Original Article

ELECTRON AND LIGHT MICROSCOPIC STUDY OF THE EPILIGAMENT OF THE LATERAL COLLATERAL LIGAMENT IN A RAT KNEE JOINT DURING EARLY POSTNATAL DEVELOPMENT

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Summary

The thin layer of connective tissue enveloping ligaments, termed the epiligament is not well described. The aim of this study was to elucidate the morphology of the epiligament in the midsubstance of the lateral collateral ligament in rat knee joint during early postnatal development using light and transmission electron microscopy. Our investigations presented different forms of fibroblasts and neuro-vascular bundles in the epiligament and high differences between the ligament and its covering tissue. The observations and their literature explanations were discussed in details.

Key words: epiligament, lateral collateral ligament, rat, early postnatal development.

Introduction

Despite the fact that ligaments of knee joint are widely studied in light and transmission electron microscopy, very little is known about the thin layer of connective tissue adherent to these ligaments, termed the epiligament (EL) [1]. In the current literature, there are only few studies describing the morphology of the EL in mature animals or comparing its characteristics between mature and immature animals [1, 2, 3]. No information about the morphological changes, which occurred during postnatal development have been described.

Therefore it is our aim to present here the dynamics of the common morphology of the EL of the lateral collateral ligament (LCL) in rat knee joint during first three weeks of postnatal life.

Materials and Methods

Animals of both sexes in four age groups (neonate, 1-, 2-, 3-week-old) were studied. All animals received humane care in compliance with the "Principles of laboratory animal care" formulated by the National Society for Medical Research and the "Guide for the care and use of laboratory animals" prepared by the National Institute of Health (NIH publication No. 86–23, revised 1996). The animals were sacrificed after overdose of ether. The LCL and surrounded EL was dissected precisely, and then the pieces were fixed in 3% glutaraldehyde and 1% osmium tetroxide, followed by embedding in Durcupan (Fluka, Buchs, Switzerland). The objects were identified on semi-thin sections (for light microscopy) stained with 1% metilene blue, azure II and basic fuchsin. The ultrathin sections (for transmission electron microscopy) 60 nm thick were contrasted with 2.5% uranyl acetate, lead nitrate, and sodium citrate. Electron microscope Hitachi-500.

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Results

The normal components of the midsubstance on the lateral side of the EL of the lateral collateral ligament (LCL) have been presented in light and electron microscopic micrographs. In newborn (Fig. 1 a, b), the light microscopy revealed single or group of cells in the EL and thin-walled blood vessels. Different types of fibroblasts-elongated, spindle-shaped, spinous-shaped and irregular in form with well-formed cytoplasmic processes, large nucleuses and high protoplasmic index were established. The cytoplasm contained considerable amounts of free ribosomes, polysomes and developing rough endoplasmic reticulum. During the first week (Fig. 2 a, b) the light microscopy revealed that number of single and groups of cells increased. Single blood vessels in the EL substance were detected. Ultrastructurally, their intima consisted of layers of endothelial cells, which rest on a well-presented basal lamina. The blood vessels and myelinated nerve fibers surrounded by different types of fibroblasts were established. Their cytoplasm consisted free ribosomes, well-developed granular endoplasmic reticulum and poorly developed Golgi apparatus. In the second week (Fig. 3 a, b) the number of fibroblasts increased. They show a large nucleus having one or two nucleolus. The small surrounded cytoplasm contained large mitochondria, free ribosomes and fragments of the rough endoplasmic reticulum membranes. In the third week (Fig. 4 a, b) a single or groups of unilocural adipose cells were established and the complex of cells in the EL increased. The myelinated and unmyelinated nerve fibres formed nerve trunks in this layer. In the intercellular space in all groups there was collagen fibres made of collagen type I with multiple orientations.

