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Original Article

The symmetry of the medial collateral and anterior cruciate ligament properties. A biomechanical study in the rat hind limb

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Abstract

The medial collateral (MCL) and the anterior cruciate ligament (ACL) of the rat's knee are frequently used in biomedical research and occasionally in ligament healing studies. The contralateral normal ligament serves as a control. In this study the presence of symmetry in the biomechanical properties of the MCL and the ACL was investigated. Bilateral femur-MCL-tibia and femur-ACL-tibia preparations were obtained from the hind limbs of sixty rats and were subjected to tensile testing to failure under the same loading conditions. Tensile load to failure, stiffness and energy absorption capacity were measured and the mode of failure was recorded. All biomechanical parameters were not significantly different between the two knees of the same animal, although significant individual variation was evident. The most common mechanism of failure was mid-substance tear. Symmetry seems to exist in the biomechanical properties of the MCL and the ACL in the rat knee. When ligament healing is evaluated, increased group size is necessary and the use of a normal control group may be advisable. The contralateral normal knee ligament may serve as a control when the properties of an injured ligament are evaluated and when the parameters of tensile testing failure under similar load conditions are applied.

Keywords: Anterior Cruciate Ligament, Biomechanics, Knee, Ligament, Medial Collateral Ligament, Rat

Introduction

It is assumed that the contralateral knee joint ligaments may be used as a control when performing experimental animal studies on ligament healing. Several studies have examined the symmetry in the biomechanical, geometric and densitometric properties of the long bones in humans and animals. In most studies no significant differences between the contralateral bones could be ascertained, despite the presence of significant variation between the animals[1-3]. The issue of symmetry in the biomechanical properties of the knee ligaments has not been sufficiently evaluated. In many ligament and tendon healing studies, the contralateral ligament or tendon is used as a normal reference arbitrarily assuming that the biomechanical properties are uniform[4]. In ligament healing studies the selection of the right or of the left side depends only on the preferences of the individual research team and the healing process is compared to the presumably normal contralateral ligament[4-9].

The purpose of this study was to examine the presence of symmetry in the biomechanical properties of both the medial collateral and the anterior cruciate ligaments in the adult, male rat knee hind limb.

Materials and methods

This study was approved by the Veterinary Directorate, in compliance with the EEC Directive 609/86. Sixty male, 10-12 month-old Wistar rats were used. With a power analysis of pilot data a sample size of 9 was calculated. With an $n=9$ sample size and the probability of type I error set at $\alpha=0.05$, the probability of a type II error was $\beta=0.19$. The animals were randomly allocated in two groups of thirty animals each. In the first group the biomechanical properties of the medial collateral ligament (MCL) and in the second group the properties of the anterior cruciate ligament (ACL) were evaluated. For this purpose, a bone-ligament-bone preparation consisting of the femur, the MCL or ACL and the tibia was dis-

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sected. The animals were killed by ether overdose. Both hind limbs were harvested and all extraneous soft tissue was removed leaving only the tibia, the MCL or ACL and the femur, creating a femur-MCL-tibia (FMT)[4,5,7] or femur-ACL-tibia (FAT) specimen[10,11]. Both menisci and the knee capsule were removed under a dissecting microscope to avoid injury to the ligaments. The femur and the tibia were transected at their mid-shaft, all soft tissues were removed, the bone ends were embedded in methylmethacrylate and the preparations were mounted using special grips onto the testing machine. Testing was performed within three hours after euthanasia. The FMT specimens were pre-loaded to 0.25 N and the MCL was tested to uniaxial failure at a constant deformation rate of 100 mm/min using a materials testing machine (Karl Frank GmbH, Germany). The measurements have been made directly from the heads of the testing system. During tensile testing the knee was fully extended to achieve straight line ligament testing and the ligament was kept moist with saline irrigation (Figure 1). The angle of flexion was defined relative to the alignment of the femur and the tibia, since all muscles and skin were stripped off the bones.

The FAT complex was tested with the knee in 60 degrees of flexion at a loading rate of 2.5 mm/sec. A higher loading rate was employed in order to examine the properties of the ligament rather than the tendon-bone interface. The load-deformation curve was recorded on a computer analysis system at a sampling rate of 50 Hz. The ultimate load at the peak of the curve, the stiffness (the slope of the linear portion) and the energy absorption (the area under the curve) were calculated from the load-deformation curve. The mode of ligament failure was recorded for each specimen (mid-substance tear and tibial or femoral avulsion). The Student's paired t-test (two-tailed) was used to detect differences between the contralateral specimens of the same animal. Statistical significance was set at $p < 0.05$. All data are presented as the mean±SD.

