J. Anat. (1994) 185, pp. 663–667, with 6 figures Printed in Great Britain

The attachments of the rabbit medial meniscus. A morphological investigation using image analysis and immunohistochemistry

JIZONG GAO[1], GUNILLA ÖQVIST[2] AND KAROLA MESSNER[1]

[1] *Department of Orthopaedics and Sports Medicine, and* [2] *Department of Cell Biology, Faculty of Health Sciences, University of Linköping, Sweden*

(*Accepted 30 June 1994*)

ABSTRACT

In the search for a suitable experimental model the rabbit has increasingly been used for investigations on the meniscus. The present study focused on the morphology and innervation of the anterior and posterior medial meniscal attachments in adolescent and adult rabbits in comparison with man. Grossly, the posterior attachment has a similar anatomical position as in man, but the anterior is inserted more anteriorly and more laterally, with a long ligament-like structure between the osseous insertion and the meniscal horn. As in man, the attachment resembles a ligamentous insertion and contains zones of uncalcified and calcified fibrocartilage and subchondral bone. The proportion of the calcified cartilaginous zone in the attachment increases during maturation as in articular cartilage. Nerve fibres were found not only at the horns but also in the uncalcified and calcified fibrocartilaginous zones and the underlying bone. The differences between rabbit and human menisci should be borne in mind when interpreting data from animal experiments.

Key words: Knee joint; fibrocartilage; joint innervation.

INTRODUCTION

The anterior and posterior horns of the human medial meniscus are attached to the tibial plateau by strong insertions that hold the meniscus in place during weight bearing (Pagnani et al. 1991). The horns and attachments also have important sensory functions (O'Connor & McConnaughey, 1978). If the horns are absent or damaged, the distribution of load across the knee joint is disturbed (Seedhom, 1976), the articular cartilage degenerates (Cox et al. 1970) and knee function ultimately becomes impaired (Clancy et al. 1984). Because of the difficulty in obtaining suitable human material, many workers have used the rabbit for experimental purposes (Zukor et al. 1990; Sommerlath et al. 1992). In contrast to other small laboratory animals in which the epiphyseal plates remain open throughout life, rabbits attain skeletal maturity at about 6 months. Further, their menisci resemble those of man in that they do not develop ossicles and because the response of the articular cartilage to meniscal resection is similar (Caputo et al. 1988). However, despite the importance of the menisci and the suitability of the rabbit, the structure and innervation of meniscal attachments in this animal are unknown. The purpose of the present study was to establish basic quantitative data on the attachments of the medial meniscus in the rabbit that can be compared with existing knowledge for man. Information is presented about the distribution of nerve fibres at the meniscal attachments.

MATERIAL AND METHODS

The structure and innervation of the medial meniscal attachments were studied in both knees of 7 adolescent, skeletally immature (ununited epiphyses), 3–4-month-old New Zealand white rabbits (1.5–2.3 kg) and in 7 mature (united epiphyses), 6–7-month-old rabbits (3.1–3.5 kg). In each group, 5 animals were used for morphological studies and 2 for immunohistochemistry.

Correspondence to Dr Jizong Gao, Department of Orthopaedics and Sports Medicine, University Hospital, 581 85 Linköping, Sweden.

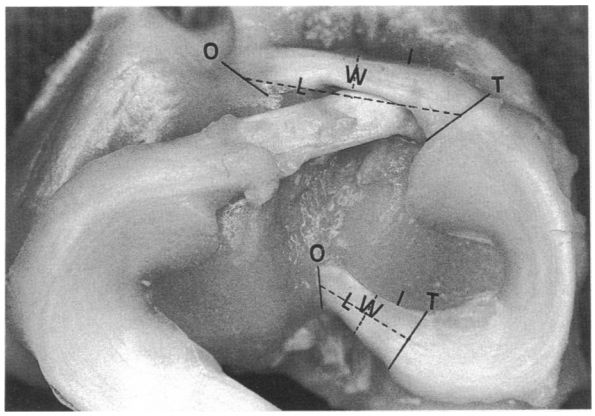

Fig. 1. The medial (right-hand side) and lateral menisci (left-hand side; the posterior attachment is detached from the femoral condyle) in situ, viewed from above (tibial tuberosity at the top of the figure). The different parts of the 2 medial meniscal attachments as described in the text are marked with the letters O (osseous), I (intermediate, ligamentous), T (transitional part), L (length), and W (width).

