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Meniscal transection rather than excision increases pain behavior and structural damage in experimental osteoarthritis in mice

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SUMMARY

Objective: To evaluate pain behavior and structural damage in mice subjected to either meniscal transection or removal.
Methods: Mice (10/group) were subjected to transection of the medial collateral and anterior cruciate ligaments (ACLT/MCLT) followed by either transection (meniscotomy) or removal (meniscectomy) of the medial meniscus. A control group was subjected only to transection of the ligaments. Pain was assessed using the electronic pressure-meter paw test. Cell influx, measured in joint exudates, and joint histopathology were assessed after 49 days. Four other groups subjected to meniscotomy received indomethacin, the inducible nitric oxide synthase (iNOS) inhibitor 1400W, morphine or the vehicles.
Results: Both meniscotomy and meniscectomy groups displayed persistent and significant increase in pain behavior as compared to controls, being significantly more severe in the former. Cell influx was more intense in the meniscotomy as compared to the meniscectomy group. Structural damage at the tibia, but not at the femur, was also more severe in the meniscotomy group. Indomethacin and 1400W partially but significantly reduced pain whereas morphine abrogated pain behavior in meniscotomized mice.
Conclusion: Meniscal transection rather than resection promotes more severe pain and structural damage in mice. Administration of opioids, cyclooxygenase and nitric oxide (NO) synthase inhibitors provide analgesia in this model. Careful description of the structures damaged is crucial when reporting experimental osteoarthritis (OA).

Introduction

The menisci are semilunar discs composed of fibrocartilaginous tissue located inside the knee joint. Their major function is to minimize damage to the articulating surfaces by absorbing shock during movement[1,2].

Meniscal damage is one of the risk factors associated with knee osteoarthritis (OA) development. Partial or complete meniscectomy ranks high among the causes for orthopedic knee surgery and is associated with OA development. However, controversy as to whether meniscal removal, meniscal repair or conservative management is the best approach to treat a clinically symptomatic torn meniscus still remains[3,4]. Presence of nerve endings in peripheral portions of the menisci may contribute to joint pain. In addition, damage to the underlying cartilage and subchondral bone could provoke bone marrow edema thus leading to pain. In these situations, preserving as much meniscus as possible could allegedly protect the joint from major damage[3,5].

Surgically-induced models are considered the best current approach to study pain development in experimental OA[6]. Previously, rats subjected to partial medial meniscectomy were shown to

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display mechanical hyperalgesia and tactile allodynia[7]. Gait changes and pain behavior were shown to be less severe in rats subjected to anterior cruciate ligament transection and partial medial meniscectomy as compared to rats subjected to the monoiodoacetate injection OA model[8]. A detailed previous study has evaluated the time course of radiographic and histological damage to the knee joint in mice exposed to four types of injury, showing that OA progression depends on the severity of the instability provoked[9]. The pain component and histological damage in the destabilizing medial meniscus (DMM) procedure in mice have been described[10,11]. In the DMM model, endogenous opioids were shown to be involved in the pain behavior[11] and the administration of a soluble nerve growth factor receptor provided analgesia[12]. However, the issue of whether surgically removing as opposed to transecting the meniscus, without removal, affects both pain behavior and histological severity has not been appreciated. Surgical procedures, especially in small animals, are aggressive thus rendering a potential risk of secondary lesions after opening the joint for complete removal of the meniscus. Therefore, special care is needed when trying to compare strategies to sever the meniscus in mice[13–15].

In the present study, we examined the differences regarding functional (pain behavior) and structural damage (histopathology) to the knee joints between mice subjected to either complete removal of the medial meniscus (meniscectomy) or complete transection without removal of the meniscus (meniscotomy) as well as the contribution of prostaglandins, nitric oxide (NO), and opioids to the pain behavior.

Materials and methods

Animals

Swiss male mice (25–30 g) were provided by the central animal house of the Federal University of Ceará, Fortaleza – CE, Brazil. Animals were housed in cages (6/cage) in temperature-controlled rooms with a 12 h light/dark cycle with free access to water and food. At the start of any experiments, mice were 2.5 months of age. All efforts were made to minimize animal suffering and the number of animals used. The protocol was approved by our local ethics committee (Comitê de Ética em Experimental Animal – Faculty of Medicine – Federal University of Ceará number 113/07) that follows the rules of the Brazilian Committee on Animal Experimentation (COBEA).

