OVER-EXPRESSION OF IL-1BETA, TNF-ALPHA, AND TGF-BETA IN TENDON FIBROBLASTS DUE TO STRESS DEPRIVATION CANNOT BE REDUCED BY STRESS RESUMPTION

*Uchida, H; +*Tohyama, H; **Matsumoto, H; ++*Toyama, Y; ***Ohba, Y; ***Nagashima, K; *Yasuda, K
++Division of Medical Bioengineering & Sports Medicine, Department of Advanced Surgery, Hokkaido University School of Medicine, Sapporo, Japan

Introduction

Biological tissues change their dimensions and mechanical properties in response to mechanical environments. Therefore, it has been an important issue to clarify the effect of mechanical environment on these tissues. We have developed a unique stress-shielding technique for the patellar tendon (PT) without immobilization of the joint [1]. Our recent study has shown that complete stress deprivation induces not only remarkable reduction of the mechanical properties of the PT matrix but also over-expression of IL-1beta, TNF-alpha, and TGF-beta in fibroblasts [2]. Yamamoto et al. [3] reported that the reduction of the mechanical properties is restored by resumption of stress. However, no studies have dealt with the effect of stress resumption on expression of these cytokines. The purpose of this study is to clarify whether over-expression of IL-1beta, TNF-alpha, and TGF-beta in PT fibroblasts due to stress deprivation can be reduced by stress resumption.

Materials and Methods

Twenty male Wistar rats aged 14 to 16 weeks old were used. In each animal, the right PT was completely shielded from stress by drawing the patella toward the tibial tubercle with a flexible stainless steel wire [2]. After the surgical treatment, the animals were randomly divided into the stress-shielded (SS) and the restressed (RS) groups for 10 animals each. In the SS group, animals were sacrificed at 2 weeks after the operation. In the restressed (RS) group, the wire installed between the patella and the tibial tubercle was cut to apply tension to the right PT at 2 weeks. All animals in the RS group were sacrificed at 6 weeks after the initial operation. No immobilization was applied after operation. In each group, five of 10 rats were used for immunohistochemical evaluation and the remaining five rats were used for biomechanical examination. To obtain normal control data, 10 PTs were randomly harvested from the left knees of all the rats. For immunostaining, the PT was excised and fixed with 10% neutral buffered formalin, and paraffin sections were prepared. The sections were incubated with each primary antibody for IL-1beta, TNF-alpha, and TGF-beta. The reaction products were detected with diaminobenzidine. The number of total cells was counted in a unit rectangular area (220 x 330 micrometers) that was randomly chosen in a microscopic visual field. Then, we defined the ratio of the number of positively stained cells to the number of total cells as "stained cell ratio". In biomechanical examination, the cross-sectional area of the PT was measured with an area micrometer. Then, each patella-PT-tibia complex was mounted on a tensile tester. After preconditioning with 10 cycles of 3% strain, the complex was stretched until failure at a crosshead speed of 20 mm/min. The strain in the tendon substance was determined using a video dimension analyzer. Tangent modulus was determined from the stress-strain curve. The one-way ANOVA and Fisher's PLSD test were used for statistical analysis.

Results

1) Immunohistochemical evaluations: The stained cell ratios on IL-1beta, TNF-alpha and TGF-beta were significantly higher in SS and RS groups than in the control tendons (Fig. 1). The IL-1beta ratio was significantly higher in the RS group than in the SS group (p<0.05), while there were no significant differences in the ratio on TNF-alpha or TGF-beta between SS and RS groups (Fig. 2). 2) Biomechanical evaluations (Fig. 3): The cross-sectional area values in SS and RS groups were significantly greater than that of the control (p<0.001), while there were no significant differences between the RS and SS groups. The tangent modulus of the RS group was significantly higher than that of the SS group (p<0.05), while those of RS and SS groups were significantly lower than the control (p>0.001).

Discussion

This study demonstrated that over-expression of IL-1beta, TNF-alpha, and TGF-beta in patellar tendon fibroblasts due to stress deprivation cannot be reduced by stress resumption. On the other hand, this study showed that resumption of stress significantly restored the mechanical properties of the patellar tendon. This result is consistent with findings of our previous study in the rabbit model [3], which reported the effect of stress resumption on the stress-shielded patellar tendon in detail. Therefore, stress resumption was considered to be sufficiently done in the present study. Nevertheless, the cytokine expression was not reduced by stress resumption. This fact suggested that there is a possible mechanism that patellar tendon fibroblasts keep or increase the expression levels of IL-1beta, TNF-alpha, and TGF-beta once these expression levels are unregulated by stress deprivation, such as autocrine or paracrine stimulation.

References

++Department of Orthopaedic Surgery, Keio University School of Medicine, Tokyo, Japan
***Department of Molecular & Cellular Pathology, Hokkaido University School of Medicine, Sapporo, Japan

49th Annual Meeting of the Orthopaedic Research Society

Paper #0149