Intra and Extra Synovial Healing of Knee Ligaments. A Comparative Morphological and Histological Study in Rabbits

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ABSTRACT : A comparative study of intra and extra-synovial healing of knee ligaments was done in 76 rabbits. The medial collateral and anterior cruciate ligaments were cut with a knife at their mid- substance and sutured with 5-0 prolene sutures. At the end of eight weeks, the healing of the medial collateral ligament (extra-synovial) was good in 60 (79%) rabbits. It was graded poor in 63 (83%) of the repairs of cruciate ligaments.

INTRODUCTION

Intra-articular repair of torn cruciate ligament or its substitution by other ligaments and synthetic materials has posed problems of healing as well as the quality of long term results. Extra-articular repair of torn collateral ligament of the knee on the other hand, gives the good results[1,2,3]. The uncertainty of healing of intra-articular ligaments is due to a number of factors namely, the type of injury, repair and rehabilitation. It may also reflect intrinsic problems in the healing of cruciate ligaments or the environment in which the healing takes place[4,5]. The cruciate ligaments is extra-synovial but once it is torn, its environment becomes intra-synovial.

Comparative experimental studies of healing of intra and extra-synovial repairs of torn knee ligaments is scarce. The aim of our study is to find out whether there is any difference in the quality of intra and extra-synovial healing of ligaments of the knee in rabbits.

MATERIALS AND METHODS

Seventy-six New Zealand white rabbits 1.5 to 2.5 kg, weigh were used. Under intravenous Sagatal general anaesthesia and sterile technique, the knee joint was exposed through a medial parapatellar approach. The medial collateral ligament of the knee was exposed and cut sharply with a knife at its midsubstance. This was repaired with the Kessler technique using 5/0 prolene suture (extra-synovial repair).

Arthrotomy was then done and the anterior cruciate ligament was cut at its midsubstance and repaired similarly with 5/0 prolene suture (intra-synovial repair).

The knee wound was closed in 2 layers with chromic catgut sutures. Plaster of Paris cast was applied from groin to toes and the rabbit was allowed unrestricted activity and diet in the cage. The animals were sacrificed at weekly intervals and the knee joints were removed for examination. They were examined with the naked eye for gross morphological features of healing at the suture line, adhesions from synovium, disruption of sutured ligament and sepsis. Morever, the sutured ligaments were taken photographs. The specimens were then prepared for histological examination. The sections of the collateral and cruciate ligaments were carefully prepared to show the healing process at the suture line. In addition, ten micron thick sections were also cut and stained with Hematoxylin and Eosin and Masson-Trichome stains for examination under the light microscope.

RESULTS

Healing of Collateral Ligament

The naked eye examination showed that the suture line was filled with vascular granulation tissue between 1 to 2 weeks. At the end of 2 weeks, there was progressive scar tissue formation which plateaued off at 8 weeks. The blue prolene sutures were often not visible and were buried in the scar tissue. In some specimens, there was a gap between the repaired ends and the scar was noted to be more translucent than the normal ligament tissues.

However, under the light microscope, the suture line was seen to be filled by a vascular inflammatory tissue at 1 week.

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The preponderence of inflammatory cells then subsided and fibroblasts dominated most of the field in 4 weeks. After 4 to 6 weeks, there was a decrease in the number of fibroblasts and the fibrous tissue was oriented in the direction of the ligament. At 8 weeks, there were mature fibroblasts and collagen and it was difficult to identify the repair zone except the presence of the sutures (Fig 1A).

Cruciate Ligament Healing

The intra-synovial healing of cruciate ligaments was poor (Fig 1B, 2A, 2B). In the first week, there was no repair seen in 3 animals except exudates around the repaired ends which looked fresh. In the second week, only 2 of the 7 repairs showed any evidence of healing. In 4 of the failures, there was only a tag of synovium noted to be holding the suture line. This was a common observation in the other specimens. In addition, only in 13 out of 76, the intra-synovial repairs showed some evidence of healing in four weeks.

The microscopic examination during the first week showed no evidence of healing and the sutures were intact. There was no soft tissue or vascular reaction at the site of repair. The adjacent synovium showed mild synovitis. In the second week, fibrin and blood clot filled the repair junction and in four cases only synovial tags connected to the two ends of the ligament. Most of the ligaments that failed to show any inflammatory reponse showed stump resorption and disruption of sutures. Two specimens that healed at 4 weeks showed presence of a few fibroblasts at the repair junction. They were few fibroblast when compared to those seen in extra-synovial healing. At 8 weeks, histologically most of them showed only a tenuous healing with scanty fibroblasts and collagen and the results were classified as poor (Fig 1B).

Table 1 gives a comparative account of the healing of medial collarteral ligament and anterior cruciate ligament. The results were graded as poor if there was no haematoma, no cellular response and no formation of fibrous tissue. If there was a haematoma, a cellular reponse and scanty fibrous tissue formation, the results were graded fair. The results were graded good if the suture line and the surrounding area were initially filled by haematoma and later by inflammatory cells and finally by scar tissue bridging the entire suture line.