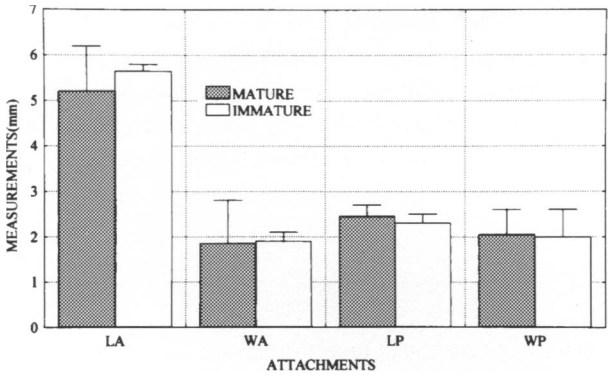

Fig. 2. The differences in length and width of the meniscal attachments in immature and mature rabbits (mean, S.D.). The anterior attachment was longer than the posterior in both age groups ($P < 0.05$). LA (length, anterior attachment), WA (width, anterior attachment), LP (length, posterior attachment), and WP (width, posterior attachment).

The rabbits were killed with pentobarbital sodium injected intravenously and the hind legs disarticulated at the hip joint. The knee joints were immediately opened and the anterior and posterior attachments of the medial menisci carefully dissected and examined by the naked eye. The attachment was defined as the part of the meniscus extending from the edge of the meniscal horn to the bony insertion on the tibia, comprising a transitional, an intermediate (ligamentous), and an osseous zone (Fig. 1). The length and width of the anterior and posterior attachments were measured with a calliper (Fig. 1). The edge of the meniscal horn was less obvious at the posterior than the anterior attachment. By bending the meniscus between the fingertips a change in material properties could be discerned, and the zone between the horn and the intermediate part of the attachment was marked accordingly. During these manoeuvres, the samples were kept moist with physiological saline.

After measurements in situ, the medial meniscus was divided at the middle to give 2 separate anterior and posterior meniscal attachment specimens. Each specimen was taken together with its attachment and underlying bone, which consisted of a square bone piece with 5 mm margins. The specimens selected for histology were fixed by immersion in formalin, decalcified in formic acid for 2 wk, and embedded in paraffin. Serial 5 μm sections were cut at 100 μm intervals parallel to the collagen fibres and perpendicular to the insertion surface (7–9 sections/attachment). The samples were stained with haematoxylin and eosin (H&E) and Alcian blue/periodic acid Schiff (AB-PAS) and evaluated by light microscopy. The thickness of the uncalcified and calcified cartilaginous zones (Benjamin et al. 1991) were measured by computerised image analysis (Image-Pro-Plus Program, Media Cybernetics, USA). The zone of uncalcified fibrocartilage was defined as the area between the tidemark to the most distally located chondrocyte emerging from a cluster or a line of chondrocytes of at least 3–5 cells. Isolated chondrocytes lying among fibroblasts were thus disregarded. The 2 borders were marked on the computer screen, and the mean distance between the 2 lines was measured. A similar line was drawn at the distinct border of the calcified fibrocartilage and subchondral bone following carefully the uneven border between these 2 tissues, and the mean distance from the tidemark representing the thickness of the calcified fibrocartilage was measured. The required borders could be clearly identified in 2–5 sections from each specimen, and the above analysis could be performed.

The immunohistochemistry specimens were excised immediately after death, rinsed in 0.1 M Millonig buffer, and fixed for 3–4 h in 4% paraformaldehyde solution, which does not prevent immunoreaction with most types of nerve fibres (Bjurholm et al. 1989). After rinsing in 0.1 M Na cacodylate buffer and decalcification in 4% EDTA in cacodylate at 4 °C for 3–4 wk, the specimens were rinsed in 0.1 Na cacodylate and then in Millonig buffer and immersed in Millonig buffer with 10% sucrose for 24–48 h. Cryostat sections (15 μm) were cut parallel to the circumferential fibres of the meniscus. The glass-mounted samples were then rinsed in phosphate-buffered saline (PBS) and incubated for 12 h at 4 °C with monoclonal mouse antihuman neurofilament protein, which reacts with the 200 kDa and the