Description of the meniscotomy and meniscectomy models

Mice were anesthetized with i.m. ketamine (50 mg/kg) and xylazine (10 mg/kg). After preparing for local aseptic surgery, the right knee joint was opened through a parapatellar medial incision with a scalpel. The patella was laterally displaced in order to expose the joint. Both the medial collateral and anterior cruciate ligaments were carefully transected in order to ease the access to the medial meniscus without damaging the articular cartilage. Following these procedures, groups of animals were then subjected to either transection of the medial meniscus, leaving a freed fragment inside the joint (meniscotomy group), or complete removal of the medial meniscus (meniscectomy group). In the meniscotomy group, the incision was made in the mid-body of the medial meniscus. In the meniscectomy group, the meniscus was freed from both the medial and lateral attachments trying to avoid damage to the underlying tibial cartilage.

The joint capsule and skin were sutured with Vycril (6-0) and mononylon (4-0) threads, respectively. A sham group was subjected to skin incision, patella displacement, and exposure of the joint without damage to ligaments or meniscus. A control group of animals was subjected to transection of both the medial collateral and anterior cruciate ligaments (ACLT/MCLT) without direct surgical damage to the meniscus. Naïve animals did not receive any manipulation. Those mice were used to compare differences concerning the pain behavior and histopathological parameters, as described below, comprising 10 animals for each of the five groups.

Assessment of pain behavior

Assessment of pain behavior (regarded as joint pain) was done daily using the electronic pressure-meter nociception paw test by an observer blinded to group allocation[16,17]. In this method, there is no direct stimulation to the affected joint. It is believed that the surgically-induced OA causes increase in sensitivity to a stimulus applied distal to that joint that would normally be innocuous. Animals were placed in acrylic cages (12 × 10 × 17 cm high) with a wire grid floor, 15 min before the beginning of the tests, in a quiet room. Stimulations were performed only when animals were quiet, without exploratory, urination or defecation movements and not resting on their paws. The electronic pressure-meter consists of a hand-held force transducer fitted with a polypropylene tip (Electronic von Frey aesthesiometer, Insight Equipamentos Científicos Ltda., Brasil). The polypropylene tip was applied perpendicularly to one of the five distal footpads of the right hind paw. The intensity of the stimulus was automatically recorded when the paw was withdrawn. The test was repeated three times, until less than a 1 g difference between measurements was obtained. Results were expressed as the mean value of three withdrawal threshold measurements (g).

Assessment of cell influx in joint aspirates

Animals subjected to the meniscotomy and meniscectomy procedures as well as sham-operated and naïve animals, as described above, were sacrificed 49 days after the surgical procedure, under terminal anesthesia. The synovial cavity of the knee joints was then washed with 0.05 ml saline containing 10 mM EDTA. The synovial fluids were collected by aspiration and total cell counts were performed using a Neubauer chamber. Differential cell counts were performed using the panoptic Instant Prov™ staining kit (New ProvBrasil™). After centrifuging (500 g/10 min), the supernatants were used for measuring the concentrations of NO using a commercially available kit (R & D Systems, São Paulo, Brazil).

Histopathology

The same groups of animals used for cell counts had their joint tissues excised for the histological study. After fixation in 10% v/v formaldehyde solution and decalcification (5% v/v formic acid in 10% v/v formaldehyde solution), the whole joint, comprising the distal femoral and proximal tibial extremities, was processed for paraffin-embedding and staining with hematoxylin-eosin (HE) and safranin-O. The material was serially sectioned at 5 μm in the sagittal plane of the articular surface, from the outer to the inner limits of the condyles. At every tenth section (50 μm apart), one section was removed for staining, with ten different sections for each sample. Analysis was done for both condyles and expressed as one result for each sample. Semi-quantitative histopathological grading was performed by two independent pathologists (VCCG, MMLP) blinded to group allocation according to the Osteoarthritis Research Society International (OARSI) histopathology grading and staging system[18]. The maximal possible final score (mean of measures made by the two pathologists) was 24. Results are expressed