of fibroblasts with positive TGFβ-R2 and R3 expression (Fig. 3). The difference between the two types of surgical intervention was not significant.

RESULTS

TGFβ receptors were expressed in the certain fibroblasts of SSCT of all rabbits (Fig. 2). The percentage of TGFβ-R1 expression in the control group was 16.0±7.7% (Fig. 3). Surgical interventions significantly increased the percentage of TGFβ-R1 expression in the fibroblasts of SSCT. We conclude that the effect of this animal model on the SSCT has some similarities to the findings in human CTS, but the difference in collagen III staining showed a mean grade of 1.70±0.36 in the control group, which was the same as the grades of 1.74±0.21 and 1.76±0.43 in the sham and open shear injury groups, respectively. Both surgical interventions resulted in the significant decrease of collagen VI comparing to the control group (1.80±0.45). But there was not significant difference between sham intervention (0.98±0.51) and SSCT shear injury (1.06±0.56).

DISCUSSION

The percentage of fibroblasts with TGFβ-R1 positive staining in the rabbit control group is similar to the percentage of 19.5±15.0% found in normal humans. Both surgical interventions resulted in a significant increase in the percentage of fibroblasts with TGFβ-R1 expression in the rabbit SSCT, to levels similar to the rate of 62.7±20.9% seen in the SSCT of patients with CTS. In contrast to the clinical situation, in which the expression of collagen III is increased in patients with CTS, the surgical interventions in this study did not alter the expression of collagen III. Meanwhile, a significant decrease of collagen VI was found in this animal model with the surgical interventions but not in CTS patients.

In this study, there was the lack of difference in the expression of TGFβ receptors and collagen VI between sham intervention and SSCT shear injury, with both sham intervention and SSCT shear injury being different from normal, suggests that even a minor surgical intervention, and thus probably a minor injury, has the potential to produce a measurable and lasting effect on SSCT structure, and possibly function.

We conclude that the effect of this animal model on the SSCT has some similarities to the findings in human CTS, but the difference in collagen III expression raise some question as to the ability of this model to perfectly mimic human CTS.

ACKNOWLEDGEMENTS

This study was funded by a grant from NIH (NIAMS AR 49823).

REFERENCES