Original article

Sensory innervation and inflammatory cytokines in hypertrophic synovia associated with pain transmission in osteoarthritis of the hip: a case-control study

Munenori Takeshita¹, Junichi Nakamura¹, Seiji Ohtori¹, Gen Inoue¹, Sumihisa Orita¹, Masayuki Miyagi¹, Tetsuhiro Ishikawa¹ and Kazuhisa Takahashi¹

Abstract

Objective. To clarify the sensory innervation and inflammatory cytokines in hypertrophic synovia associated with pain transmission in OA of the hip.

Methods. A piece of the synovium was extracted during reconstruction surgery in 50 patients with OA of the hip as an inflammatory synovium and in 12 patients with femoral neck fracture as a normal synovium. Each sample was immersed in fixative solution, sectioned on a cryostat, and then processed for immunohistochemistry using antibodies as follows: neuron-specific class III β-tubulin (TuJ-1) as a general marker for nerve fibres, calcitonin gene-related peptide (CGRP) for sensory nerve fibres, nuclear factorκB (NF-κB) for the protein complex controlling the transcription of DNA in cellular responses to painful stimuli, and TNF-α for cytokines involved in acute inflammation. The number of immunopositive cells and fibres were counted using a fluorescence microscope.

Results. In the inflammatory synovium of OA of the hip, TuJ-1 was positive in 46% (23 hips). Of those positive for TuJ-1, 78% (18 hips) were also positive for CGRP, but 22% (5 hips) were negative for CGRP. NF-κB was positive in 68% (34 hips). Of those positive for NF-κB, 76% (26 hips) were also positive for TNF-α, but 24% (8 hips) were negative for TNF-α. In normal synovia, all four substances were negative.

Conclusion. We suggest sensory innervation and inflammatory cytokines in hypertrophic synovia are associated with nociception in OA of the hip.

Trial registration: University Hospital Medical Information Network, www.umin.ac.jp, UMIN000001335.

Key words: sensory innervation, inflammatory cytokine, synovitis, hip pain, osteoarthritis of the hip.

Introduction

Osteoarthritis is a painful disorder characterized by degeneration of articular cartilage, synovitis and joint deformity. OA is one of the most common diseases among musculoskeletal disorders and is estimated to affect nearly 27 million adults in the USA [1]. OA of the hip, which is often secondary to developmental dysplasia of the hip and acetabular dysplasia [2], causes restrictions in the range of motion and gait disturbances. OA of the hip causes deterioration in the quality of life and impairs activities of daily living of middle-aged people. NSAIDs are sometimes ineffectual for chronic pain of the hip, in part because our understanding of the pathogenesis of hip pain regarding sensory innervation and pain-transmitting substances in the hip joint is limited. Although total hip arthroplasty (THA) is effective in treating painful hips, the
medical cost of this surgical approach is estimated to be more than hundreds of millions of dollars in Japan [3]. Aseptic loosening and the need for revision surgery are other drawbacks of THA. The cost to society of surgical treatment potentially could be sharply reduced with the development of new, more effective anti-inflammatory drugs based on a better understanding of the pathogenesis of painful OA of the hip.

Traditionally, it has been widely accepted that the hip joint is innervated by the femoral nerve, the obturator nerve, the superior gluteal nerve and the sciatic nerve [4]. Referred pain to the knee is transmitted by the obturator nerve, which innervates the medial aspect of the hip joint. However, the distribution and the dominance of each nerve remain unknown. Clinically, pain originating in the hip is present most frequently in the groin (85%), followed by descending frequency in the knee (34%), the buttock (31%), the anterior thigh (18%) and the lower back [5]. Hip pain initially seems to be derived from acetabular labral lesions [6, 7], followed by degeneration of acetabular cartilage [8].

Recently pain research has been advanced with immunohistochemical studies. Several basic science studies have been conducted on the characteristics of dorsal root ganglia and sensory innervation of the hip in rats [9, 10]. Immunoreactive sensory nerve fibres in the labrum and synovium of the human hip joints have also been reported [11]. However, the small sample size and mixed patient conditions encouraged our further study. The purpose of this study was to differentially characterize the sensory innervation and pain-transmitting substances between OA of the hip and the normal hip as a means of improving our understanding of the pathogenesis of hip pain.

Patients and methods

The study was in compliance with the Helsinki Declaration, was approved by our institutional review board (review board of Graduate School of Medicine, Chiba University) and was registered with the University Hospital Medical Information Network (UMIN000001335). All participants were required to provide written informed consent to participate in the study.

