The Change of the Mechanoreceptor of Anterior Cruciate Ligament in Fixed Knee and Injured Anterior Cruciate Ligament in the Rabbit

Seong Rae Cho, M.D., Hyoung Min Kim, M.D., Kee Haeng Lee, M.D., Youn Soo Kim, M.D., Joo Hyung Lee, M.D., and Yoon Jong Jahng, M.D.

Department of Orthopaedic Surgery, Holy Family Hospital, The Catholic University of Korea, Bucheon, Korea

Purpose: To identify the change of mechanoreceptor of anterior cruciate ligament (ACL) in fixed knee and injured ACL.

Materials and Methods: Eighteen rabbits were divided into three groups as control, fixed knee, and injured ACL groups. Both knee of all fixed knee group were divided into three groups as control, fixed knee, and injured ACL groups. Both knee of all fixed knee group were fixed with K-wire on the first day. The ligaments of the injured ACL group were cut at tibial attachment site on the first day. Two rabbits of each group were sacrificed for the evaluation of mechanoreceptor at the 1st, 3rd, and 5th week. Histologic studies were performed by modified gold-chloride stain for the mechanoreceptors.

Results: The types of mechanoreceptor in normal ACL were type I, type II, and type III. There was no difference in the appearance rate of each type. In the fixed knee group, the frequency of appearance decreased significantly at 5th week compared with that of the control group. In the injured ACL group, the frequency of appearance decreased at 1st week and showed no difference at 3rd week in comparison with 1st week, and zero at 5th week. In morphology the degree of degradation of mechanoreceptor increased with time in both experimental group, and showed more severe in the injured ACL group than in the fixed knee group. Most of all mechanoreceptors were located in the subsynovial region, and degraded with synovial degeneration and synovial inflammation.

Conclusion: Mechanoreceptors were changed with the change of intra-articular condition and ligament itself. Therefore the duration of immobilization should be shortened if possible and the torn ACL should be repaired anatomically, if indicated, as soon as possible to preserve the mechanoreceptors.

Key Words: Mechanoreceptor, Anterior cruciate ligament (ACL)

Injury of the anterior cruciate ligament (ACL) is one of the most important athletic injuries. Histological studies have shown the existence of mechanoreceptors and free nerve endings in the ACL. Furthermore, various authors have suggested that the ACL functions as a sensory organ, not only providing proprioceptive information but also initiating protective and stabilizing muscular reflexes. According to Freeman & Wyke’s classification, four categories of nerve endings can be found in the cruciate ligaments: type I (Ruffini endings), type II (Pacinian corpuscles), type III (Golgi tendon organlike endings), and type IV (free nerve endings) and each of these mechanoreceptors response to different kind of stimulation. Also it has been known that the proprioceptive function of the knee decreased in the aged person, and increased in the well trained athlete. These represent reversible changes of the sensory function of mechanoreceptors. So we supposed that it was worthwhile to identify the change of mechanoreceptors of ACL in fixed knee and injured ACL.

MATERIALS AND METHODS

Eighteen white rabbits of New Zealand that were three to five months old and weighed between 2.0 and 3.0 kilograms were divided into three groups as control, fixed knee, and injured ACL group. They were kept in the same circumstances with the same feed and place one week before this experiment began. 50 mg/kg of ketamine hydrochloride was infused into the auricular vein for anesthesia for six rabbits of fixed knee group and ACL injured group each on...
The first day of experiment. They were placed in supine position. Both of knees were fixed with a 0.062 inch Kirshner’s wire which were inserted from the proximal tibia to the distal femur with 45 degrees flexion in fixed knee group. The ACLs were exposed through the anteromedial approach and were cut at the tibial attachment site in ACL injured group. Wound were sutured layer by layer. 10 mg/kg of kanamycin was injected intramuscularly for three days after operation to prevent wound infection. Two rabbits of each group were sacrificed and the knee joints were exposed with the same approach as mentioned above at the 1st, 3rd, and 5th week of operation. Gross findings such as status of ACL and synovial membrane were observed. Then whole ACL including femoral attachment site was harvested and modified gold-chloride stain was performed for the histologic evaluation of the mechanoreceptors\(^7\). The staining procedure was as follows: 1) The ACL segment was placed in one part 88% formic acid to three parts filtered, freshly squeezed lemon juice and placed in the dark for 15 minutes. Hereafter, the tissue was handled with paraffin-coated forceps. 2) The lemon juice was decanted off the tissue and the tissue blotted. A 1% solution aqueous gold chloride was added and the tissue returned to the dark for 20 minutes. 3) The gold chloride solution was decanted off and 25% formic acid added. The tissue was kept in the dark overnight, 15-16 hours. 4) The tissue was washed in water and stored in the refrigerator. 5) Frozen sections were cut longitudinally into 8 pieces per segment on a sliding microtome at 40 \(\mu\)m and stored in water. 6) The sections were floated on alcoholic gelatin, mounted on slides and allowed to dry. The slides were immersed in absolute alcohol for 2 minutes, two changes of xylene, 3 minutes each, and coverslipped. Serial sections were studied with a light microscope (Olympus AHBS-514) under \(\times 40\), \(\times 100\), and \(\times 200\) magnification. We classified the mechanoreceptor by Freeman & Wyke’s classification\(^7\), and measured the type and frequency of appearance of the mechanoreceptors in every section. A mechanoreceptor was considered to be present when the positively stained one was observed at the same position on 2-3 adjacent serial sections or when the morphology of it was definite and it was connected with axon even on one section. We add up the mechanoreceptors from 32 sections and verified the data with Kruskal-Wallis methods. The level of significance was set at \(p=0.05\).