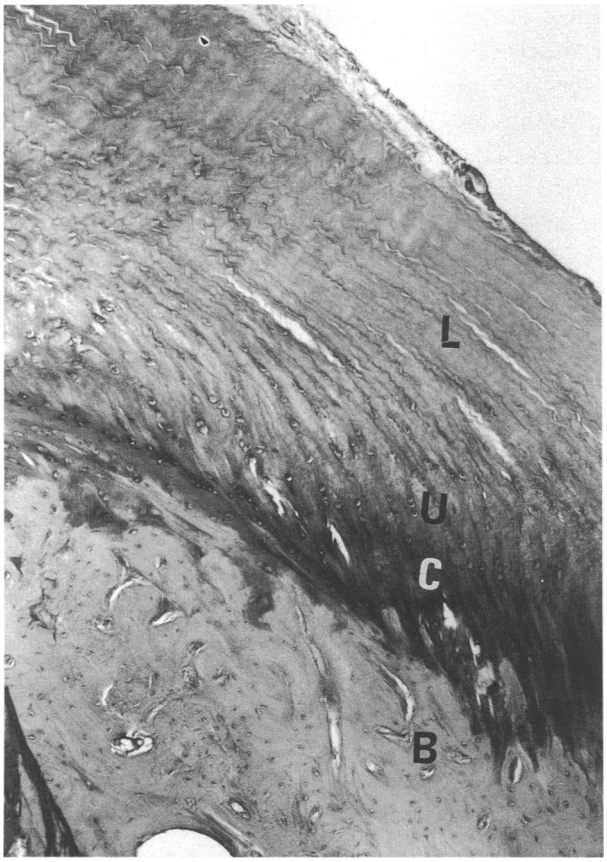

Fig. 3. The 4 zones of the anterior meniscal attachment (immature animal) are marked with the letters L (ligamentous zone), U (uncalcified fibrocartilage), C (calcified fibrocartilage), and B (subchondral bone) (AB/PAS, × 12.5).

70 kDa components of the 3 major polypeptide subunits (concentration 1:600, Dakopatts, Denmark). The antibody is specifically raised against neurofilament purified from human brain and shows cross-reactivity with neurofilaments from other species, e.g. mouse, rat and rabbit, but does not recognise other intermediate filament proteins. Its specificity has been documented (Osborn et al. 1986). After careful rinsing with PBS, the sample was incubated for 30 min with rabbit antimouse antibody conjugated with fluorescent isothiocyanate (concentration 1:20, Dakopatts, Denmark) at room temperature, rinsed again, and then mounted with glycerin/PBS. The samples were examined in a Zeiss Axiophot fluorescence microscope and photographed with Kodak Tri-X pan 400 ASA black and white film.

The incubation with primary antibody was omitted in the controls.

Statistics

Paired t tests were used for intra-animal comparisons; t tests for independent samples were chosen to compare interanimal measurements. For both tests a 5% significance level was required. All calculations were performed using commercially available software (STATISTICA, Statsoft Inc, USA).

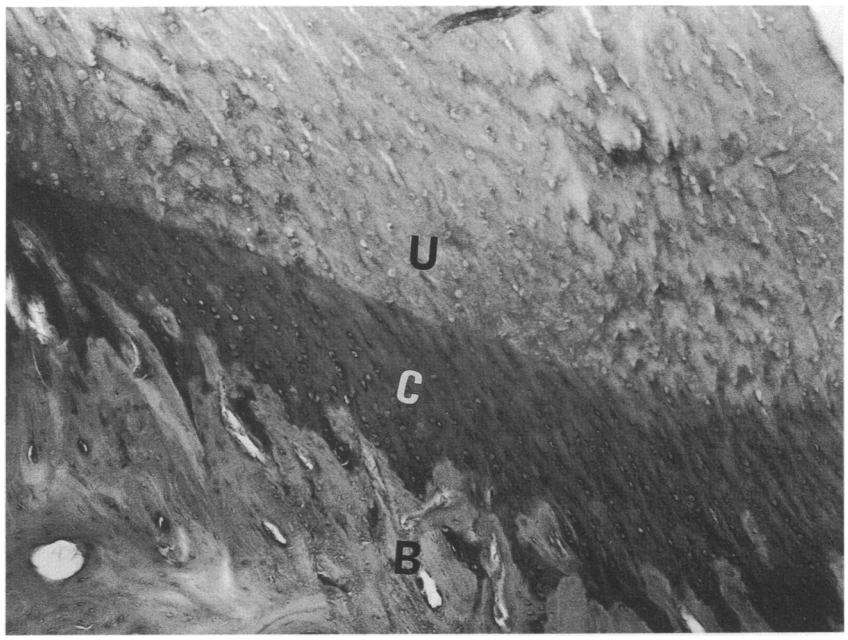

Fig. 4. The 3 zones of the posterior meniscal attachment (mature animal) are marked with the letters U (uncalcified fibrocartilage), C (calcified fibrocartilage), and B (subchondral bone) (AB/PAS, × 12.5).

