The Effect of Alpha-2 Macroglobulin on the Healing of Ruptured Anterior Cruciate Ligament in Rabbits

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To investigate the effect of modification of biological environmental conditions, one of the factors influencing the healing of anterior cruciate ligament rupture, we performed experimental anterior cruciate ligament ruptures on New Zealand rabbits. After experimental rupture, intra-articular alpha-2 macroglobulin was injected into the knees of the rabbits in the experiment group to prevent structural changes resulting from the enzymatic reactions in the ruptured anterior cruciate ligament. At the end of 10th day of the experiment, we observed that the anterior cruciate ligaments in the experiment group had retained their prerupture brightness and volume when compared with the control group in which intra-articular alpha-2 macroglobulin had not been injected. We also noted that the anterior cruciate ligaments in the experiment group had not retracted or swollen, the incision sites were regular and clean, and they did not show any signs of degeneration. In the histological examination, the anterior cruciate ligaments in the control group showed disruption of the collagen network and a significant diminution in number of fibroblasts and fibrocytes ($p < .001$). At the end of this study, we concluded that the necessary conditions for the healing and repair of ruptured anterior cruciate ligament could exist if the enzymatic and biological environments were under control.

Keywords Alpha-2 Macroglobulin, Anterior Cruciate Ligament Rupture, Rabbit.

INTRODUCTION

In many clinical experimental studies, the anterior cruciate ligament was shown to have a very low potential for primary healing [1–4]. Although the extra-articular ligaments are known to heal under suitable biological conditions, the anterior cruciate ligament, which is an intra-articular structure, does not show such a healing process, it does not because of decreased vascularity, a decreased number of undifferentiated mesenchymal cells, and characteristics of the intra-articular environment that have negative enzymatic and biomechanical effects on the healing of ruptured anterior cruciate ligaments [3, 5, 6].

Collagenase is an enzyme from the metalloproteinase group. It was secreted from cells that are stimulated by growth factors and cytokines; in the form of procollagenase then it was converted to active collagenase by plasmin. Collagenase release has been documented from articular structures such as anterior cruciate ligament, synovium, and articular cartilage [5]. This enzyme disturbs ligament healing by affecting collagen network stabilization. Experimental studies showed that collagenase levels increased in the joint fluid after rupture of the anterior cruciate ligament causing some structural changes in the ligament [3–5].

Alpha-2 macroglobulin is an inhibitor of metalloproteinase enzymes and its distribution to the tissues by diffusion is limited because of its high molecular weight (725 kD) [7, 8]. Its half-life is 1–2 days in tissues [8]. In inflammatory conditions, its serum level increases and its concentration in the synovial fluid increases due to increased capillary permeability. Then, it inhibits collagenase that is a metalloproteinase enzyme [9, 10]. In the current study, we used an experimental animal model to study the effects on the healing of the anterior cruciate ligament of an increased concentration of alpha-2 macroglobulin, which is low in the joint cavity.

We investigated the possibility of providing suitable conditions for the ruptured anterior cruciate ligament to heal primarily or optimum conditions for its surgical repair by administration of alpha-2 macroglobulin that blocks the enzymatic environment produced by collagenase in the anterior cruciate ligament rupture.

MATERIALS AND METHODS

Twenty New Zealand-type white rabbits that received humane care (age 8–10 months and weight 2.2 ± 0.2 kg) were
used in the study. The animals were anesthetized by xylazine (Rompun) 5 mg/kg and ketamin (Ketradol) 35 mg/kg, their right knees were shaved and aseptically prepared, and arthotomy was done by medial parapatellar incision. The anterior cruciate ligament was exposed and transected at mid-portion of the ligament with a blade. Sefazoline sodium (30 mg/kg) was injected intra-muscularly for infection prophylaxis. Wound closure was done by suturing joint capsule and fascia at the same time. Next, long leg cast was performed to the operated extremity. Then 1 ml/day sheep derived alpha-2 macroglobulin was injected into the right knees of the 10 rabbits in the experiment group for 3 consecutive days. For the control group of 10 rabbits, 1 ml saline solution was injected.

