Preliminary Evaluation on Meniscal and Different Functional Splitting of Anterior Cruciate Ligament Injuries of Knee Joint in Rabbit Models

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Abstract. The diagnosis, treatment and efficacy evaluation of anterior cruciate ligament (ACL) partly injury is controversy. We established the models of different damage to functional bundle, which is used on the ACL of rabbit knee, and analyzed the impact of different damage to functional bundle on ACL. 45 New Zealand, white rabbits were randomly divided into three groups, anteromedial bundle (AMB) fracture group, posterolateral bundle (PLB) fracture group and no fracture group. When finishing building the models, 8 rabbits were killed randomly on the second, fourth and eighth week respectively. Finally, we can observe the general form of the meniscus through experimental HE staining. The two groups were similar to the general form. In addition, the surface was unsmooth in the HE staining experiment and the tissues were loose as well as collagen fibers of varying thickness. It is believed that it will cause meniscus regression along with the ruin of different functional bundle on the ACL.

Introduction
With the development of mass sports and the increase of traffic accidents, anterior cruciate ligament (ACL) splitting is an extremely common sports injury, accounting for about half of the knee ligament injury, the incidence rate is increasing year by year trend in China [1]. ACL part of the damage, such as single bundle of injury, because of their physical examination and imaging performance, arthroscopy is also difficult to assess its functional retention, so the diagnosis of the ACL part injury, treatment and efficacy evaluation is controversy [2]. When the ACL injury developed, poor knee stability resulted in greatly improving the chance of joint injury. Therefore, treatment of ACL after different functional bundle injury can be related to the patient's knee joint, such as articular cartilage, meniscus and other structures of acute or long-term damage [3].

This study was designed to establish a model of different functional bundle injury in rabbits with knee joint, and to observe whether the differences in the morphology and histological morphology of meniscus and to analyze whether the effects of different functional bundle injury on knee meniscus. Meanwhile, we would like to further serve as a theoretical basis for the selection of clinical treatment methods.

Materials and Methods

Animals and Groups
This experiment was approved by the Animal Experiments Ethics Committee of the first hospital of national petroleum industry Co. Ltd, Jilin. The study was performed on 45 mature New Zealand rabbits weighing between 2.5-3.0 kg, which obtained from the Experimental Animal Breeding and Research Centre, Bethune Medical College Animal Experimental Center, Jilin University. 45 rabbits...
were randomly allocated into three groups, anteromedial bundle (AMB) fracture group, posterolateral bundle (PLB) fracture group and no fracture group with 15 rabbits in each group.

**Plan-operative Procedure**

The experimental knee model was set up by cutting the ACL of the rabbits as previously described [7]. The right legs were utilized to make AMB fracture models, PLB fracture models and no fracture models respectively. In brief, anesthesia was administered intramuscularly at dosages of ketamine (100 mg/kg). An adequate opening was obtained through the medial incision of the knee into the joint cavity. The ACL was directly cut off. The anterior drawer experiment confirmed that the ACL had been completely cut off, and then sutured with 3/0 absorbable sutures. The additional 10 as a sham operation control, the same procedure as the medial incision of the knee into the joint cavity exposed before the cruciate straight comb without cutting off the joint cavity directly. After surgery for 4 days, all postoperative rabbits were injected intramuscular with 400,000 U penicillin to prevent infection. Rabbit's leg does not make any fabrication, sub-cage feeding, each cage 1, the cage size of 60 cm × 60 cm × 40 cm.

**General Observation of Knee Joint and Meniscus**

Eight rabbits were randomly sacrificed in the experimental group at the 2nd, 8th and 10th weeks after operation respectively. After the death of the experimental animal knee dissection, the meniscus general shapes were observed, which included the meniscus and the edge of the existence of broken, color, elasticity and surface smoothness.

**Histopathological Evaluation**

The operated knee joints including the femoral and tibial joint surfaces only was removed by cutting at a distance of 2 cm from the joint. The meniscus histological section of operational side was taken for haematoxylin-eosin stain. Histological changes in the cartilage tissue were evaluated according to the method as described as previously [4].

**Results**

**Characteristics of Knee Joint and Meniscus**

The articular cartilage showed smooth, bright color without significant joint effusion and synovial hyperplasia in the control group (Fig. 1 A). Two weeks in the experimental group, the articular cartilage showed that the main surface rough, dark gray, small laceration, and superficial joint erosion (Fig. 1 B). At 4, 8 weeks, cartilage changes are still concentrated in the femoral condyle joint surface, formation of ulcers and joint effusion and synovial hyperplasia (Fig. 1 C and 1D).

**Pathological Morphology of the Atrial Tissue**

The knee articular cartilage HE staining characteristics exhibited that the surface was dense and smooth in the control group. Chondrocyte cells and collagen fibers were arranged neatly and well-defined. Non-clustered chondrocytes and inflammatory cells were found in the normal rabbit groups. According to the morphologic characteristics, the cartilage can be divided into four layers: the surface cells flat, cross section was spindle-shaped, similar to the horizontal arrangement; the middle layer of cells, smaller, round, scattered in the distribution. Columnar layer of cells was arranged in distinct lines. Calcification in the subchondral bone surface, thin, large cells, as scattered in the distribution (Fig. 2 A).

For two weeks after the anterolateral bundle injury group, the staining was consistent and the surface structure was complete. The collagen fibers were arranged in a compact area, and only the surface cells were enlarged (Fig. 2 B). 4 weeks after the anterolateral bundle injury group, the staining depth was changed, the surface was uneven, the tissue was loose, and the cells were arranged irregularly (Fig. 2 C and 2 D). 8 weeks after the anterolateral bundle injury group, the smoothness of the
meniscus was significantly reduced, showing that the local slag formation, collagen fiber tissue sequence changes significantly, a small amount of inflammatory cell infiltration (Fig. 2 E and 2 F).