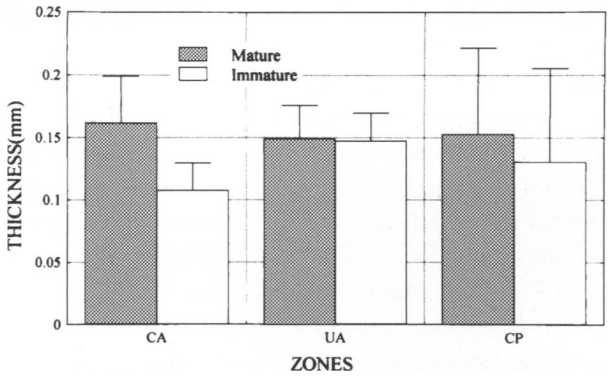

Fig. 5. Differences in thickness of the uncalcified and calcified cartilaginous zones of the attachments (means ± S.D.) in mature and immature rabbits. In the mature animal the calcified zone of the anterior attachment was thicker than the uncalcified zone ($P < 0.05$); in the immature, the situation was reversed. The calcified zone of the anterior attachment was thicker in the mature than in the immature animal ($P < 0.05$). CA (calcified zone, anterior attachment), UA (uncalcified zone, anterior attachment), and CP (calcified zone, posterior attachment).

Fig. 6. Arrows indicate nerve fibres in the uncalcified cartilaginous zone of the posterior meniscal attachment (fluorescence microscopy, × 30).

RESULTS

Both meniscal attachments were similar in width, but the anterior was twice as long and more distinct than the posterior. There was no measurable increase in dimensions from adolescence to maturity (Fig. 2).

The attachments had similar structural characteristics in both age groups. The anterior attachment comprised 4 zones: (1) the ligamentous zone, (2) uncalcified fibrocartilage, (3) calcified fibrocartilage, and (4) bone (Fig. 3); the posterior consisted only of 3, and lacked the ligamentous zone (Fig. 4). The junction between calcified fibrocartilage and bone was rough; the uncalcified and calcified fibrocartilaginous zones of the meniscal attachment continued uninterruptedly into the corresponding structures of the adjacent articular cartilage. The size of the uncalcified cartilaginous zone remained unchanged from adolescence to maturity, but the calcified zone increased in size. The proportions between uncalcified and calcified zones thus changed, the latter coming to predominate over the former (Fig. 5).

Nerve fibres were found in the meniscal horn, in the uncalcified (Fig. 6) and calcified cartilaginous zones, and in the subchondral bone underlying the attachment, but not in the ligamentous zone of the anterior attachment. Nerve fibres were also found in the meniscal–capsular junction, usually occurring as sparse structures. Nerve fibres were more numerous in the subchondral bone than in the other areas.

The controls were negative.

DISCUSSION

As a mainly squatting animal the rabbit loads the knee joint differently from man, and the medial and lateral menisci differ in gross anatomy from human menisci. In this respect, the rabbit medial meniscus resembles the human meniscus more closely than the lateral. As in man, the posterior horn is attached at the middle of the posterior aspect of tibia, between the attachments of the cruciate ligaments. The anterior horn, however, is attached differently. In the rabbit, it is attached solely on the anterior aspect of the lateral tibial condyle; in contrast, the anterior horn in man is attached more centrally, medial to the insertion of the anterior ligament. On histological examination, the rabbit meniscal attachments showed osseous and cartilaginous zones similar to those in human ligaments and menisci (Benjamin et al. 1986, 1991). However, at the site of the anterior attachment of the rabbit meniscus, a long ligament-like structure is interposed between the osseous attachment and the meniscal fibrocartilage of the horns. Further, the fact that the anterior attachment is placed in front of the joint, away from compressive forces, may explain its mainly ligamentous character. Except for the small portion of the transverse ligament, no comparable features have been reported for the human meniscus (Benjamin et al. 1991). The more central attachment of the posterior horn within the area of contact between the femoral and tibial condyles resembled meniscus-horn tissue with indistinct borders between the horn and its attachment. Because of its location and the fact that it lacks a ligamentous zone, it could be inferred that the posterior attachment resists both compressive and tensional forces. The gradual tran-

