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Distribution of Substance P Nerve Fibers in Osteoarthritis Knee Joint

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The objective of the study was to evaluate the distribution of substance P-immunoreactive nerve fibers in osteoarthritis knee and to determine whether a degenerative disease has any influence on the occurrence of neuropeptide-containing fibers, positively stained for substance P. Twenty consecutive patients, 16 females and 4 males, with gonarthrosis participated in the study. For comparison we used the group of 20 patients, 14 females and 6 males, operated on because of traumatic lesion of the knee. The medial and lateral retinaculum, medial compartment synovium and infrapatellar fat pad of these two groups of patients were evaluated using monoclonal antibody to substance P (PEPA40, Serotec Ltd, UK). The slices were examined semi-quantitatively for nerve fibers showing substance P expression. The values were analyzed with ONE WAY ANOVA test, which was then corrected with LSD test, at the level of significance $p<0.05$. There were no statistical differences in distribution of substance P nerve fibers in the fat pad, lateral and medial retinaculum or synovium between both groups, as well as in the each study group ($p<0.05$). The results allow us to speculate that different biomechanical axial disturbances of the knee could have the same influence on substance P-positive mechanoreceptors of the soft tissues around the joint modulating the pain pathway in knee osteoarthrosis.

Introduction

Degenerative joint disease involves numerous degenerative, cellular and basal substance changes that cause the decrease in the amount and efficiency of articular cartilage, with reparative reactions in cartilage and bone structural changes. There are both histological and clinical data indicating an enhanced inflammation of soft tissues in degenerative joint disease. The inflammatory reaction is mediated by cytokines, with the major role of interleukin 1β, interleukin 6, tumor necrosis factor alpha (TNFα) and granulocyte-macrophage colony stimulating factor (GM-CSF) [1, 2]. It has been also demonstrated that interleukin 1β induces substance P (SP) release from C afferent neural fibers [3, 7, 8]. Substance P activity within the external stratum of the spinal dorsal horn, connected with nociceptive afferent fibers suggests its role in pain sensing transmission [13, 15]. Substance P is a neuromediator that stimulates postsynaptic potential, influences blood vessel permeability and reactivity, induces lymphocyte proliferation, displays chemotactic activity for phagocytes. Thus, its activity is closely related to inflammatory state [7, 9, 14]. A recent study of ruptured anterior cruciate ligament (ACL) provides immunohistochemical evidence suggesting that rupture of this ligament leads to increase of the mean number of neuropeptide-containing fibers at the side of injury [18]. Also adolescent patients with anterior knee pain syndrome demonstrate differences in distribution of SP-immunoreactive nerve fibers in soft tissues around the knee [16, 17]. The prior findings and above observation prompted the study, the objective of which was to evaluate the distribution of SP-immunoreactive nerve fibers in osteoarthritis knee and to determine whether a degenerative knee disease has any influence on the occurrence of neuropeptide-containing fibers, positively stained for substance P.

Material and Methods

Twenty consecutive patients, 16 females and 4 males, average age 64.5 years, with osteoarthritis in all compartments of the knee joint, participated in the study (group A). For further comparison we used the group of 20 patients, 14 females and 6 males, mean age 18.6 years, operated on be-

cause of a traumatic lesion of the knee, like meniscal tear or ACL rupture (group T). The tissues including medial and lateral retinaculum, medial compartment synovium and infrapatellar fat pad of these two groups of patients were evaluated. The material for investigation was collected during arthroscopic procedures or total knee arthroplasty. The patients were informed about the research study and agreed to participate in the investigation.

The biopsies were prepared for routine light microscopic observations. The tissue samples were fixed for 24 h in 4% solution of paraformaldehyde and embedded in paraffin. Afterwards, 5 m-thick sections were stained with hematoxylin and eosin (HE) and examined using Carl Zeiss (Jena) microscope.

Immunohistochemistry

Paraffin sections were mounted onto superfrost slides, deparaffinized and streptavidin-biotin complex technique was applied. After re-hydration, sections reacted for 5 minutes with 3% hydrogen peroxide in distilled water and were rinsed in Tris-buffered saline (TBS). Then slides were incubated with rabbit anti-human substance P antibody (PEPA40, Serotec Ltd, UK) in dilution 1:2000 overnight at 4°C in a moist chamber. Afterwards, sections were rinsed in TBS, and the DAKO LSAB+/HRP Universal Kit (DAKO A/S, Glostrup, Denmark) was used according to the instructions of the manufacturer. The positive immunoreactivity was visualized with DAB as chromogen. After washing in distilled water, sections were counter-stained with hematoxylin and coverslipped. Negative controls were carried out by incubation in the absence of the primary antibody and always yielded negative results. The observation of substance P reactivity was made using Carl Zeiss (Jena) microscope.

