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Correlation between neuropeptide distribution, cancellous bone microstructure and joint pain in postmenopausal women with osteoarthritis and osteoporosis

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ABSTRACT

Objectives: To explore the relationship between the distribution of neuropeptides, cancellous bone microstructure and joint pain in postmenopausal women with osteoarthritis (OA) and osteoporosis (OP).
Methods: Cancellous bone of the femoral head was obtained at the time of hip arthroplasty from 20 postmenopausal women, 10 with OA and 10 with OP. Pain intensity was evaluated using the visual analog scale (VAS) before the operation. The microstructural parameters were measured with micro-CT and the neuropeptides of the cancellous bone were stained by an immunohistochemical method.
Results: We observed that BV/TV, Tb.Th and Th.N values in the OP were significantly decreased compared to those in the OA. Immunohistochemical analysis revealed that the mean optical density (MOD) values for SP, CGRP, and VIP in the OA group were significantly higher than those in the OP, and the MOD value for NPY in the OA was significantly lower than that in the OP. We also observed that the MOD values for SP were positively correlated with AD, BV/TV, Tb.Th, Tb.N and Conn.D and negatively with MD, Tb.Sp and SMI in all patients. The MOD values for CGRP were positively correlated with AD, BV/TV and Tb.Th. MOD values for VIP were positively correlated with BV/TV and Tb.Th and negatively with SMI. The VAS score was correlated positively with the MOD values for SP, CGRP, VIP and negatively with NPY in all patients.
Conclusions: Neuropeptides play an important role in the pathogenesis of OA and OP, which may cause pain and influence the bone microstructure.

1. Introduction

Over the last decade studies have shown that regulation of the neurological system plays an important role in many physiological and pathological processes in bone remodeling (Franquinho et al., 2010; Lee and Herzog, 2009; Yoo et al., 2014; Yu et al., 2015). A number of neuropeptides, such as substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY) are synthesized in unmyelinated sensory neurons and sympathetic nerves and released from their peripheral terminals which were found in the bone and periosteum tissue. These neuropeptides have been implicated in the regulation of local bone turnover in addition to nociception, inflammation, angiogenesis, and cellular proliferation (Ballica et al., 1999; Offley et al., 2005).

Studies have shown that spinal cord injury can cause damage to bone mass, bone structure and bone metabolism in the sublesional area in animals and humans (Jiang et al., 2006; Liu et al., 2008), and these may be attributable to the changes of local neuropeptide levels in the microenvironment, such as SP, CGRP, VIP and NPY (Lerner and Persson, 2008). Also, dietary magnesium intake has been linked to osteoporosis (OP), and bone loss may be secondary to the increased release of substance P and TNF-α (Rude et al., 2004). Conflicting data have also been reported. Unilateral sciatic neurectomy caused the rapid loss of cancellous bone in the proximal and distal femur and tibia in the ipsilateral hindlimb of the rats. This study showed that a widespread reduction in SP content in bone contributes to the osteoporotic effects of sciatic neurectomy and that residual SP signaling maintains bone integrity after nerve section in both the denervated and contralateral intact hindlimb (Kingery et al., 2003). Above all, accumulating evidence suggests that these neuropeptides are directly involved in the regulation of bone remodeling (Lee and Herzog, 2009). They can be released and exert paracrine biological effects on bone cells present close to nerve endings expressing these signaling molecules (Lerner and Persson, 2008). And, as the neuropeptides are present throughout the bone

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marrow, mineralized bone and the periosteum, they may also be involved with the mechanisms that drive bone pain (Mach et al., 2002).

Pain is the major symptom of osteoarthritis (OA), while the mechanism is still not clear. There is a remarkably rich and heterogeneous sensory and sympathetic innervation of the bone marrow, mineralized bone and periosteum that is sensitive to seemingly innocuous physicochemical stimuli such as neuropeptides which can activate these nerve fibers leading to the sensation of joint pain (Mach et al., 2002). For OA, there are several likely effects of subchondral ischaemia: the degradative changes in the cartilage and apoptosis of osteocytes in regions of the subchondral bone, which would initiate osteoclastic resorption and reduce the bony support for the overlying cartilage (Findlay, 2007). Substantial pain mediators such SP and CGRP are released from the exposed bone into the joint cavity. In addition, local ischemia and increased venous pressure in the subchondral bone combine with the pain mediators to cause joint pain. One study showed that peripheral application of certain neuropeptides causes increased knee joint allodynia and secondary hyperalgesia (McDougall et al., 2006). Furthermore, antagonists that inhibit the activity may prove beneficial in the alleviation of OA pain (McDougall et al., 2006). Thus, neuropeptides are not only involved in the development of OA (Iwasaki et al., 1995), but are also closely related to the generation of OA pain.

Bone metabolic disorders are closely linked to the development of both OP and OA. Neuropeptides also play an important role in pathologies while the exact mechanisms are unknown. The aim of this study is to explore the relationship between the distribution of neuropeptides, cancellous bone microstructure and joint pain in postmenopausal women with OA and OP. Additionally, it may be important to recognize the role of these neuropeptides in the development of bone disorders and to find more effective treatments to control bone pain and inhibit the progression of OA and OP.