sition from fibrocartilage to full mineralization (Benjamin et al. 1986) at the attachment, as in tendon insertions, could enhance the ability of this structure to dissipate force evenly during loading, and therefore reduce the risk of failure of the unit. This idea is corroborated by clinical experience, ruptures of the attachment being very rare, in contrast to the common meniscal tissue ruptures. Thus the change in proportion between the attachment zones during maturation, giving predominance of calcified fibrocartilage in the mature animal, could reflect strengthening of the unit. A similar increase in the calcified fibrocartilage has been noted in growing articular cartilage in several species (Oegema & Thompson, 1992).

The presence of mechanoreceptors in the horns of human menisci (O'Connor & McConnaughey, 1978) indicates important sensory functions over and above the obvious mechanical function of an intact meniscus. An interesting finding in the present investigation was that nerve fibres were also found in the structures making up the gradual transition from soft tissue to bone, and which are subject to high stresses. The presence of nerve fibres in subchondral bone has been demonstrated by Reimann & Bach Christensen (1977), but the specific character of these nerve fibres and of those described in the different cartilaginous and osseous zones of the meniscal attachment remains to be elucidated.

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (project nos 10396 and 3761) and the Swedish National Centre for Research in Sports. It was approved by the local ethics committee.

REFERENCES

BENJAMIN M, EVANS EJ, COPP L (1986) The histology of tendon attachments to bone in man. *Journal of Anatomy* **149**, 89–100.

BENJAMIN M, EVANS EJ, DONTHINENI RAO R, FINDLAY JA, PEMBERTON DJ (1991) Quantitative differences in the histology of the attachment zones of the meniscal horns in the knee joint of man. *Journal of Anatomy* **177**, 127–134.

BJURHOLM A, KREICBERGS A, SCHULTZBERG M (1989) Fixation and demineralization of bone tissue for immunohistochemical staining of neuropeptides. *Calcified Tissue International* **45**, 227–231.

CAPUTO CB, SYGOWSKI LA, PATTON SP, WOLANIN DJ, SHAW A, ROBERTS P et al. (1988) Protease inhibitors decrease rabbit cartilage degradation after meniscectomy. *Journal of Orthopaedic Research* **6**, 103–108.

CLANCY WG, KEENE JS & GOLETZ TYH (1984) Symptomatic dislocation of the anterior horn of the medial meniscus. *American Journal of Sports Medicine* **12**, 57–64.

COX JS, NYE CE, SCHAEFER WW, WOODSTEIN IJ (1975) The degenerative effects of partial and total resection of the medial meniscus in dogs' knees. *Clinical Orthopaedics and Related Research* **109**, 178–183.

O'CONNOR BL, MCCONNAUGHEY JS (1978) The structure and innervation of cat knee menisci and their relation to a sensory hypothesis of meniscal function. *American Journal of Anatomy* **153**, 431–442.

OEGEMA TR, THOMPSON RC (1992) The zone of calcified cartilage. Its role in osteoarthritis. In *Articular Cartilage and Osteoarthritis* (ed. K Kuettner) pp. 319–331. New York: Raven Press.

OSBORN M, DIRK T, KÄSER H, WEBER K, ALTMANNSBERGER M (1986) Immunohistochemical localization of neurofilaments and neuron-specific enolase in 29 cases of neuroblastoma. *American Journal of Pathology* **122**, 433–442.

PAGNANI MJ, COPPER DE, WARREN RF (1991) Extrusion of medial meniscus. *Arthroscopy* **7**, 297–300.

REIMANN I, BACH CHRISTENSEN S (1977) A histological demonstration of nerves in subchondral bone. *Acta Orthopaedica Scandinavica* **48**, 345–352.

SEEDHOM B (1976) Load-bearing function of the menisci. *Physiotherapy* **62**, 223–226.

SOMMERLATH K, GILLQUIST J (1992) The effect of a meniscus prosthesis on knee biomechanics and cartilage. *American Journal of Sports Medicine* **20**, 73–82.

ZUKOR DJ, RUBINS IM, DAIGLE MR, RUDAN JF, ROY I, DURVAL P et al. (1990) Allotransplantation of frozen irradiated menisci in rabbits. *Transactions of the Orthopaedic Research Society* **15**, 219.