**RESULTS**

On gross findings, the ACLs of control group were white, slick, and glitter with approximately 3 mm in thickness and 7 mm in length. In the fixed knee group, most of knee joints showed swelling without relation of time and had synovial hypertrophy and synovitis aggravated with time. In the injured ACL group, severe degenerative change of the synovium and ACL was found from the first week of operation.

On microscopic findings, all types of mechanoreceptors except type IV were found at 1st, 3rd, and 5th week (Fig. 1) and there were no differences in the appearance rate of

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**Fig. 1.** Mechanoreceptors of the normal anterior cruciate ligament (modified gold-chloride stain). (A) Most of all mechanoreceptors are located at subsynovial region (\(\times 40\)). (a) Type 1 mechanoreceptor. (b) Type 2 mechanoreceptor. (B) Various mechanoreceptors (\(\times 100\)). (a) Type 1 mechanoreceptor. (b) Type 2 mechanoreceptor. (c) Type 3 mechanoreceptor. (C) Intrasyovial location of type 1 mechanoreceptor (\(\times 100\)). (D) Type 2 mechanoreceptor and its connection axon (\(\times 200\)).
Each type in the control group (Table 1). At 1st week, there were three type I, three type II, and one type III mechanoreceptors in the fixed knee group and one type I, one type II, and two type III in the injured ACL group. At 3rd week, we found two type I, one type II, and three type III mechanoreceptors in the fixed knee group and two type I, three type II, and zero type III in the injured ACL group. There was no significant difference between the 1st and 3rd week in both experimental groups (Table 1). At 5th week, zero type I, one type II, and four type III mechanoreceptors were found.
in the fixed knee group and no mechanoreceptors in the injured ACL group (Table 1). The frequency of appearance of mechanoreceptors decreased with time in fixed knee group and showed significant difference at 5th week compared with that of the control group (p<0.05). And there was also a significant decrease in the frequency at 5th week in the injured ACL group compared with that of the control group (p<0.05).

Mechanoreceptors were mostly located in superficial layer of the ACL and subsynovial region (Fig. 1A-C), and closer to the femoral insertion (Fig. 1A). In morphology the mechanoreceptors were degraded with time in the fixed knee group (Fig. 2). The degradation of mechanoreceptors was observed from the 1st week (Fig. 3A, B) and the degree of degradation increased at 3rd week (Fig. 3C, D), and mechanoreceptors were not seen at 5th week in the injured ACL group. The degree of degradation showed more severe in the injured ACL group than in the fixed knee group. The mechanoreceptors, were degraded earlier as they were located in superficial region of ACL and degraded at the almost same time with degeneration of synovium surrounding ACL. However, type IV mechanoreceptors were not found in all groups through the entire this study.