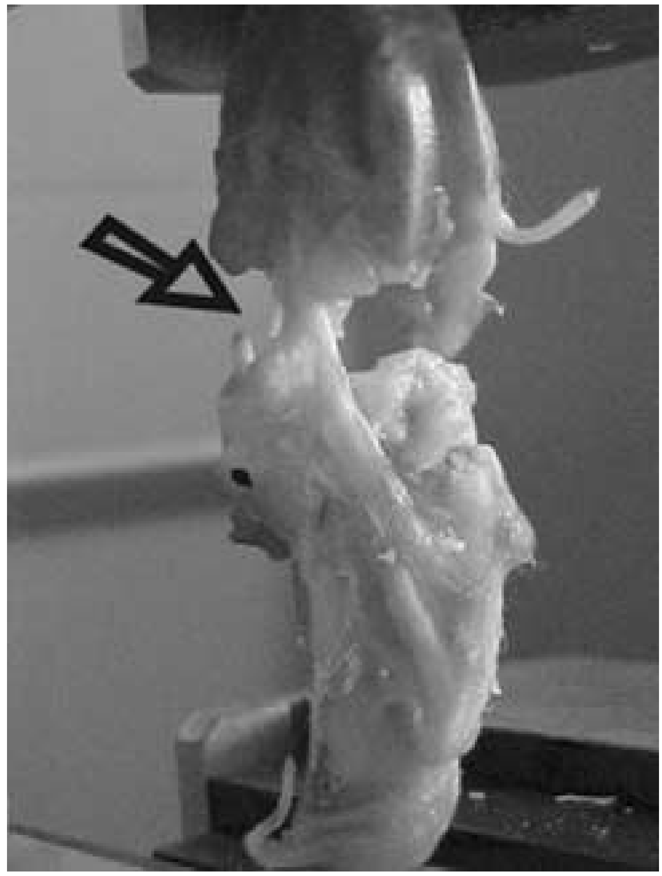

Figure 1. Uniaxial tensile testing of the rat MCL (arrow) with the knee in extension.

Results

Biomechanical testing demonstrated no statistically significant difference in any parameter examined between the contralateral MCL and ACL when all specimens were examined as groups. Nearly all specimens, FMT and FAT, failed at the mid-substance of the MCL or the ACL. The ultimate load in the left and the right knee regarding the MCL was 29.3±4.7 N and 31.4±5.3 N, respectively, the stiffness was 19.5±3.2 N/mm and 20.6±2.5 N/mm, respectively and the energy absorption was 18.2±3.1 Nmm and 18.6±4.3 Nmm, respectively (Figure 2). Regarding the ACL properties, the ultimate load in the left and the right knee was 34.6±4.2 N and 32.4±5.1 N, respectively, the stiffness was 14.4±5.1 N/mm and 16.3±3.5 N/mm, respectively and the energy absorption was $6.1 \pm 2.5\ 10^{-2}$ Nmm and $6.3 \pm 3.5\ 10^{-2}$ Nmm, respectively (Figure 3). However, in 9 cases (15%) the difference between the two sides was more than 20% in favour of one side or the other. Considerable variation was noted in the absolute values of the biomechanical parameters between the different animals.

Discussion

Symmetry is a relationship of characteristic equivalence among the two sides of an entity. In the human or animal body, symmetry refers to the equivalence in the geometric and structural properties of the contralateral bones[12]. It reflects the orderly and similar distribution of the body parts, such that an animal may be divided into two parts which are structurally symmetrical. Symmetry is a relative term. In bilateral symmetry, the body can be divided into symmetrical halves by a vertical plane passing through the middle. In this study the presence of symmetry between the MCL and ACL in the rat knee was evaluated and biomechanical symmetry between the ligaments of the two knees of the same animals was found. As a result, in animal ligament healing studies, using the same experimental conditions, the contralateral, uninjured ligament may serve as a control for the injured one[4]. The body side where a surgical or other intervention is planned to be undertaken should be chosen randomly and

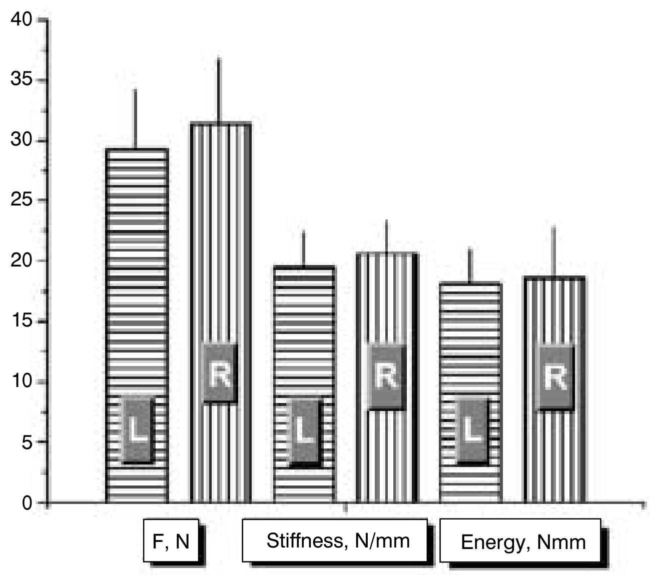

Figure 2. The biomechanical properties (ultimate load, stiffness, energy absorption) of the rat MCL. Comparison between the left and the right knee.

