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Brief Report

Abnormal perfusion in patellofemoral subchondral bone marrow in the rat anterior cruciate ligament transection model of post-traumatic osteoarthritis: a dynamic contrast-enhanced magnetic resonance imaging study

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SUMMARY

Objective: Although anterior cruciate ligament (ACL) injury is a well-recognized risk factor for developing knee post-traumatic osteoarthritis (PTOA), the process in the patellofemoral (PF) joint after ACL injury is still under-researched. Our aim was to investigate the perfusion changes in PF subchondral bone marrow in the rat ACL transection (ACLX) model of PTOA using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI).

Design: Eighteen male Sprague Dawley rats were randomly separated into three groups ($n = 6$ each group): a normal control group and groups receiving ACLX and sham-surgery, respectively, in the right knee. Perfusion parameters in the patellar and femoral subchondral bone marrows of all rats were measured on DCE-MRI at 0, 4, 8, and 16 weeks after respective treatment. After the last MRI at week 16, the rats were sacrificed and their right knees were harvested for histologic examination. In addition, to observe the long-term histologic change in PF joints, 9 additional rats ($n = 3$ in each group) were included and sacrificed at week 32 for histologic examination.

Results: In the ACLX group vs the sham and control groups, the perfusion parameters were significantly changed in both patellar and femoral subchondral bone marrows at week 16. Histologic examination revealed cartilage defects in ACLX rats at 32 weeks after surgery.

Conclusions: These data point to a possible functional relationship between subchondral bone marrow perfusion abnormalities and cartilage breakdown in PTOA. Moreover, the perfusion parameters derived from DCE-MRI can potentially serve as biomarkers of early OA.

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Introduction

Post-traumatic arthritis (PTA) is a broad clinical term used to describe articular degeneration after any kind of joint injury, such as joint fractures, joint dislocations, ligament injuries, and cartilage injuries[1]. The progression of PTA may give rise to OA-like changes of joint cartilage, referred to in the literature as post-traumatic osteoarthritis (PTOA)[2]. Anterior cruciate ligament (ACL) injury is by far the most well known risk factor for developing PTOA. ACL insufficiency after the initial trauma causes chronic instability of the knee and leads to OA, most recognizably in the tibiofemoral (TF) joint where the major stress develops. TF joint PTOA after an ACL tear has been studied extensively; in contrast, the patellofemoral (PF) joint PTOA associated with ACL injury is relatively under-researched. However, this disease entity may be more common and important than once thought[3]. In one prospective cohort study[4], PF joint OA was present in 16% of knees 15 years after the acute ACL injury. PF joint PTOA is associated with increased functional deficits (i.e., flexion and extension) in ACL-deficient patients[4]. PF joint OA is even more prevalent (47%) than TF joint OA (31%) in patients receiving autograft ACL reconstruction[5,6]. The long-term symptoms and functional deficits in these patients correlate with PF joint OA severity but independent of the TF joint OA severity. In a broader sense, regardless of the etiology, PF joint OA is an important source of anterior knee pain[7].

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) provides a valuable noninvasive method to measure bone marrow hemodynamics and derive several crucial perfusion parameters. A previous DCE-MRI study successfully demonstrated significant alteration of perfusion in a spontaneous TF joint OA animal model[8]. A similar change can be anticipated in TF joints after ACL injury based on the radiographic similarity of PTOA and primary OA. A preliminary study[9] has already shown that the DCE-MRI derived hemodynamic parameters change significantly in the TF joints of ACL-injured human subjects. Nonetheless, it would be more intriguing to assess hemodynamic change in the more subtle, late onset, yet equally important PF joint OA after ACL injury using DCE-MRI, in the hope of developing hemodynamic change as an early biomarker for PF joint PTOA. Therefore, the purpose of present study is to use DCE-MRI to investigate the subchondral bone marrow hemodynamics of the PF joints in rat model of knee OA induced by ACL transection (ACLX) and to correlate DCE-MRI findings with histologic examination.