Figure 1. Morphous of meniscus of rabbits at the 2th, 4 th and 8 th week after the operation in the experimental groups and control group (dissecting microscope, ×8). A. The rabbit was killed at two weeks and meniscus was imaged in the control group. B. The rabbit was killed at two weeks and meniscus was imaged in the experimental group. C, D. The rabbit was killed and meniscus was imaged in the experimental group at four and eight weeks respectively.

Figure 2. Morphous of knee articular cartilage HE staining of rabbit at the 2nd, 4 th and 8 th week after the operation in the anterolateral bundle injury group (HE staining, ×400). A. The rabbit was killed at two weeks and knee articular cartilage HE staining was imaged in the control group. B. The rabbit was killed at two weeks and knee articular cartilage HE staining was imaged in the experimental group. C, D. The rabbit was killed and knee articular cartilage HE staining was imaged in the experimental group at four and eight weeks respectively.

As regarding to the posterolateral bundle injury group, the meniscus was structurally intact, the surface was poorly formed, the pale yellow, the elasticity was worse and there was relaxation. After 2 weeks, there were differences in staining deep, poor surface flatness, loose tissue and irregular arrangement of cells (Fig. 3 A and 3 B). After 4 weeks of posterolateral injury, the flatness of the meniscus was poor, and the local slag formation was observed. The sequence of the collagen tissue was changed obviously, and a small amount of inflammatory cells were infiltrated (Fig. 3 C and 3 D). After 8 weeks in the posterolateral bundle injury group, the smoothness of the meniscus was worse, the tissue was loose, the sequence of the collagen tissue was altered obviously, and the thickness was uneven, and a large number of inflammatory cells were infiltrated (Fig. 3 E and 3 F).
Figure 3. Morphous of knee articular cartilage HE staining of rabbits at the 2\textsuperscript{nd}, 4\textsuperscript{th} and 8\textsuperscript{th} week after operation in the posterolateral bundle injury groups (HE staining, ×400). A. The rabbit was killed at two weeks and knee articular cartilage HE staining was imaged in the control group. B. The rabbit was killed at two weeks and knee articular cartilage HE staining was imaged in the experimental group. C, D. The rabbit was killed and knee articular cartilage HE staining was imaged in the experimental group at four and eight weeks respectively.

Discussion

The anterior cruciate ligament is an important stable structure in the knee joint. Its principal role is to limit the tibial advance and adjust the stability of the knee joint rotation function, but it is also the most easily damaged structure in the knee joint. Most scholars believe that the ACL can be divided into anterior medial bundle (AMB) and posterolateral bundle (PLB) [5]. Each function bundle affects the tibial advancement and rotation in different tension modes over the entire flexion and extension of the knee joint. If any of these bends, it is bound to affect the distribution of stress at all parts, can lead to damaging to the corresponding parts [6, 7].

Knee joint ACL injury can be divided into complete injury and incomplete injury, the current complete injury to ACL surgery should have reached a consensus, but for the ACL part of the damage that is different bundle injury diagnosis, treatment has been controversial. ACL part of the damage is a common ACL injury model, the treatment of such patients, there is no uniform standard. Foreign orthopedic experts reported that none of the ACL patients who had been followed up for 9 to 15 years had developed a complete rupture but had only 32% of those who had recovered to pre-injury levels. 48% of patients with poor prognosis, 86% of patients with symptoms persist [8]. Whether the complete breakdown of ACL single bundle injury depends on the number of damaged fibers, the type of injury and the presence of secondary injury, especially after posterolateral bundle injury, the greater the stability of the knee joint. If the ACL injury is not timely treatment, knee joint secondary injury will be progressive development, sustained increase. Due to conservative treatment of uncertainty, scholars have reserved AMB or PLB to enhance the reconstruction of the ACL and achieved good clinical results in recent years [9, 10].

The meniscus lies in the articular space of the knee joint and is an essential structure of the knee joint. Meniscus with a transfer load, absorption shocks, to maintain joint stability and other vital functions for the collagen tissue composed of fibrous tissue [11]. The medial meniscus and the medial collateral ligament contacted deeply, and the fiber and the diaphragm muscle connected [18]. Meniscus on the knee maintained the stability and flexibility of the normal physiological function of the crucial role. Some researchers have demonstrated that the knee joint can change the mechanical properties of the medial meniscus of the knee after injury of the knee in different angles. After the meniscus angle is significantly higher than the anterior horn, the knee joint tomography suggesting
that the meniscus of the posterior horn is more likely to damage, can lead to secondary knee medial meniscus damage, this situation should be as soon as possible ACL reconstruction [12].

In this study, we established a part of the rabbit knee joint injury model, observed on the meniscus systematically observed at 2, 4, 8 weeks as a time point. the control side of the meniscus structure is complete, the surface showed a bright white, very smooth, smooth, flexible. If the ACL before the inner beam injury, the injury time is shorter for half months, the plate did not change significantly with the prolonged injury time, the part of meniscus slowly silted, the meniscus surface becomes gradually rough and yellow, and the meniscus elasticity deteriorated and got relaxed. And after the lateral bundle injury, with the passage of time, the same will cause meniscus injury. By HE staining, the damage of the different functional bundles (anterior medial and posterolateral bundles) of the ACL can be clearly found to cause damage to the meniscus, and with the prolongation of the anterior cruciate ligament injury, the more severe meniscus damage.

Summary
We successfully established the models of distinctive damage to functional bundle on ACL in rabbit knee, and found that the surface was unsmooth and the tissues were loose as well as collagen fibers of varying thickness. It proved that meniscus regression was associated with the damage of discrete functional bundle.

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References