Introduction

The incidence of anterior cruciate ligament (ACL) rupture in female athletes is two to eight times that in males. Although many anatomical and biomechanical gender differences have been described, evidence is beginning to accumulate that female hormones are responsible for alterations in ligament laxity and strength. The ACL injury rate has been shown to increase during the luteal phase of the menstrual cycle, corresponding to peak relaxin levels in the non-pregnant female. Relaxin is a peptide hormone found in the sera of pregnant and non-pregnant females, yet has never been detected in male serum. This hormone is thought to be responsible for connective tissue remodeling secondary to its collagenolytic effects. This restructuring occurs at the maternal-fetal interface, allowing for elongation of the pubic symphysis during late pregnancy and parturition. Ligamentous laxity of the knee has also been reported in pregnant women who have high relaxin titers. These effects are largely mediated by the release of collagenase, several matrix metalloproteinases, and plasminogen activator, which lead to a marked local decrease in collagen content. Since collagen is the main load-bearing component in ligamentous tissue, alterations in collagen content and organization could lead to changes in ligament integrity.

Methods

After obtaining IRB approval and informed consent, ACL remnants were harvested from 5 female and 5 male patients undergoing routine ACL reconstruction. Women with a history of pregnancy, or those on estrogen replacement therapy or oral contraceptives were excluded from the study. The specimens were frozen to -80°C and sectioned. Relaxin was biotinylated using a standard protocol and analyzed for biologic activity using the mouse interpubic ligament bioassay. Immunohistochemical localization of the relaxin receptors was accomplished by incubating the sections in four different reagents. The first group was incubated with 4-µg/ml of biotinylated relaxin to localize relaxin receptors. The second group was incubated with unmodified relaxin as a negative control. The third group was incubated with biotinylated relaxin and a 2000-fold excess of human insulin, the structural homologue of relaxin, to determine hormonal specificity of the biotinylated relaxin. The fourth group was incubated with biotinylated relaxin and a 2000-fold excess of unmodified relaxin to determine receptor saturability. One half of the sections were then incubated in anti-biotin IgG conjugated to colloidal gold and silver intensified. The remaining sections were incubated in anti-biotin IgG bound to an FITC marker.

Results

All 5 female ACL sections incubated with biotinylated relaxin and stained with silver intensified colloidal gold or FITC showed uniform, specific binding, limited to cells within the synovial lining of the ligament, fibroblasts in the ligament stroma and cells lining blood vessel walls (figures 1 and 2). No signal was seen in any of the 5 male sections (figure 1), nor was signal detected in sections incubated with unmodified relaxin. Sections treated with biotinylated relaxin showed specific binding in the presence of a 2000-fold excess of human insulin, but not in the presence of a 2000-fold excess of relaxin.

Conclusion

Labeled relaxin exhibits a specific, saturable binding pattern in the human female ACL, which was competitively inhibited only by unmodified relaxin. This supports the conclusion that specific relaxin receptors are present on the human female ACL, which may, in part, help explain the high incidence of ACL rupture in female athletes.