Method of assessment of SP-immunopositive fibers

Slices were examined semi-quantitatively for nerve fibers showing substance P expression. The measurements were made in ten consecutive high power (400× magnification) fields (HPF). In each field the number of SP-positive fibers was determined using the three-grade scale: 0 – lack of nerve fibers showing expression of substance P, 1 – one immunopositive nerve fiber in the HPF, 2 – two or more fibers with substance P expression in the HPF (Table 1). Subsequently in each case the arithmetic mean of SP-immunopositive fibers was assessed for ten HPF.

The obtained values were submitted to statistical analysis with ONE WAY ANOVA test, which was then corrected with LSD test, at the level of significance $p<0.05$ (Table 2).

TABLE 1

The values in each visual field of substance P expression in slices from Hoffa body (H), lateral retinaculum (LR), medial retinaculum (MR) and medial compartment synovium (MS) of patients with gonarthrosis (group A) and traumatic lesion of the knee (group T)

	Gr. A H	Gr.T H	Gr. A LR	Gr. T LR	Gr. A MR	Gr. T MR	Gr. A MS	Gr. T MS
1	0.0	0.4	0.4	0.1	0.0	0.2	0.2	0.0
2	0.4	0.3	0.0	0.0	0.3	0.1	0.4	0.0
3	0.4	0.0	0.0	0.0	0.5	0.0	0.0	0.0
4	0.5	0.2	0.1	0.0	0.1	0.0	0.1	0.0
5	0.5	0.1	0.1	0.2	0.2	0.1	0.1	0.1
6	0.4	0.4	0.2	0.3	0.2	0.0	0.4	0.2
7	0.0	0.1	0.2	0.2	0.5	0.0	0.1	0.0
8	0.3	0.1	0.0	0.3	0.1	0.0	0.0	0.2
9	0.1	0.3	0.5	0.3	0.0	0.2	0.2	0.1
10	0.4	0.0	0.1	0.2	0.0	0.1	0.0	0.1
11	0.0	0.1	0.1	0.0	0.2	0.4	0.2	0.0
12	0.2	0.0	0.1	0.0	0.0	0.2	0.0	0.2
13	0.5	0.1	0.1	0.2	0.0	0.2	0.0	0.2
14	0.2	0.0	0.1	0.3	0.1	0.1	0.1	0.0
15	0.3	0.0	0.1	0.1	0.5	0.0	0.2	0.0
16	0.4	0.0	0.2	0.2	0.1	0.1	0.0	0.1
17	0.1	0.0	0.4	0.2	0.3	0.0	0.2	0.1
18	0.4	0.2	0.5	0.3	0.2	0.0	0.0	0.1
19	0.5	0.1	0.2	0.3	0.5	0.1	0.2	0.0
20	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0

TABLE 2
The minimum and maximum values, mean value, standard deviation and standard error from 10 consecutive visual fields showing substance P expression in slices from Hoffa body (H), lateral retinaculum (LR), medial retinaculum (MR) and medial compartment synovium (MS) of patients with gonarthrosis (group A) and traumatic lesion of the knee (group T)

Variable	Valid number	Minimum	Maximum	Mean	Standard deviation	Standard error
Group A H	20	0.000000	0.500000	0.280000	0.1932180	0.061101
Group T H	20	0.000000	0.400000	0.122222	0.148137	0.049379
Group A LR	20	0.000000	0.500000	0.170000	0.163639	0.051747
Group T LR	20	0.000000	0.300000	0.155556	0.133333	0.044444
Group A MR	20	0.000000	0.500000	0.190000	0.191195	0.060461
Group T MR	20	0.000000	0.400000	0.111111	0.136423	0.045474
Group A MS	20	0.000000	0.400000	0.120000	0.131656	0.041633
Group T MS	20	0.000000	0.200000	0.066667	0.086603	0.028868

Results

In osteoarthritis patients (group A) HE staining revealed hyalinized synovium and retinaculum with few calcifications. Lymphocytes and plasma cells were scattered in the fibrotic synovium. Infrapatellar fat pad consisted of fat lobules separated by connective tissue. In group T the synovium was edematous and scattered infiltrate composed of plasma cells and lymphocytes was seen. Retinaculum disclosed hyalinization. Infrapatellar fat pad was congested edematous, and with focal hemorrhages.