**DISCUSSION**

Morphologic studies have revealed that four categories of nerve endings can be found in the cruciate ligaments. According to the Freeman and Wyke’s classification, these are type I, II, III and IV endings and the difference in the structure and variability of receptors of some species of mammals had reported. The type I, Ruffini afferent fibers are slowly adapting, and have low mechanical thresholds. These receptors are able to signal static joint position, intra-articular pressure, and amplitude and velocity of movement. The type II, Pacinian corpuscles are rapidly adapting and have low thresholds to mechanical stress. They are inactive in the immobile joint and when the joint is rotated at constant speed but become active at acceleration and deceleration. Accordingly, Pacinian corpuscles are regarded solely as dynamic mechanoreceptors. The type III, Golgi tendon organlike endings are slowly adapting, have high thresholds, and are completely inactive in immobile joints. These receptors measure the tension of the ligaments most efficiently. Type IV, free nerve endings which were not a real mechanoreceptors constitute a pain-receptor system for the tissues of the joint and the paravascular neural fibers have been thought to be concerned with vasomotor control. In this study, type IV, free nerve endings were not found and all types of mechanoreceptors, type I, II, and III were found and were decreased in frequency of appearance in two experimental groups compared with control group.

The human ACL seems to accommodate most of the receptors near the bone attachments, while few receptors are located in the midparts of the ligament. However, there are disagreements about the prime locations for the receptors. Kenned, et al. and Schutte et al. reported that they were mainly located closer to the tibial insertion whereas Schultz et al. and Girgis et al. reported that they were closer to the femoral insertion. Authors cut the tibial end of ACL and harvested the end of the femoral insertion for the experiment because the tibial end of the ligaments were harder to preserve during dissection and the main trunk of the nerve bundle enter and exits from the femoral end of the ligament.

Since 1984, when Schultz et al. firstly reported the existence of mechanoreceptors in the human ACL, many related reports have appeared about the function of these mechanoreceptors. Barrack et al. reported that ACL-deficient knee had impaired proprioception and suggested that stabilizing reflexes originating in ACL receptors might be altered. Miyatsu et al. reported that ACL loading has an excitatory effect on the thigh muscles through a multimotor neurone output, and that the posterior articular nerve is one of the afferent routes from the mechanoreceptors of the ACL. The ACL-muscle reflex may therefore play a physiological role in maintaining knee kinematics. To summarize the function, various authors have suggested that the ACL functions as a sensory organ, not only providing proprioceptive information but also initiating protective and stabilizing muscular reflexes.

The proprioceptive ability declines as part of normal aging and this was found to occur secondary to the arthritic process. It was also noted that the relative absence of mechanoreceptors in arthritic knees compared to disease-free knees. Symptoms such as functional instability, feelings of giving way, and muscular weakness sometimes persisted after the various surgical treatment of injured ACL. Freeman et al. proposed that such symptoms might be the result of partial deafferentation of the capsule and ligaments in associa-
tion with the injured joint. In contrast, studies on athletic populations indicated that extensive training might improve proprioceptive ability\(^2\). Therefore, mechanoreceptors are influenced by the conditions of the knee joint.

In the fixed knee group, knee joint swelling persisted regardless of the period of fixation and it was thought that the causes were synovitis developed by immobilization and K-wire irritation. The frequency of appearance of receptors was significantly decreased at 5th week and the degree of degradation was progressed with time. In the injured ACL group, there were severe inflammatory change in the synovial tissue and marked degradation of mechanoreceptors from the 1st week. And no receptors were found at 5th week. As our results, mechanoreceptors of ACL were decreased in number and degraded in morphology with time when the knee joint was fixed and tension of ACL was removed. It was thought that the normal physiologic environment was destroyed, so proprioceptive function declined and the normal sensory feedback was altered by a deafferentation. Adachi et al.\(^1\) reported that a positive correlation between the number of mechanoreceptors and accuracy of the joint position sense, suggesting that proprioceptive function of the ACL is related to the number of mechanoreceptors. Kennedy et al.\(^13\) reported that the loss of mechanoreceptors due to injury leads to unstable knee function. Krauspe et al.\(^14\) demonstrated that electrophysiological afferent responses from the ACL were activated by movement of the knee.