Patient characteristics

We prospectively registered 50 patients (4 males and 46 females) with OA of the hip as the painful hip group (having inflammatory synovium) and 12 patients (3 males and 9 females) with femoral neck fracture as the normal hip group (having normal synovium). Inclusion criteria for the painful hip group were as follows: (i) OA of the hip secondary to developmental dysplasia of the hip; (ii) patients with severe pain and severe disability before surgery; and (iii) patients who underwent primary THA. Exclusion criteria for the painful hip group were as follows: (i) trauma, osteonecrosis of the femoral head, RA or rapid destructive OA; (ii) previous hip surgery and (iii) compromised host who suffered from heart failure, pulmonary failure, liver failure or renal failure. Inclusion criteria for the normal hip group were as follows: (i) asymptomatic patients without OA of the hip before femoral neck fracture; (ii) patients who underwent primary hemiarthroplasty of the hip joint; (iii) no signs of OA in X-ray images and (iv) no signs of OA in the macroscopic aspect of the femoral heads during surgery. The mean age was 61.3 years (range 48–80 years) in the painful hip group and 79.0 years (range 63–90 years) in the normal hip group. In the painful hip group, the Kellgren and Lawrence classification [12] was grade 4 in all hips, the mean preoperative Harris hip score [13] was 39.7 points (range 12–62 points), and the mean preoperative visual analogue score (VAS) was 75 mm (range 50–100 mm).

Immunohistochemistry

Tissue samples were taken from the synovium of the hip joint during hip reconstruction surgery both in the painful hip group and in the normal hip group. The size of tissue samples was ~1–2 cm in diameter. Each specimen was immersed in 4% paraformaldehyde fixative solution overnight at 4 °C and incubated in 0.01 M PBS (pH 7.4) containing 20% sucrose for 20 h at 4 °C. The specimens were cut horizontally into 10-μm thick sections using a cryostat and mounted on poly-L-lysine-coated slides. The specimens were then treated for 90 min in blocking solution comprising 0.01 M PBS containing 0.3% Triton X-100 and 3% skimmed milk at room temperature. The specimens were then prepared for immunohistochemistry using the following antibodies: (i) neuron-specific class III β-tubulin (TuJ-1, Covance, Princeton, NJ, USA), a general marker of nerve fibres (Fig. 1A); (ii) calcitonin gene-related peptide (CGRP, Chemicon, Temecula, CA, USA), a marker of thinly myelinated or unmyelinated peptidergic sensory nerve fibres (Fig. 1B); (iii) nuclear factor κB p65 (NF-κB; Santa Cruz Biotechnology, Santa Cruz, CA, USA), a protein complex that controls the transcription of DNA in cellular responses to painful stimuli (Fig. 2A); and (iv) TNF-α (Calbiochem, San Diego, CA, USA), a cytokine involved in acute inflammation (Fig. 2B). Sections were then incubated with a goat anti-rabbit Alexa 594 (Texas red fluorescent dye, 1:100; Molecular Probes, Eugene, OR, USA) fluorescent antibody conjugate for TuJ-1 immunoreactivity (IR) and NF-κB IR and a goat anti-mouse Alexa 488 (FITC, 1:100; Molecular Probes, Eugene, OR, USA) fluorescent antibody conjugate for CGRP IR and TNF-α IR. After each step the sections were rinsed three times in 0.01 M PBS. The sections were then observed with a fluorescence microscope (Eclipse E600; Nikon, Tokyo, Japan). Each sample was independently judged by three observers who were blinded to the patient information. IR was considered positive when two of the three observers or all three judged a sample to be positive.

Histopathological analysis of synovium

Haematoxylin and eosin staining was performed for histopathological analysis of the synovium using the synovitis score of Krenn et al. [14]. This grading is based on the three synovial membrane features (synovial lining cell...
layer, stroma cell density and inflammatory infiltrate), the ranking of alterations being on a scale of none (0), slight (1), moderate (2) and strong (3). The values of the parameters were summarized and interpreted as follows: 0/C150/C 1, no synovitis; 2/C150/C 4, low-grade synovitis; and 5/C150/C 9, high-grade synovitis.

Statistical analysis
The numbers of IR cells and IR nerve fibres, counted with a fluorescence microscope, were compared between the painful hip group and the normal hip group using Fisher’s exact probability test. Synovitis score was compared using a Mann–Whitney U test. Preoperative and postoperative Harris hip scores in the painful hip group were compared using a Mann–Whitney U test. Preoperative and postoperative VASs in the painful hip group were compared using a Mann–Whitney U test. P < 0.05 was considered to be significant.

