Original Article

EPILIGAMENT CHANGES AFTER INJURY OF KNEE

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Summary

The purpose of this study was to determine changes, occurring in the epiligament tissue after injury of the lateral collateral ligament in rat knee joint. On the 16th day after surgery, the animals were sacrificed and the isolated ligaments were studied using light and transmission electron microscopy. The results from histological and ultrastructural examinations were compared, and **their literature explanations were discussed.**

Key words: epiligament, lateral collateral ligament, injury, rat

Introduction

In 1990 Bray et al. [1] in his work "*Fine vascular anatomy of adult rabbit knee ligaments*" defined for the first time the term "epiligament" (EL). This structure has been described as a "surrounding adherent connective tissue removed simultaneously with the ligament but which was grossly distinguishable from ligament tissue proper". The EL is characterized as a hypercellular structure and different variant in form and size fibroblasts, fibrocytes, adipocytes, mast cells have been established [2, 3, 4]. Due to the ultrastructural characteristics of the fibroblasts in the EL, as typical synthesizing cells and the fact that the EL contains most of the blood vessels in the ligament, some authors speculate the possible role of this structure in ligament healing [2, 3, 4]. However, little information concerning the EL changes through the early healing of the ligaments existed in the literature [5, 6].

Accordingly, the aim of this study is to investigate the histological and for the first time the ultrastructural changes occurring in the EL, on the 16th day after grade III injury in an experimental rat model.

Materials and Methods

Ten male Wistar rats aged between 8-10 months were used for this study after approval was obtained from the University Committee on Animal Resources. Seven rats were anesthetized by

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intraperitoneal injection using a mixture of 5mg/kg b.w. of Xylasine (Bioveta, Czech Republic) and 45mg/kg b.w. of Calypsol (Ketamine, Gedeon Richter, Hungary). The left lateral collateral ligament (LCL) was cut with scalpel in aseptic conditions, and the gap was not sutured. The damaged end of the ligament was marked with catgut and the wound was closed. The remaining three rats were used as unoperated normal controls. The experimental animals had unrestricted cage activity and were under control of a veterinarian.

On the 16th day after surgery the animals were sacrificed with an overdose of Thiopental. The LCL and the surrounding EL was finely dissected, and the marked ligament zone was isolated. The specimens were fixed in 3% glutaraldehyde, processed routinely and embedded in Durcupan (Fluka, Buchs, Switzerland). Then semi-thin sections for light microscopy and ultrathin sections for transmission electron microscopy were made.

Results

Histological examination of the EL revealed that the untreated LCL scar was, at first, extremely disorganized and consisted of plump fibroblasts, which removed damaged debris and produced new but disorganized matrix (Fig. 1, 2). Moreover, the fibroblasts and the progenitor cells in the EL migrated in the endoligament, covering the collagen fibres of the ligament. In the regenerative zone of the EL, clusters of adipocytes could be seen; a finding also detected in the EL of the control animals. However, after ligament injury, the adipocytes were irregular in shape and varied in size. Ultrastructurally, the fibroblasts in the scar region had large and in some cells picnotic nuclei. Their cytoplasmic organization had also changed. The cisterns of the granular endoplasmic reticulum were larger, and an increased number of lysosomes and complex lysosomes were seen (Fig. 3). The collagen fibers in the EL were organized in bundles. These bundles had different orientations, as in the control animals. However, there were damaged collagen fibers between them. Moreover, these collagen fibers were included in the bundles of regularly orientated collagen fibers.