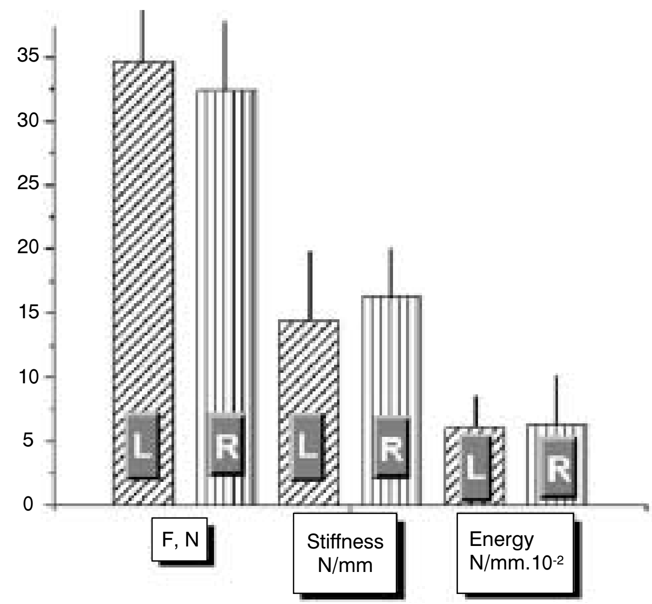

Figure 3. The biomechanical properties (ultimate load, stiffness, energy absorption) of the rat ACL. Comparison between the left and the right knee.

the constant use of the one or the other body side should be avoided to minimize the selection bias.

The presence of symmetry in the biomechanical, anatomical and densitometric properties of the bone in humans and in animals is generally accepted[1,8,13]. The symmetry of the mechanical properties of tendons and ligaments has not been extensively examined. Tipton et al.[8] compared the separation force between the left and the right MCL in male and female rats with different body weights and of presumably variable age, without noting any significant difference, despite the fact that the correlation co-efficient between the two sides was 0.732. They found significant differences between male and female rats that persisted despite normalisation with body weight. Frank et al.[7] evaluated the biomechanical properties of the normal rabbit MCL and directly compared the right with the left ligament and found no difference in the load and stress to failure, in the cyclic load relaxation and in the first cycle load.

The biomechanical properties of a ligament or a tendon depend on many factors such as the animal model, the tendon or ligament under evaluation, the age, the sex, the weight, the skeletal maturity, accompanying diseases, the testing conditions and parameters, etc[2,14,15]. Control of as many variables as possible decreases the possibility of a systematic error. In this study only male rats of similar age were used, employing the same loading rate trying to minimize the effect of the above mentioned parameters on the biomechanical properties of the ligaments. Additionally, ligament testing was performed soon after euthanasia to avoid any preservation-related negative effect.

However, this study has several limitations. The results of this study are valid in the male rat knee hind limb as there may be differences (hormonal etc.) between male and female animals, while hind limbs experience different forces than front limbs in quadrupeds. Only aged, male rats have been examined employing a high strain rate. The conclusions of this study can be expanded by using female rats, increasing the age range and comparing high and low strain rates. Another limitation to this study is that only one loading rate for each ligament and one angle of flexion were employed, which may have influenced the results. Additionally, only mature male rats were tested, as to avoid inferences with respect to female sex or immaturity. Furthermore, symmetry of the mechanical and viscoelastic properties of the knee ligaments should be examined in several other animal species used in biomedical research, e.g., goats, rabbits, dogs, etc. The presence of symmetry in several disease states and the effect of a ligament injury to the contralateral ligament also need to be evaluated.

Frank et al.[7] used a rabbit MCL injury model and compared the biomechanical properties of the contralateral, normal MCL with the properties of a normal rabbit MCL population 3, 6, 14 and 40 weeks after the surgical trauma. The cross-sectional area of the MCL mid-substance, the stress at failure and the cyclic load relaxation did not differ significantly between the contralateral MCL and the normal ligaments. The ultimate tensile force of the contralateral ligament was 16-20% lower than that for the normal ligament, while its laxity was increased. Using electron microscopy, they found that the contralateral to the injured ligament

MCL had a different fibril profile when compared with the normal ligaments, with reduction of large diameter fibres and increase in the small diameter fibres.

The animal MCL model is commonly used to evaluate ligament healing[4]. The injured ligament is compared biomechanically, histologically or biochemically with the contralateral, uninjured ligament, which is regarded as a normal, internal control. The difference between similar ligaments in the same animal species may sometimes be significant. In our study, differences up to 25% have been encountered. As a result, studies using small number of animals may face significant problems in the interpretation of their results. This fact should be taken into account when contemplating studies where the contralateral knee ligament will be used as a control. Increasing the sample size in experimental studies may significantly improve the sensitivity of the experiment.

In conclusion, this study confirmed the presence of symmetry between the two knees of the rat when performing mechanical experiments on the MCL or ACL. The contralateral knee ligaments in the rat are equivalent in tensile testing and can be used as a control of each other.

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