The location of the receptors near the surface of the ligaments suggested that such a location allowed greater sensitivity to their deformation\(^17\). The change of intra-articular condition such as an knee effusion can lead to decline the proprioceptive function. Schutte et al.\(^18\) insisted that this was due to the responses of mechanoreceptors located in the capsule or menisci but not in the ACL itself. Because the cruciate ligaments are intraarticular but are extrasynovial, so receptors within the cruciate ligaments might be unaffected by intraarticular condition and affected only when they were injured directly. Fahrer et al.\(^9\) also reported that the knee instability which might be occurred in the case of severe knee effusion was due to the impairment of quadriceps muscle contracture. In this study, however, when the knee had hemarthrosis or effusion caused by injury to the ACL, or was fixed, mechanoreceptors in ACL were degraded or disappeared as well as the destruction of synovial tissue because of the subsynovial location of them. It was supposed that the number of mechanoreceptors declined earlier in case of the absence of tension to the ligament and hemarthrosis at the same time as in injured ACL group, which was due to the severe degeneration of both synovial tissue and ACL. These results suggested that the mechanoreceptors of ACL were progressively degraded due to not only the alteration of tension or direct injury to the ACL but also synovial degeneration surrounding ACL, which led to both morphological and functional loss. It was considered that these process was similar to the effect of age and arthrosis on the knee. Clinically the repair of ACL is usually performed within two weeks of injury and it is not suitable for direct repair in three weeks later because of degenerative change of ACL. Therefore, we decided to carry out an experiment on ACL at 1st, 3rd, and 5th week. The results of this study showed that there was significant decrease in number of mechanoreceptors in both fixed and injured ACL group at 5th week. Morphologically, there was gradual degradation of the ACL with time in the fixed knee group and severe degradation of both synovial tissue and ACL at 3rd week in injured ACL group.

Authors could not find any articles that reported about regeneration of mechanoreceptors after suture for ruptured ACL on medline. Reconstructive surgery for the treatment of ACL injury is currently widely performed using tendon autografts such as bone-patellar tendon-bone grafts and hamstring grafts, with the objective of an early return to sports. Denti, et al.\(^4\) found mechanoreceptors histologically in human ACL remnants obtained arthroscopically. If the ACL remnants could be preserved during reconstruction, the mechanoreceptors of the remnants might also be preserved to some extent, which may help maintain proprioception after reconstruction. Georgoulis et al.\(^8\) and Adachi et al.\(^1\) recommended that remnants of the ruptured ACL as a possible source of re-innervation of the ACL autograft should be preserved during ACL reconstruction as much as possible. However, if ACL remnants play a substantial role in proprioceptive function of the injured knee, it seems likely that a normal intact ACL may be still more important.

**CONCLUSION**

In conclusion, mechanoreceptors were changed with the change of intra-articular condition and ligament itself. There-
fore, the duration of immobilization should be shortened if possible and the torn ACL should be repaired anatomically, if indicated, as soon as possible to preserve the mechanoreceptors. In addition, inflammation of synovial tissue should be treated enthusiastically.

REFERENCES


목적: 손상 받은 전방십자인대와 슬관절을 고정했을 때의 전방십자인대에서의 기계적 수용기의 변화를 알아보고자 하였다.
대상 및 방법: 18마리의 토끼를 각각 6마리씩 대조군, 관절고정군, 전방십자인대손상군으로 나누고 실험첫날, 관절고정군은 K-강선으로 고정하였고, 십자인대손상군은 신인의 경골부착부위를 절단하였다. 실험 제1주, 3주, 5주에 각 군에서 2마리씩 도살한 후 조직학적 관찰을 위해 대퇴골부착부를 포함한 전방십자인대를 절취하였다. 기계적 수용기에 대한 조직학적 관찰은 수정된 gold-chloride 염색법을 이용하였다.
결과: 대조군의 정상 전방십자인대의 기계적 수용기는 제1, 2, 3형으로 각 형간 출현 빈도에 차이가 없었다. 관절고정군에서는 대조군에 비해 제5주에 기계적 수용기의 빈도가 유의하게 줄었다. 십자인대손상군에서는 수술 후 제1주에 기계적 수용기의 수가 대조군에 비해 감소하였고 수술 후 제3주에는 수술 후 1주와 차이를 보이지 않았다가 수술 후 5주에는 기계적 수용기가 보이지 않았다. 기계적 수용기의 형태학적 변화에는 실험군 모두에서 시간이 경과함에 따라 붕괴되었으며, 관절고정군에 비해 전방십자인대군에서 그 정도가 더 심하였다. 기계적 수용기의 대부분은 표층 및 활액조직의 직하부층에 위치하였으며, 전방십자인대를 감싸고 있는 활액막의 변성 및 염증과 함께 거의 동시에 붕괴되었다.
결론: 전방십자인대의 기계적 수용기는 관절내 조건에 따라 변화하였다. 따라서 기계적 수용기를 보존하기 위해서는 관절의 고정이 필요한 경우 가능한 최소한의 기간만 고정시키고, 전방십자인대의 손상이 있는 경우에는 적응이 된다면 가능한 조기에 해부학적 수복을 해야 한다.
색인 단어: 기계적 수용기, 전방십자인대