Results
TuJ-1 IR nerve fibres were observed in 46% (23 of 50 hips) in the painful hip group, but were not evident (0%) in the normal hip group. There was a significant difference between the two groups (P = 0.002). CGRP IR sensory nerve fibres were observed in 54% (27 of 50 hips) in the painful hip group, but were not evident (0%) in the normal hip group. There was a significant difference between the two groups (P = 0.001). Of 23 hips with TuJ-1-positive IR, 78% (18 hips) were also positive for CGRP, but 22% (5 hips) were negative for CGRP.

NF-κB IR cells and fibres were observed in 68% (34 of 50 hips) of the inflammatory synovia in the painful hip group, but were not evident (0%) in the normal hip group. There was a significant difference between the two groups (P = 0.003). Of 34 NF-κB IR cells, 97% (33 cells) in the painful hip group were also positive for TuJ-1, but 3% (1 cell) in the normal hip group were positive for TuJ-1.
group, but were not evident (0%) in the normal hip group. There was a significant difference between the two groups ($P = 0.001$). TNF-α IR cells and fibres were observed in 58% (29 of 50 hips) in the painful hip group, but were not evident (0%) in the normal hip group. There was a significant difference between the two groups ($P = 0.001$). Of 34 hips with NF-κB-positive IR, 76% (26 hips) were also positive for TNF-α, but 24% (8 hips) were negative for TNF-α.

The mean synovitis score was 2.4 points (range 1–4 points) in the painful hip group and 0.2 points (range 0–1 points) in the normal hip group. There was a significant difference between the two groups ($P = 0.001$, Mann–Whitney U test). In the painful hip group, the synovitis score was not significantly different between both TuJ-1 and CGRP double-positive hips and the other hips (2.3 points vs 2.4 points, $P = 0.721$). The synovitis score was not significantly different between both NF-κB and TNF-α double-positive hips and the other hips (2.6 points vs 2.4 points, $P = 0.105$). All the patients with OA who underwent THA obtained pain relief, significantly improving the mean postoperative Harris hip score of 85.5 points (range 70–100 points) and the mean postoperative VAS of 5.8 mm (range 0–25 mm) compared with preoperative values ($P = 0.001$ and $P = 0.001$, respectively).

**Discussion**

Sensory innervation in hyperplastic synovia was observed with inflammatory cytokines in patients with OA of the hip. The most significant aspect of this study was the use of immunohistochemistry of the human hip joints on a larger scale than in previous reports. We believe that our findings elucidate the nature of the pathogenesis of pain transmission in OA of the hip, because all the patients with OA who underwent THA obtained pain relief and because neither sensory innervation nor inflammatory cytokines were detected in the normal hip.

The use of immunohistochemistry in pain research has been widely accepted [15–17]. However, to our knowledge, few basic science and translational studies of human hip pain have been published. In mice, Mach et al. [18] have examined the sensory and sympathetic innervation of the femoral bone using immunohistochemistry. In rat, Nakajima et al. [9] reported that CGRP IR neurons play an important role in the perception of pain in the hip joint. Nakajima et al. [9] also reported that Fluoro-Gold-labelled neurons were distributed throughout the left dorsal root ganglia from T13 to L5, primarily at L1, L2, L3 and L4 in the hip joint. In contrast, in the inguinal skin, Fluoro-gold-labelled neurons were distributed throughout the left dorsal root ganglions from T13 to L3, primarily at L1, L2 and L3 [10]. This characteristic sensory innervation pattern would explain the referred pain from the hip joint. In humans, Saxler et al. [19] observed upregulation of substance P-positive and CGRP-positive neurons in the capsules and the synovia of the hip joints in three patients with arthritis, but not in three normal controls. Shirai et al. [11] hypothesized that the hip pain occurred following the invasion of blood vessels and nerve fibres from inflamed synovial tissue to the labrum following labral degeneration in the hip joint affected by OA. Unfortunately the number of patients used in these previous studies was too small to draw any definite conclusions.

TNF-α is a well-recognized mediator of the inflammatory response in the peripheral nervous system [20]. TNF-α is primarily synthesized and released by macrophages, Schwann cells and activated T cells during inflammation and nerve injury. TNF-α is also released from sensory nerve fibres, where the cytokine plays a crucial role in the pathophysiology of injury-related pain [20]. Furthermore, TNF-α induces neural ingrowth, which is associated with pain via nerve growth factor (NGF) and its receptor [21, 22]. As a result, TNF-α and the p55 TNF receptors are upregulated in the glia and neurons of primary sensory nerves and result in neuropathic pain [20]. TNF-α in nerve itself has several effects, including the transmission of pain, induction of tissue damage and the development of secondary hypersensitivity to pain [20–22]. Saxne et al. [23] found TNF-α in the synovial fluid of 6 (50%) of 12 patients with RA and 3 (27%) of 11 patients with ReA, suggesting that TNF-α might be involved in a common pathogenic mechanism in inflammatory joint diseases. In the current study, TNF-α was observed in 63% of synovia in patients with OA of the hip, but in none with a normal hip. Thus we suggest that TNF-α promotes the development of a painful hip joint in OA and that TNF-α released from inflammatory cells facilitates nerve ingrowth into the hip joint affected by OA.