Materials and methods

Ethics statement

The experiments were performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The applied protocol was approved by the Institutional Animal Care and Use Committee of the National Defense Medical Center (Permit Number: IACUC-06-103).

Animal preparation

Eighteen male Sprague Dawley rats aged 8 weeks and weighing around 300 g were randomly separated into three groups ($n = 6$ for each group): the normal control group without intervention, the ACLX group where the right ACL was transected as previously described[10] while the left ACL was left intact, and the sham-control group where the skin of the right knee was surgically wounded, while the left knee was left intact. The rats were housed (two rats per cage) in a sanitary ventilated room on a 12-h light–dark cycle within a temperature range of 21 ± 2°C, with free access to tap water and standard chow. The time when the rats received respective treatment was designated as week 0. The right knees of all rats were assessed using DCE-MRI at week 0, 4, 8, and 16. After the last MRI, all the rats were sacrificed and their right knees harvested for histologic examination. In addition, to observe the long-term histologic change of PF joint, 9 supplementary rats ($n = 3$ in each group) were included in the study, with DCE-MRI performed at week 0, 4, 8, 16, and 32 and sacrifice performed at the end of the MRI experiments for histologic examination.

DCE-MRI

The rats were first anesthetized by inhalation of an isoflurane–oxygen mixture. A birdcage coil with an inner diameter of 72 mm was used as the transmitter coil, and a separate quadrature surface coil (Bruker, Ettlingen, Germany) was placed above both knee joints to achieve maximum signal reception. The entire device was placed in an Oxford Instruments (Bruker) 200/300 magnet (4.7 T, 33 cm clear bore) equipped with an actively shielded Oxford gradient coil (16 cm inner diameter, 18 G/cm, 200 μs rise time).

After three-plane tripilot imaging, 10 contiguous sagittal T_2-weighted images (T2WIs) were acquired for the purpose of later slice positioning. In the subsequent imaging, four contiguous axial imaging planes (slices) were placed nearly perpendicular to the patella cartilage with the prior obtained right knee mid-sagittal T2WIs as reference images [Fig. 1(a)].

DCE-MRI was performed as dynamic T_1-weighted images (T1WIs) using a fast gradient echo sequence with repetition time = 100 ms, echo time = 3.5 ms, slice thickness = 1 mm, matrix size = 128 × 128 (zero-filled to 256 × 256), in-plane resolution = 156 × 156 μm^2, number of excitation = 1, flip angle = 90°, bandwidth = 37.879 kHz, and acquisition time = 6 min 24 s. The rats received a bolus injection of gadolinium-diethylene triamine pentaacetic acid (Gd-DTPA) (Magnevist; Bayer Healthcare Pharmaceuticals, Wayne, NJ, USA) via jugular vein at a dose of 0.2 mmol/kg during the 10th image acquisition. The temporal resolution is 6.4 s.

Data analysis

The Gd-DTPA-enhanced kinetic signals were analyzed based on the Brix two-compartment pharmacokinetic model[11–13]. Regions of interest (ROIs) were drawn manually on the patella and femoral subchondral bone marrows with reference to the first DCE-MRI image acquired at the middle level of the PF joint as shown in Fig. 1(b). To minimize manual discrepancies in the positioning of the ROIs, the ROIs were drawn by two image analysts well trained in knee MRI (PHT, CYW) and their positions were confirmed by an experienced musculoskeletal radiologist (GSH). Results shown in this study are the mean of two measurements. Mean signal intensities of the ROIs at each imaging frame were calculated. Three perfusion parameters were obtained from the time–intensity curve fitting: amplitude (A), rate constant (k_{ep}), and elimination constant (k_{el}) as previously described[13].

Histologic examination

All rats were sacrificed at the end of the MRI experiments (18 rats at week 16 and 9 rats at week 32). Right PF joints were removed, fixed in neutral formalin, and decalcified in a rapid decalcifier (Nihon Shiyaku Industries, Osaka, Japan). After decalcification, the PF joint tissues were cut in half along the mid-axial plane. Samples from each joint were paraffin-embedded, and cut into 5-μm sections for hematoxylin and eosin (H&E) staining.