Nerve fibers immunoreactive for SP, most commonly associated with blood vessels, were found in fat pad, retinaculum and synovium (Fig. 1). Some free SP fibers, which were not associated with any vascular profiles, have also been observed. Commonly immunopositive nerve fibers coursed parallel to the vascular structures or the longitudinal axis of connective tissue fibers, and were located in larger nerve bundles among SP-negative fibers.

There were no statistical differences in distribution of substance P nerve fibers in the fat pad, lateral and medial retinaculum or medial compartment synovium between both groups, as well as within each comparative group ($p>0.05$).

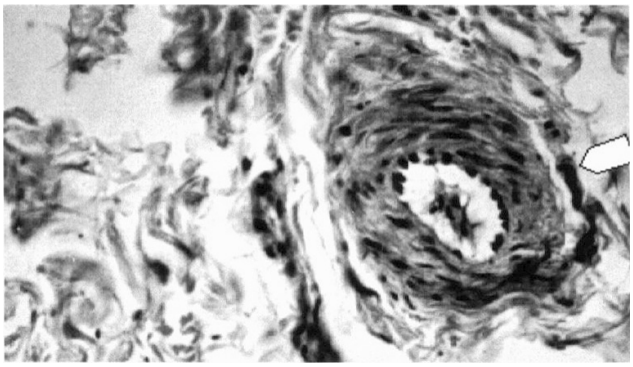

Fig. 1. Substance P immunoreactive nerve fibers (arrow) associated with blood vessel in the infrapatellar fat pad. Magn. 400×.

Discussion

At the initial stage of studies on degenerative joint disease, it was assumed that inflammation is mediated solely by cellular elements with predominant role of polymorphonuclear leukocytes. Current data support significant role of numerous mediators as cytokines, prostaglandins, complement factors and lyzosomal enzymes [1, 4, 6]. Lyzosomal enzymes released by polymorphonuclear leukocytes, with protease, collagenase and elastase properties, damage articular cartilage and activate other inflammatory mediators such as complement. Inoue et al. proved interleukin 1β influence on the release of substance P from afferent neurons and suggested that this phenomenon may be responsible for enhanced nociception in the course of inflammation [3]. Pritchet demonstrated increased level of substance P in articular fluid in 73% of patients with pain symptoms in the course of knee osteoarthritis [10]. The level of substance P was normal in healthy subjects and in patients with no pain symptoms. Articular fluid substance P level in patients after complete alloplastics of the joint was significantly decreased or reached normal values. Marshall et al. demonstrated the increase in substance P level in knee joints after trauma and in the course of osteoarthritis along with its normal level in blood serum [5]. Onuoha and Alpar found increased level of substance P and calcitonin gene-related peptide in patients with traumatic damage of connective tissue [8]. Saito and Koshino in their studies on the synovial innervations of the knee with medial compartmental osteoarthritis found that substance P and

calcitonin gene-related peptide (CGRP) appeared more frequently in the medial than in the lateral and suprapatellar areas [11, 12]. Previous investigations revealed that some abnormal biomechanical conditions of the knee or rupture of anterior cruciate ligament could lead to an increase in number of SP-positive nerve fibers. However, they do not always increase pain when comparing patients with anterior knee pain or ruptured anterior cruciate ligament [16, 17, 18]. Although in the degenerative joint disease enhanced inflammation has been observed, nociceptive afferent nerve supply is not different from the knee without osteoarthrosis.

Our studies indicated that there was no significant difference in the distribution of nociceptive mechanoreceptors with substance P expression (SP-immunopositive neural fibers) in the soft tissues around the knee joint between the patients with osteoarthritis and the patients with traumatic knee joint damage, as well as within each comparative group. These results prove that higher level of substance P in articular fluid and nociception in the course of osteoarthritis are not associated with changed number of SP-immunopositive nerve fibers in osteoarthritis knees that has been observed in other disease syndromes of the knee joint [16, 17, 18]. Based on these observations, it may be assumed that the increased level of substance P in articular fluid is a secondary phenomenon.

Conclusions

Clinical relevance of this study, revealing lack of different distribution of SP-positive nerve fibers of osteoarthritis knee, is that we could speculate that different biomechanical axial disturbances of the knee could have the same influence on SP-positive mechanoreceptors of the soft tissues around the joint modulating the pain pathway in knee osteoarthrosis. This hypothesis merits complex research with modern investigation tools, and the study results are considered as preliminary.

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