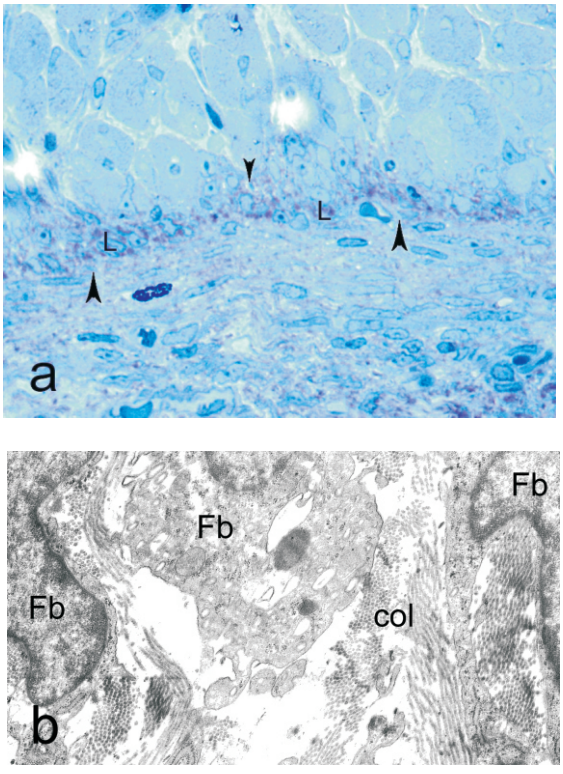

Figure 1 a, b. A – ligament (L), epiligament is indicated with arrowheads x 100. B – electron micrograph of fibroblast (Fb), collagen fibers (col) in the intercellular matrix in newborn rat x 12 000.

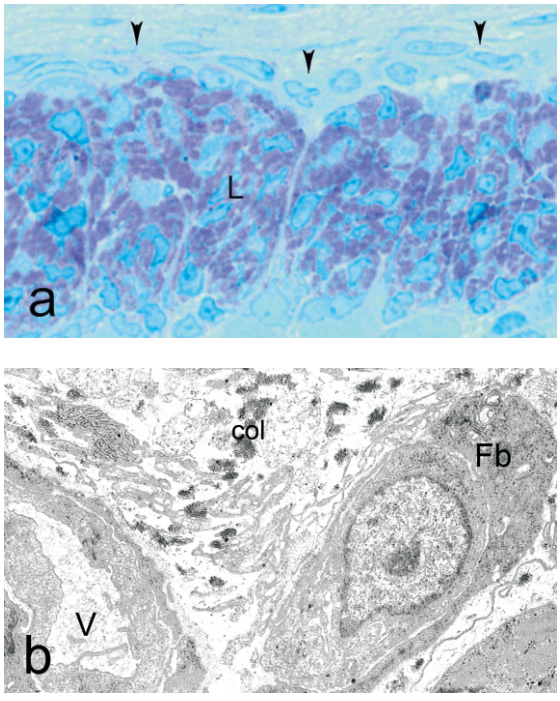

Figure 2 a, b. A – ligament (L), epiligament is indicated with arrowheads x 100. B – electron micrograph of fibroblast (Fb), collagen fibers (col) in the intercellular matrix in first week rat, vessel (V) x 4 300.

Discussion

The data from the present study gave a detail examination of the morphological changes in the midsubstance of the lateral side of the EL of the LCL in the first three weeks of postnatal life. During this period the number of cells increased and well-formed neuro-vascular bundles was detected.

Regarding the EL, the data from the present study differed significantly from the data reported by other authors [1, 2, 3]. According to Chowdhury et al., who described for the first time the external surface of the medial collateral ligament in rabbits, there were only two types of cells – spinous-shaped and fat cells (adipocytes), which differed from ligament cells [2]. On the contrary, according to our data there were different types of fibroblasts – with spinous-shaped,