The animals were sacrificed on the 10th day of experiment by an overdose pentothal injection. The right knees were exposed; joint fluid and anterior cruciate ligaments were examined. The gross appearances of joint fluids were evaluated. The anterior cruciate ligaments were fixated in 10% tamponized formalin solution, stained with H & Eosin dye, and the transected ends were examined under light microscope. In histological examination, the organization of tissue matrix and the number of fibroblasts and fibrocytes were evaluated. The cellularity was stated as the number of cells on 100 power fields in light microscope. The numbers of the cells counted were compared, and the Mann Whitney U test was used for statistical analysis.

RESULTS

No infection or wound problems were detected in the rabbits during the study. The macroscopic examination of the knees of the rabbits in the control group showed light brown colored joint fluid of decreased viscosity together with swollen and retracted anterior cruciate ligaments. The cut ends of the anterior cruciate ligaments showed degeneration and an appearance of tuft due to the disruption of fiber structure. On the other hand, the rabbits in the experiment group showed preservation of normal colored and viscous joint fluid. The anterior cruciate ligaments conserved their brightness and volume after cut. We detected that they did not show swelling and/or retraction and the cut ends had no degeneration (Figure 1).

Microscopic examination revealed that collagen network stabilization was disturbed and fibroblast and fibrocyte count had decreased in the control group rabbits (Table 1). In contrast, the collagen network was organized in the experiment group, and fibroblasts and fibrocytes were visible on every power field on light microscopy (Figure 2). The difference in fibroblast and fibrocyte count between the experiment and the control groups was statistically significant ($p < .001$).

DISCUSSION

There is no primary healing potential of anterior cruciate ligament after rupture [1–3]. The retraction of the ligament after rupture and several structural changes in tissue matrix are responsible for the prevention of healing shown in many studies [5, 6, 11]. Amiel et al. [5] stated that the collagenase activity increased in cut meniscus and anterior cruciate ligament, the collagen network in matrix was disturbed, the cell count decreased, and some structural changes occurred in the ligament as a result of his experiments carried out on rabbits. Dye [3] said that some tissues such as extra-articular ligaments (medial collateral ligament) may heal rather rapidly given the appropriate biological environment, whereas other structures such as the intra-articular ligaments may not heal at all. The intra-articular environment of the knee can be particularly harsh, both mechanically and biochemically on structurally damaged ligaments and cartilage [3]. From an evolutionary perspective, it makes sense that there are biochemical processes to minimize the possibility of loose tissue impingement as in the presence of the enzymatic process designed to dissolve exposed intra-articular collagen.

A basic concern about the interpretation of any experiment on animals is whether the conclusions are clinically applicable or not. The role of the anterior cruciate ligament in rabbits seems to be more important than in humans, because isolated loss of the ligament inevitably produces severe osteoarthritis. Biologically, however, the similarities are much more important [12]. In our experimental model, the enzymatic effect was minimized by applying intra-articular alpha-2 macroglobulin and the biomechanical effect was prevented by immobilization of the legs of the rabbits in a long leg cast. The structural disruption was prominent in the cut ends of the ruptured anterior cruciate ligament in the control group. The findings observed in histological examinations were similar to the results reported by Helti et al. [12].

The aim of our study was not to achieve healing of the transected anterior cruciate ligament. We aimed to block the negative enzymatic processes happening in the joint when the anterior cruciate ligament was cut.