NF-κB, a nuclear transcription factor composed of p50 and p65 subunits, is activated by several cytokines including TNF-α [24, 25]. NF-κB was recently reported to play a crucial role in regulating proinflammatory cytokine gene expression and the transfer of nociceptive information [26, 27]. NF-κB is activated in the dorsal root ganglion after partial sciatic nerve injury and is crucial for hyperalgesia [28]. Inoue et al. [29] observed that NF-κB decoy reduced mechanical allodynia and thermal hyperalgesia in the rat inflammatory pain model, suggesting that NF-κB may represent a key mechanism for inflammatory pain. Fujisawa [25] reported that TNF-α caused synovial hyperplasia through NF-κB activation. In this study, NF-κB was observed in 68% of synovia in patients with OA of the hip, but in none of those with a normal hip. Moreover, NF-κB and TNF-α coexist in most patients with OA (Fig. 2). We believe that our findings support the previous reports.

CGRP is a marker of sensory neurons typically involved in pain perception [15, 16]. It has been reported that CGRP IR nerve fibres are present within the lumbar muscle, intervertebral discs and facet joints [15, 16]. TuJ-1, a mammalian B-tubulin gene product, is predominately expressed in neurons, but not in glia or non-neural cell types [30]. In this study CGRP was observed in 61% and TuJ-1 was observed in 50% of synovia in patients with OA of the hip. Of TuJ-1-positive nerve fibres, 78% were double-positive sensory nerve fibres with CGRP.

---

www.rheumatology.oxfordjournals.org
However, neither CGRP nor TuJ-1 was observed in normal hip synovia. Thus we hypothesize that hip joint pain occurs following invasion of blood vessels and nerve fibres from the inflammation of synovial tissue, which is activated by TNF-α and NF-κB.

A better understanding of the pathogenesis of painful OA of the hip should in turn facilitate development of new and more effective anti-inflammatory therapy with fewer and less severe side effects compared with conventional drugs. The development of such agents should hopefully reduce the number of THAs, resulting in reductions in medical costs and improvements in patient care. Indeed, several TNF-α inhibitors have already been applied in clinical use and have improved the quality of life in patients with RA [31]. Recently a randomized clinical trial of inhibitor of NGF in OA of the knee was reported to reduce joint pain [32]. Shigemura et al. [33] reported that ~6% of patients with OA of the hip showed neuropathic pain, therefore inhibitors of NGF should be effective for hip pain.

There were several limitations to this study. First, patients with femoral neck fracture were regarded as controls for OA of the hip based on previous work [19]. The mean age was significantly higher in the normal hip group than in the painful hip group (P = 0.001). This was because the normal group consisted of patients with femoral neck fracture, which often occurs in older people. Cartilage degeneration might be observed in such a population. However, we confirmed no signs of OA in clinical, radiological and surgical findings. It is ethically difficult to obtain synovium from healthy volunteers. Thus we believe that our study design is valid. Secondly, evaluation of immunohistochemistry is qualitative. We expect that a quantitative analysis using ELISAs will be able to confirm the upregulation or downregulation of each substance detected by immunohistochemistry. Third, although we studied the synovium, we did not study every component of the hip joint, such as the acetabular labrum, the articular cartilage and the subchondral bone in the acetabulum and the femoral head. Dirmeier et al. [34] reported that the density of synovial nerve fibres positive for CGRP relative to substance P was higher in OA than in RA. Further study is needed to document the characteristics of the dominant parts for hip pain.

Conclusion

Sensory innervation and inflammatory cytokines in hypertrophic synovia are associated with pain transmission in OA of the hip.

Acknowledgements

M.T. and J.N. made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data and drafted the manuscript. S.O. was involved in drafting the manuscript and revising it critically for important intellectual content. K.K. and G.I. were involved in drafting the manuscript and revising it critically for important intellectual content. M.M. participated in the design of the study and performed the statistical analysis. K.T. gave final approval of the version to be published. All authors read and approved the final manuscript.

Funding: This study was supported by the Hip Joint Foundation of Japan, Mitsui Sumitomo Insurance Welfare Foundation and Grants-in-Aid for Scientific Research (11019119).

Disclosure statement: The authors have declared no conflicts of interest.

References

Sensory innervation and inflammatory cytokine in hip OA