2. Materials and methods

2.1. Study subjects

Twenty postmenopausal Chinese women who underwent hip arthroplasty were included in the study. Ten of them for primary OA and the others for OP fracture of the femoral neck. The method of patient recruitment has been described previously (Li et al., 2012; Zhang et al., 2010). Briefly, we matched the OA and OP patients as closely as possible in terms of their age (age range 57–67 years in the OA patients and 56–68 years in the OP patients). All patients were more than 5 years postmenopausal at the time of recruitment for study. Patients who had bone diseases other than OA and OP, and those who took medicines that affect bone metabolism (e.g., calcitonin, denosumab, estrogen, raloxifene, teriparatide, and corticosteroids, etc.) were excluded. All OA patients had grade III–IV disease according to the Outerbridge classification (Uhl et al., 1998), whereas all OP patients had femoral neck fracture. Pain intensity was evaluated using the visual analog scale (VAS) before the operation (Bolognese et al., 2003). The OP patients, who had fractures, had their pain evaluated on the unfractured, non-operative side; the OA patients had their pain evaluated on the operative side.

Informed consent was obtained from each patient. The study was approved by the Institutional Review Board of Shanghai Renji Hospital.

2.2. Specimen preparations

The specimen of subchondral cancellous bone (20–20 mm in cross-section and 10 mm long) was collected from the load-bearing area of the femoral head and divided into two parts using a pendulum saw. One part was sectioned into 15 mm–15 mm in cross section and 10 mm long along the trabecular bone which was used for micro-computed tomography (micro-CT) examination (Li et al., 2012). The other part of the specimen was used for immunohistochemical analysis. The specimens were wrapped in saline-soaked gauze and stored at −80 °C. Specimens were thawed at room temperature before examination.

2.3. Micro-CT imaging

Twenty OA and OP specimens, 10 in each group, were examined using a micro-CT system (μCT 80, Scanco Medical AG, Switzerland) (Zhang et al., 2010). Each specimen was scanned continuously at a slice thickness and slice increment of 36 μm. After scanning, a constant volume of interest (VOI) centered over the specimen was selected for analysis of all study samples. Three-dimensional (3-D) images were reconstructed based on the VOI. The bone volume fraction (BV/TV; %), trabecular thickness (Tb.Th; μm), trabecular number (Tb.N; mm^{-1}), trabecular spacing (Tb.Sp; μm), connectivity density (Conn.D; mm^{-3}), degree of anisotropy (DA), and structure model index (SMI) were calculated using the software provided with the instrument (Li et al., 2012). SMI is a topological index for estimating the characteristic form in terms of the plates and rods that compose the 3-D structure. This index assumes integer values of 0 and 3 for ideal plates and rods.

Based on the standard curve of bone mineral density (BMD), the μCT system could calculate the mean density of the selected three-dimensional VOI. Apparent density (AD) represented mean density of total volume (TV), whereas material density (MD) represented that of bone volume which was segmented with the method of binarization (BV). The unit of measurement was mg HA/cm^3.

2.4. Immunohistochemical staining

The samples were embedded with paraffin after decalcification and sectioned along the longitudinal axis with a thickness of 5 μm. After dewaxing and hydration, antigen retrieval was performed on the sections using heat retrieval by boiled citrate. After antigen retrieval, the sections were rinsed in phosphate-buffered saline (PBS, 0.01 mol/L, pH 7.4) for 15 min. The immunohistochemistry experiment was performed using the S–P method. Endogenous peroxidase activity was blocked by incubating the sections with 3% H_2O_2 in methanol for 10 min. The non-specific reaction was blocked with normal goat serum prior to overnight incubation at 4 °C with the primary antibody separately (Anti-SP antibody, 1:1000, Santa Cruz, Biotechnology, Inc. USA; Anti-CGRP antibody, 1:1000, Abcam, Cambridge, USA; Anti-VIP antibody, 1:500, Abcam, Cambridge, USA; Anti-NPY antibody, 1:1000 Abcam, Cambridge, USA). After being rinsed in PBS, the sections were incubated for 15 min at 37 °C with biotinylated goat anti-rabbit IgG serum for all antisera, while biotinylated goat anti-mouse IgG serum was used for insulin. They were then washed in PBS and incubated for 15 min with S–P. Peroxidase reaction was carried out in a solution of DAB containing 0.01% H_2O_2 in Tris–HCl buffer (0.05 mol/L, pH 7.6).

Table 1
Comparison of the microstructural parameters of cancellous bone from OA and OP patients.

Parameters	OA ($n = 10$)	OP ($n = 10$)	*P* value
BV/TV (%)	31.71 ± 9.71	17.00 ± 4.15	0.001#
Tb.Th (μm)	236.31 ± 66.85	150.36 ± 26.11	0.003#
Tb.N (mm^{-1})	1.35 ± 0.18	1.13 ± 0.14	0.008#
Tb.Sp (μm)	521.38 ± 125.18	748.38 ± 106.96	0.000#
Conn.D (mm^{-3})	3.43 ± 0.91	2.66 ± 0.76	0.058
SMI	0.82 ± 0.44	1.72 ± 0.45	0.000#
DA	1.48 ± 0.13	1.61 ± 0.30	0.244

BV/TV, bone volume fraction; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular spacing; Conn.D, connectivity density; SMI, structure model index; DA, degree of anisotropy.
Value are the mean ± SD. OA, osteoarthritis; OP, osteoporosis.
# $P < 0.05$ vs. OA.