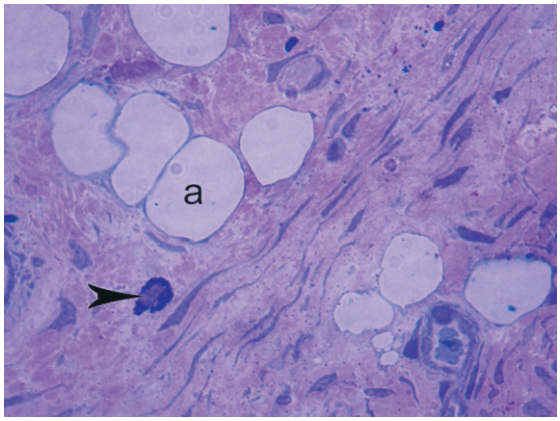

Fig. 1. Epiligament scar tissue composed of plump fibroblasts and disorganized matrix: a – adipocytes; arrow head – mast cell.

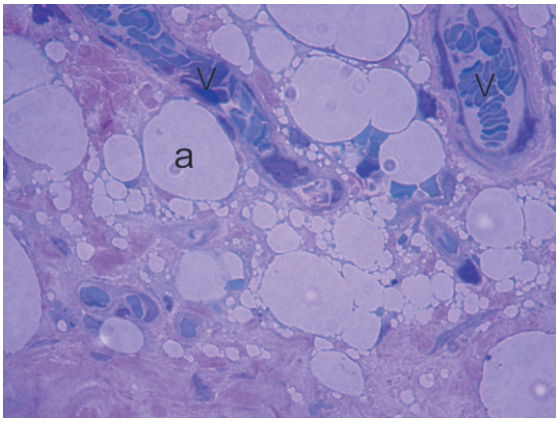

Fig. 2. Epiligament scar tissue composed of plump fibroblasts and disorganized matrix: a – adipocytes; v – blood vessels.

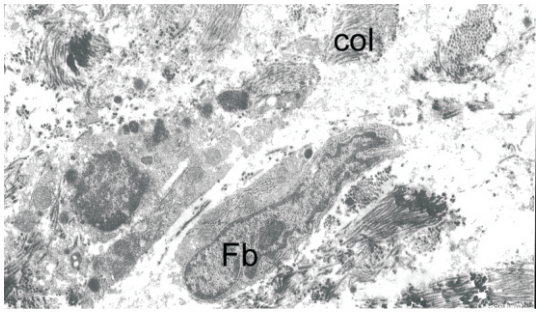

Fig. 3. Electron micrograph of fibroblast (Fb) and collagen fibers (col) in the intercellular matrix on the 16th day after injury. x 6 400.

Discussion

Ligaments injuries do not heal by regeneration but by the formation of scar tissue, similar to healing in other soft connective tissues [3]. Numerous studies investigated the healing process of the collateral ligaments of the knee in animal models [3, 7, 8, 9, 10, 11, 12]. However,

very little is known about the changes, which occurred in the EL after ligament rupture.

The EL structure is quite different from the ligament substance [2, 3]. The ligaments are described as poorly vascularized connective tissue, composed of fascicles [3]. These fascicles are formed by longitudinal groups of collagen fibers [3]. Each fascicle appears hypocellular, and the cells are aligned interspersed between bundles of collagenous fibers [3]. In contrast, the EL is more cellular, composed of different types of cells, and is abundant in blood vessels and nerves [2, 3, 4]. Due to these characteristics Lo et al. [3] supposed that the EL is perhaps the major source of cells that formed the ligament scars during ligament healing. According to Chowdhury et al. [2] the EL cells more closely resembled the fibroblastic cells that compose ligament scar tissue. Our ultrastructural investigations revealed that on the 16th day after rupture of the ligament, the EL fibroblasts had undergone different changes, including picnotic nuclei, expanded cisterns of the granular endoplasmic reticulum, lysosomes and complex lysosomes. We consider that due to the ultrastructural characteristics of the fibroblasts in the EL, they may be involved in phagocytosis and collagen synthesis and thus take part in ligament healing. Moreover, these fibroblasts proliferate and migrate in the ligament scar and this determines the role of the EL as a donor of cells during reparative process.

Conclusion

We consider that detailed knowledge of the ultrastructural changes in the EL during ligament healing is essential to get a better understanding of the normal healing process. To present more detailed information on this topic during different intervals of time, a future study is necessary.

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