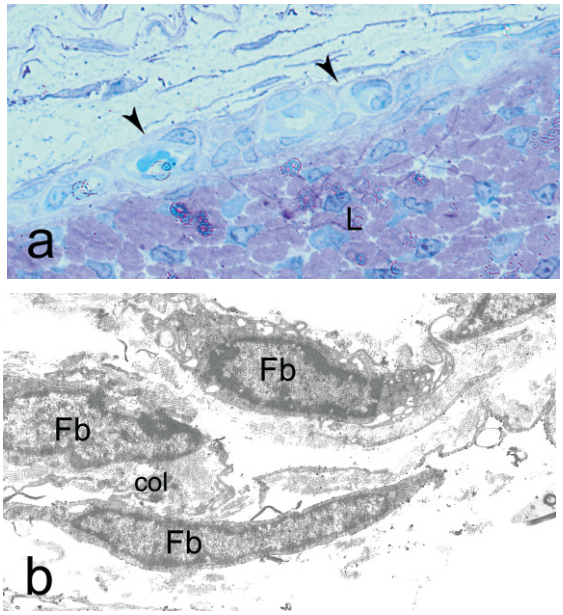

Figure 3 a, b. A – ligament (L), epiligament is indicated with arrowheads x 100. B – electron micrograph of fibroblast (Fb), collagen fibers (col) in the intercellular matrix in second week rat x 4 300.

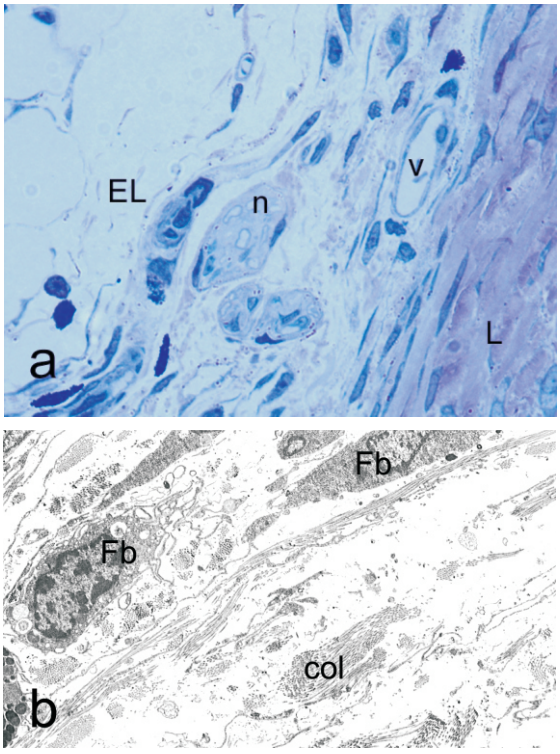

Figure 4 a,b. A – ligament (L), epiligament (EL) x 100. B – electron micrograph of fibroblast (Fb), collagen fibers (col) in the intercellular matrix in third week rat x 4 300.

spindle-shaped, elongated and with irregular form. The Chowdhury et al. speculated that the spinous-shaped cells are primarily responsible for the synthesis of the collagen fibers [2]. The cells described by ours, also had the characteristics of the synthesizing cells and we proposed that they could also be responsible for collagen synthesis.

The fat cells in the EL appear to be typical adipocytes that make up white adipose tissue [4]. According to Chowdhury et al. these cells metabolize and store lipids and may function as a packing material that could confer distinct material properties to the EL [2].

It is well known that both small and large collagen fibers in the ligament were aligned parallel to its long axis [5, 6, 7, 8]. In this study, transmission electron microscopy observations revealed that the collagen fibers in the EL have uniform small diameters and were organized in bundles with multiple orientations, as described by other authors [2, 5, 9].

The EL layer appeared to contain relative abundance of blood vessels and nerves, in contrast to the ligament tissue [5]. Vessels in the EL were randomly dispersed in a loose connective tissue matrix and nerve bundles often accompanied the blood vessels in the EL, but apparently not all blood vessels formed part of a neuro-vascular bundle [2, 5].

Conclusions

There are numerous differences between the ligament structure and the EL tissue in morphology of the cells, the orientation of collagen fibres, the areal fraction of blood vessels and nerve bundles. However, this investigation examined only the early changes in the EL in postnatal development. To present more detailed information on this topic, a future study is necessary.

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