Abstract The structure and vascularization of the human anterior and posterior cruciate ligament were investigated by light microscopy, transmission electron microscopy, injection techniques and by immunohistochemistry. The major part of the anterior and posterior cruciate ligament is composed of bundles of type I collagen. Type III collagen-positive fibrils separate the bundles. The major cell type is the elongated fibroblast, lying solitarily between the parallel collagen fibrils. The histologic structure of the cruciate ligaments is not homogeneous. In both ligaments there is a zone where the tissue resembles fibrocartilage. In the anterior cruciate ligament the fibrocartilaginous zone is located 5–10 mm proximal of the tibial ligament insertion in the anterior portion of the ligament. In the posterior cruciate ligament the fibrocartilage is located in the central part of the middle third. Within those zones the cells are arranged in columns and the cell shape is round to ovoid. Transmission electron microscopy reveals typical features of chondrocytes. The chondrocytes are surrounded by a felt-like pericellular matrix, a high content of cellular organelles and short processes on the cell surface. The pericellular collagen is positive for type II collagen. The major blood supply of the cruciate ligaments arises from the middle geniculate artery. The distal part of both cruciate ligaments is vascularized by branches of the lateral and medial inferior geniculate artery. Both ligaments are surrounded by a synovial fold where the terminal branches of the middle and inferior arteries form a periligamentous network. From the synovial sheath blood vessels penetrate the ligament in a horizontal direction and anastomose with a longitudinally orientated intraligamentous vascular network. The density of blood vessels within the ligaments is not homogeneous. In the anterior cruciate ligament an avascular zone is located within the fibrocartilage of the anterior part where the ligament faces the anterior rim of the intercondylar fossa. The fibrocartilaginous zone of the middle third of the posterior cruciate ligament is also avascular. According to Pauwel's theory of the "causal histogenesis" (1960) the stimulus for the development of fibrocartilage within dense connective tissue is shearing and compressive stress. In the anterior cruciate ligament this biomechanical situation may occur when the ligament impinges on the anterior rim of the intercondylar fossa when the knee is fully extended. Compressive and shearing stress in the center of the middle third of the posterior cruciate ligament may result from twisting of the fiber bundles.

Key words Posterior cruciate ligament · Anterior cruciate ligament · Fibrocartilage · Type II collagen · Laminin

Introduction

The cruciate ligaments are the main stabilizers of the knee joint in the sagittal plane and they are secondary stabilizers in the frontal plane. Tears of the anterior and posterior cruciate ligament are frequent knee injuries in younger individuals and athletes because of increased sporting and outdoor activities.

In spite of the great clinical significance of the cruciate ligaments, little data about their structure in human beings is available in the literature. In classical textbooks of anatomy and histology (Bloom and Fawcett 1975; Cormack 1987; Williams and Warwick 1989), of pathology (Riede and Schaefer 1995) and orthopedic surgery (Buckwalter and Weinstein 1994) ligaments have typically been considered as dense connective tissue with few morphologic features beyond their gross anatomic characteristics. Some original articles, however, have highlighted structural differences between particular ligaments of the rabbit knee (Amiel et al. 1983; Lyon et al. 1991). The cells of the rabbit anterior cruciate ligament

Dedicated to Professor Dr. Benno Kummer on the occasion of his 75th birthday.

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have a round cell shape and resemble chondrocytes; the elongated fibroblast is the major cell type of the rabbit medial collateral ligament (Lyon et al. 1991). Results from animal models, however, can not be extrapolated to humans because of differences in gross anatomy.

Previous studies on the vascularization of the cruciate ligaments were performed by injection techniques (Pfab 1927; Davies and Edwards 1948; Zahm 1965; Scapinelli 1968; Alm and Strömberg 1974; Rubin and Marshall 1976; Arnoczky et al. 1979; Arnoczky 1985; Arnoczky et al. 1993). Injection techniques have to be interpreted carefully because of false negative and false positive results (Rudert and Tillmann 1993; Petersen and Tillmann 1995). A false positive result may be observed when the injection medium is pressed into the paravasal space because of a high injection pressure. A negative filling of a vessel may be caused by an insufficient injection pressure, microemboli or arteriosclerosis. Light-microscopically, small collapsed blood vessels are often hardly visible in dense connective tissue. The immunohistochemical proof of laminin as a component of the basement membrane (Timpl et al. 1979) is a reliable method for the detection of blood vessels in dense connective tissue (Rudert and Tillmann 1993; Petersen and Tillmann 1995).