There was no regeneration after complete transection of the anterior cruciate ligament in any animals. After this procedure had been done, transected anterior cruciate ligaments appeared swollen and retracted. Occasionally, a joint effusion of corroded

| TABLE 1 |
| Number of fibroblast and fibrocytes on 100-power fields in light microscope in experiment and control groups. |

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Experiment group</th>
<th>Control group*</th>
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<tbody>
<tr>
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<td>34</td>
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<tr>
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<td>9</td>
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<td>33</td>
</tr>
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<td>10</td>
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<td>27</td>
</tr>
<tr>
<td>Mean</td>
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<td>38.0</td>
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* $p < .001$. 


Figure 1. The macroscopic examination of the knees of the rabbits in the control group (A) showed light brown colored joint fluid of decreased viscosity and swollen and retracted anterior cruciate ligaments. However, the anterior cruciate ligaments in rabbits in experiment group (B) conserved their brightness and volume after having been cut.
Figure 2. On microscopic examination, collagen network stabilization was disturbed and fibroblast and fibrocyte count decreased in the control group of rabbits (A). In contrast, the collagen network was organized in the experiment group and fibroblasts and fibrocytes were visible on every power field on light microscopy (B), (H&E × 100).
viscous fluid was noted. Probably, at the end of 3 months, severe osteoarthritis can develop. Marchall and Olsson [13] observed complete disappearance of a severed anterior cruciate ligament and marked osteoarthritis changes in a dog model. They concluded that degenerative changes were inevitable in a joint in which the anterior cruciate ligament has been severed. Kleiner et al. [14] reported that healing in a rabbit model was extremely poor after primary suture of partially transected anterior cruciate ligament.

Anterior cruciate ligament healing involves remodeling in which structural proteins (such as collagen) are synthesized and degraded. And provided that the capacity for tissue synthesis is maintained, regulation of the degradative activity of tissues may play an important role in determining healing capacity. Treatment methods or agents that limit these processes of ligament degeneration may be useful in the clinical injuries of the anterior cruciate ligament. If the degeneration of the anterior cruciate ligament by collagenase is prevented by enzymatic routes, the ligament can heal primarily or it can be more suitable for primary surgical repair. Agents like alpha-2 macroglobulin provide this enzymatic effect and can be injected intra-articularly in to the traumatic knees.

Anatomically, the anterior cruciate ligament shows different histological characteristics from femoral insertion to the tibial end. The insertion of the normal anterior cruciate ligament into bone occurs through complex transition zones at fibrocartilage and calcified cartilage. Arnoczky et al. [15] showed this anatomic difference in dogs. For this reason, to detect the structural changes, the end portions of the ligament should be examined. Our findings related to changes in cellularity and collagen structure showed that alpha-2 macroglobulin had a preventive effect on degradation caused by collagenases. Lindy et al. [16] stated that collagenase could originate from menisci shown in the knees of patients with primary osteoarthritis and rheumatoid arthritis. Amie et al. [5] showed that the anterior cruciate ligament also could secrete collagenase. The injured ligament is thus degraded by collagenase that is secreted by the ligament itself.

The rupture of the anterior cruciate ligament causes inflammation in the knee joint. The increased alpha-2 macroglobulin concentration is observed in synovial fluid due to increased synovial tissue permeability in inflammatory conditions [9, 10]. The intra-articular administration of alpha-2 macroglobulin is very effective in achieving a higher concentration for a long period of time, as its passage from extracellular environment to intracellular environment is difficult due to its large molecular size.

In comparison to the saline-treated ligaments, the fibroblasts in the end transected area of alpha-2 macroglobulin–treated tissues were more numerous.

**SUMMARY**

Our animal model resembles the complete rupture of anterior cruciate ligament in human beings. When the anterior cruciate ligament is ruptured, it will heal primarily or be repaired. It is impossible that it can heal primarily after complete rupture. In this study, we concluded that blocking the effect of collagenase by alpha-2 macroglobulin has multifactorial effects on anterior cruciate ligament healing and may be offered as a stimulus to the fibroblasts. The macroglobulin also may contribute to the structural scaffold necessary to initiate repair of the anterior cruciate ligament.

**REFERENCES**


