Origin of Osteoarthritic Knee Pain: Immunohistochemical Analysis of Subchondral Bone -second report-

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Introduction

Osteoarthritis of the knee joint (OA) is the most common form of joint disease and its incidence has been increasing with an aged society. Deformity and walking difficulty could be a chief complaint of the patients but the most common and biggest chief complaint is knee pain. But a mechanism of OA knee pain is poorly understood. OA knee pain has been thought to originate from deformation of periarticular tissues and secondary synovitis. Several authors have reported that pain in the joint mainly caused by free nerve endings which existed in the capsule or synovium. Subchondral bone (SB) that obviously changes histologically as OA progresses appeared to have been neglected as a source of pain until we showed that SB was a potent source of pain with immunohistochemical analysis (1). TNF-α and Cox-2 positive cells were proved to be found in affected knee compartment. In the present study, further evidence that attribution of SB to OA knee pain is presented.

Materials and Methods

Medial-type OA knees that received total knee arthroplasty (TKA) in our institution from April 2000 to March 2005 were involved in the present study. Their age, gender, X-ray grading, and knee functional score employing Japanese Orthopedic Association score (JOA score) were recorded at the time of operation. All patients were underwent X-ray and MR imaging (Siga, GE medical systems, 1.5T) of the affected knee joints. Knees with none or minimum changes in lateral compartment as well as patello-femoral compartment on MRI were included in the present study. At the time of TKA, weight-bearing area of medial and lateral femoral condyles (MFC and LFC) was obtained. Specimens were immediately fixed in 4% paraformaldehyde in phosphate-buffered saline, and demineralized in 20% ethylenediaminetetra-acetic acid (EDTA) and embedded in paraffin. Sections were cut and mounted on glass slides. Six-micrometer sections were made and mounted on glass slides. They were stained with Mayer’s Hematoxylin solution and 1% Eosin alcohol solution (HE stain). Next, immunohistochemical examinations were performed using anti-cyclooxygenase 2 (Cox-2), anti-tumor necrosis factor-alpha (TNF-α), anti-substance P (SP), anti-CD34 and neural class 3 beta-tubulin (TUJ1). The localization of the antigens was visualized by peroxidase-labeled strept-avidin-biotin staining kit (Histofine; Nichirei, Tokyo, Japan). After washing, tissue sections were dehydrated and mounted under coverslips with Permount (Fisher Chemicals, New Jersey, U.S.A.). Lateral femoral condyles, that were unaffected joint compartment, were used as control. The numbers of cysts that evaded SB or calcified zone were counted according to previous report (2) and cell population forming those cysts were analyzed.

Results

Two were male and 21 were female with ages ranging from 62 to 79 years (mean 67.7 years old). An average of JOA score was 49.6pts (range 20 - 65). X-ray showed that all the knees had OA changes in medial compartment and were graded 4 on Kellgren and Laurence grading scale. HE staining revealed that articular cartilage in weight-bearing area of MFC was worn out and part of the surface was covered with fibrous tissue in all cases (Fig.1). The average number of cystic changes is 22.2/10mm in MFC and 4.2/10mm in LFC. Total cell numbers of each cyst in MFC were 94.6cells/cyst and 55.2cells/cyst in LFC. A proportion of osteoblast to each cystic lesion was 78.5% in MFC, and 76.1% in LFC. Percentage of osteoclast and endothelium to each cystic lesion were 1.4% and 2.1% in MFC, and 0.7% and 2.3% in LFC. No significant difference about cell population in cystic lesions was found between MFC and LFC. Immunohistochemical examination revealed that certain cells in cystic lesions in subchondral bone plate observed in MFC were positively stained with Cox-2 (Fig.2-a), TNF-α (Fig.2-b), TUJ1 (Fig.2-c) and SP (Fig.2-d). 100% specimens were positive for Cox-2 as well as for TUJ1, 91.3% for TNF-α, and 67 % for SP. Although more or less cysts with the same kinds of cell composition were found in LFC, no cell was positive for these four antigens.

Discussion

In 2006, we reported the new concept that subchondral bone plate contributes to OA knee pain when OA progressed beyond certain stage. In that report, several cystic lesions in subchondral bone plate in the affected knees expressed Cox-2 and TNF-α with significant high rates. Good correlation between knee score and the pathology of subchondral bone plate was also reported. In the present study, we found that numbers of cystic lesions per unit area in MFC were about five times larger than that of LFC, and no significant difference about cell composition was found in cystic changes between MFC and LFC. Cystic changes observed both in MFC and LFC might be related to bone remodeling. But, considering that TNF-α, cox-2, TUJ1, and SP were only positive for MFC, character of cysts were totally different between MFC and LFC. Especially, existence of substance P and TUJ1 positive cells in cystic lesions of MFC is direct evidence that subchondral bone plate is pain generator. Substance P is particularly suggested as one of the most important neuropetides in the modulation of the inflammatory process of arthritis. Levine showed that the intraarticular injection of substance P increased the severity of arthritis in animal model. Existence of TUJ1 indicates the existence of neural fibers. So the existence of this pain-related molecule in certain cells in cystic lesions along with neural fibers strongly support our assumption that subchondral bone plate is one of the sources of OA knee pain. Immediate pain reduction accomplished by TKA might be decrement effects of pain-generating subchondral bone.

References


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Fig.1 MFC stained with HE
cystic lesion in subchondral bone

Fig.2 immunohistochemistry of subchondral cystic lesions, arrow head indicates positively stained cells

a) Cox-2
b) TNF-α

c) TUJ1
d) substance P