In the present study the structure and vascularization of the human cruciate ligaments have been investigated. For this purpose, the following investigations were performed: (1) light and transmission electron microscopic examination of the anterior and posterior cruciate ligament at defined segments, (2) immunohistochemical proof of type I, II and III collagen, (3) conventional injection techniques and immunohistochemical proof of laminin in the basement membrane for the detection of blood vessels.

**Materials and methods**

**Light microscopy**

Anterior and posterior cruciate ligaments and their bony attachment sites were obtained from 25 subjects of different ages (13 females, 12 males; 23–77 years) during autopsy within 24 h of death. All knee joints were free of any kind of degenerative joint disease. Both ligaments were sharply separated by dissection of the surrounding synovial tissue. For light microscopy, each ligament-bone complex was fixed in 4% formalin, decalcified in 10% EDTA, and embedded in paraffin. Ten anterior cruciate ligaments and 10 posterior cruciate ligaments were cut in a longitudinal direction; the other 15 anterior cruciate ligaments and 15 posterior cruciate ligaments were divided into proximal, middle and distal segments. Serial transverse sections were made of each segment. Sections (8 µm) were mounted on gelatin-coated slides. The following stainings have been performed: toluidine blue (pH 5.8), Goldner staining, Gomori staining. The slides were examined with a Zeiss-Axiophot microscope.

**Transmission electron microscopy (TEM)**

For transmission electron microscopy 5 anterior cruciate ligaments and 5 posterior cruciate ligaments (2 females, 3 males; 23–56 years) were obtained. The ligaments were sharply separated from the bone close above the level of the ligament insertions. Samples of the proximal, middle and distal segment were fixed in 3.5% glutaraldehyde after initial treatment with 0.1 M Soerensen phosphate buffer solution at pH 7.4 and embedded in Araldite. Ultrathin sections were cut with a microtome and contrasted with uranyl acetate and lead citrate. Examination was carried out with a Zeiss EM 900 electron microscope.

**Immunohistochemistry**

For immunohistochemistry, 42 posterior cruciate ligaments (20 females, 22 males; 23–78 years) and 25 anterior cruciate ligaments (13 females, 12 males; 23–82 years) were obtained at routine autopsy. The ligaments were divided into proximal, middle and distal segments. Samples were snap-frozen in liquid nitrogen. Longitudinal frozen sections were cut with a cryostat at −30°C and mounted on gelatin-coated slides. For immunohistochemistry frozen sections were pretreated with testicular hyaluronidase (Boehringer, Mannheim) in TRIS-buffered saline (TBS) in a moist chamber at 37°C for 30 min. The sections were washed three times with TBS and incubated with goat serum for 45 min at room temperature. Incubation with the primary antibody was carried out for 60 min at room temperature. Antibodies used in the investigations were: Mouse anti-collagen type I (gift from Prof. P. K. Müller, Lübeck University, Germany), type II collagen monoclonal antibody (gift from Dr. R. Holmdahl, Uppsala University, Sweden), type III polyclonal antibody (Bio-science, Hamburg); laminin polyclonal antibody (Medac, Hamburg). Sections were labeled with the respective secondary antibody, fluorescein thiocyanate (FITC)- conjugated goat anti-rabbit IgG for 45 min. Control sections were incubated only with the FITC-conjugated antibody. Positive controls, including tissues with defined antigen sites of the same individual (collagens: human cartilage, biceps tendon and spleen; laminin: skeletal muscle and skin), were used. The slides were examined with a Zeiss-Axiophot microscope equipped for epifluorescence.

**Injection techniques**

Femoral arteries of five lower limbs of fresh frozen cadavers (2 females, 3 males; 60–81 years) were injected with India ink gelatin solution under continuous manual pressure. The injection was finished when the subcutaneous capillaries became visible through the intact skin. For the investigation of the intraligamentous and intraosseous vascularization the knee joints were prepared according to the method of Spalteholz (1914).

**Results**

**Light microscopic findings in the anterior cruciate ligament**

The major part of the anterior cruciate ligament is covered by a layer of synovial tissue (Fig. 1a, b). The surrounding synovium consists of loose connective tissue and is rich in blood vessels. The extracellular matrix of the anterior cruciate ligament consists of parallel bundles of collagen that are separated by small reticular fibers. The diameter of the collagen fibril bundles varies between 70 and 150 µm. In all anterior cruciate ligaments two different types of cells can be distinguished. The major cell type of the anterior cruciate ligament consists of spindle-shaped fibroblasts that lie solitarily between the parallel collagen fibrils (Fig. 2e).

In the anterior portion of the anterior cruciate ligament approximately 5–10 mm proximal of the tibial in-