Table 1

Results of repair	Good	Fair	Poor	Total number of Ligaments
Anterior cruciate ligament	0 0%	13 17%	63 83%	76
Medial collateral ligament	60 79%	16 21%	0 0%	76

DISCUSSION

Healing of the medial collateral ligament of the rabbit in this series occurred by scar formation and remodelling was similar to healing of soft tissues in other species. There was an orderly process of haemorrhage, inflammation, proliferation of fibrous tissue and remodelling in and around the medial collateral ligament defect. The continuity of the old ligament was thus restored. The junction between the scar and the old ligament was indistinct after some time and it was difficult to identify the site of the scar histologically from the intact ligament except by the presence of the non-absorbable nylon sutures. The overlying subcutaneous tissue and skin also contributed to the healing by invasion of blood vessels. The healing process was very rapid and it occurred within 3 to 4 weeks in most specimens.

Healing of anterior cruciate ligament was uniformly poor. Most repairs were incomplete and a common observation was the presence of synovial exudates around the repaired ends. Even at 4 weeks, the repair site looked fresh without any inflammatory or repair tissue bridging it. O'Donoghue[6] postulated that the haematoma following an anterior cruciate ligament tear is washed away by the synovial fluid. They said that there was no haematoma for organisation of repair. They also suggested that the exposed ends of the torn cructiate ligaments were vulnerable to the action of synovial fluid and were therefore, dissolved. There is evidence from our study that the haematoma formation was poor in the group of animals with anterior cruciate ligament repairs. Whether organisation of a haematoma is necessary for ligament repair or not it cannot be answered from this study for repair may occur even in the absence of haematoma. A number of anterior cruciate ligament repairs in our study showed resorption of the cut ends. This may be due to the action of some enzymes in the synovial fluid. There was also a possibility that the sutures gave way without the action of synovial fluid. At the time of the surgical repair, it was made sure that the suture line was intact and the knees were immobilised in a plaster cast while the animals were under anaesthesia. It is therefore, unlikely that the movement of the knee disrupted the suture line. Whether the disruption of the suture line and resorption of the cut ends of the cruciate ligaments were due to action of synovial fluid or not they could not be concluded from this study except us to draw the attention of its possibility. Our study shows that the rate and the quality of medial collateral ligament and anterior cruciate ligament repair are different. Anterior cruciate ligament cut in its mid-substance does not heal at all or heals poorly.

There were no biomechanical studies done in this study for the end result of the anterior cruciate ligament repair was a non-union of the ligament.

Figure 1A : Longitudinal sections comparing the healing of medial collateral ligament

Figure 1B : (MCL)(Fig 1A) and the anterior cruciate ligament (ACL) (Fig 1B) eight weeks after repair. Good healing by mature collagen in MCL and poor healing of ACL by a few strands of immature collagen H&E X25.

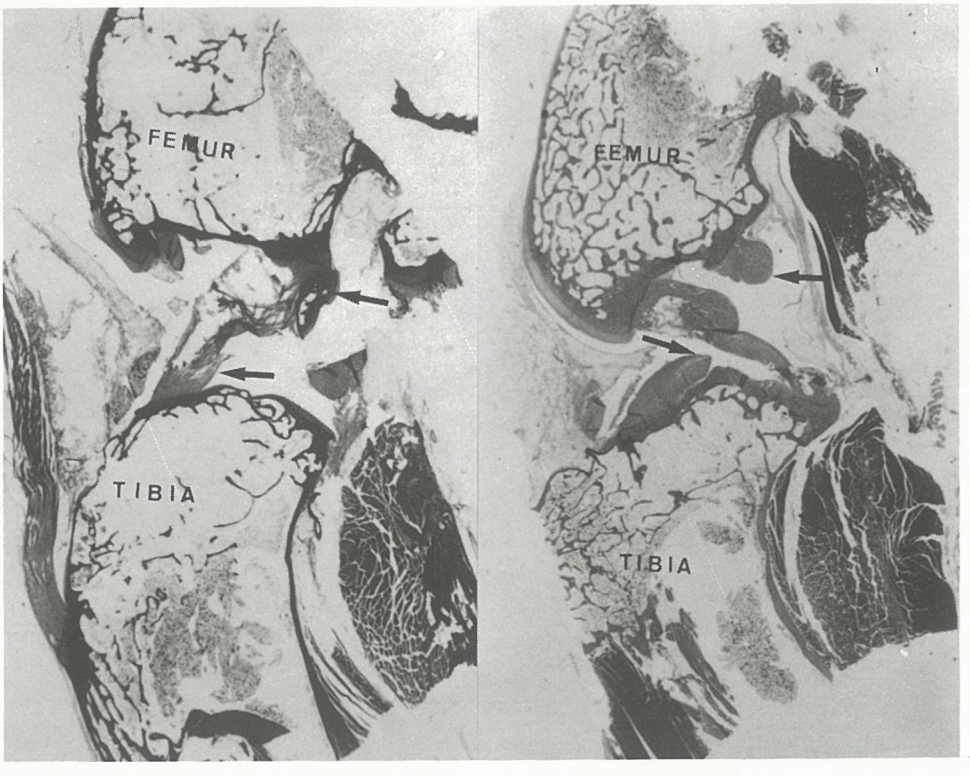

Figure 2A : Longitudinal sections of two knees showing femur, tibia, and anterior cruciate ligament.

Figure 2B : Cut ends of anterior cruciate ligament resorbed in one (arrows)(Fig 2A) and the gap is filled with a thin strand of fibrous tissue in the other (Fig 2B) H&E X25.

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