Neural growth factor expression in the lateral retinaculum in painful patellofemoral malalignment

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ABSTRACT – We studied 7 samples of lateral retinaculae excised at the time of surgical realignments. They were obtained from patients with isolated symptomatic patellofemoral malalignment resistant to conservative treatment and were evaluated with immunohistochemistry and immunoblotting. We found that neural growth factor is higher in patients with pain than in those with instability as the main symptom. Neural growth factor is related to neural proliferation in vessels and perivascular tissue and to the release of neuroceptive transmitters, such as substance P. We postulate that both mechanisms are involved in the pathogenesis of pain in isolated symptomatic patellofemoral malalignment.

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Patellofemoral malalignment (PFM) (Insall 1979, Insall et al. 1983, Sanchis-Alfonso et al. 1994, Sanchis-Alfonso 1995, Gresalmer and McConnell 1998, Sanchis-Alfonso et al. 1999a) is one of the leading causes of anterior knee pain and patellofemoral instability in active young people. Some studies have implicated neural damage and hyperinnervation of the lateral retinaculum as a possible source of the pain (Fulkerson et al. 1985, Mori et al. 1991, Sanchis-Alfonso et al. 1997, Sanchis-Alfonso et al. 1998, Sanchis-Alfonso and Roselló-Sastre 2000). Hyperinnervation is thought to be involved in the pathophysiology of pain in other orthopedic pathologies (Coppes et al. 1997, Freemont el al. 1997, Sanchis-Alfonso et al. 1999b). However, the mechanisms producing this neural proliferation in the lateral retinaculum are not known.

In a previous paper, we studied the roles of neural growth factor (NGF) and substance P (SP) in the hyperinnervation of the lateral retinaculum of patients with isolated symptomatic PFM, using histochemistry and found that both molecules were expressed more in patients with painful PFM (Sanchis-Alfonso and Roselló-Sastre 2000). NGF is a polypeptide with two biologically active precursors: a long form of ~ 34 kD and a short form of 27 kD (Dicou et al. 1997). NGF is a cytokine neurotrophin which is released during axonogenesis and inflammation, and stimulates neural sprouting. This is involved in pain mechanisms by stimulating the release of neuroceptive mediators, such as SP, attracting lymph cells and mastocytes, which can release more cytokines, including NGF, and thus perpetuate the circle (Malcangio et al. 1997, Sanchis-Alfonso and Roselló-Sastre 2000). In the present paper, we continue the study of NGF expression in the lateral retinaculum of patients with isolated symptomatic PFM.

Patients and methods

Tissue samples from 7 lateral retinaculae excised at realignment surgery, performed in 7 patients' knees (all women) with isolated symptomatic PFM, were used for this study. Our diagnostic criteria of isolated symptomatic PFM and therapeutic indications have been well-defined in previous studies (Sanchis-Alfonso et al. 1994, 1999a, Sanchis-Alfonso 1995). In 4 knees, pain was the main symptom, while 3 patients complained of instability as the main symptom, with little, if any, pain between instability episodes. The average age of the patients at the time of surgery was 19 (17–23) years. Surgery was performed after a mean of 25

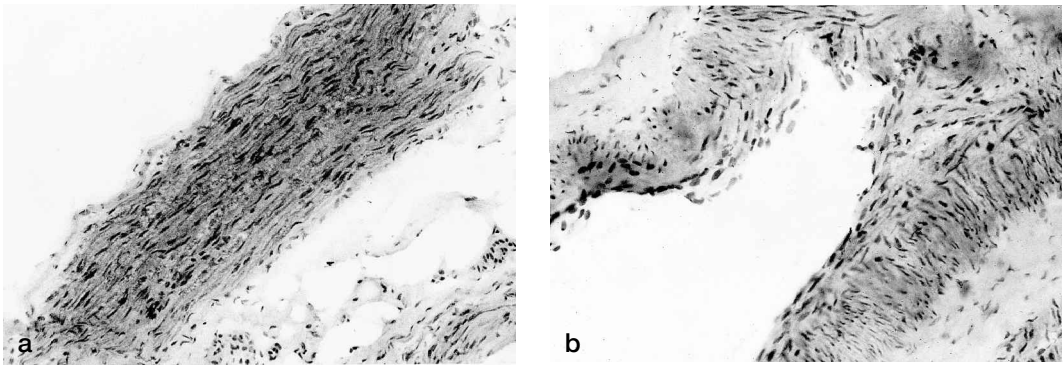

Figure 1. Immunohistochemistry shows that NGF is present not only in the thick nerves in the axons in a granular fashion and in the cytoplasm of the Schwann cells (a), but that it is also detected in the vessel walls, after its release by the nerves (b). NGF, Hematoxylin counterstained, 400×.

(12–30) months following the onset of symptoms. To obtain an homogeneous group, we included in this study, as also in previous papers (Sanchis-Alfonso et al. 1998, Sanchis-Alfonso and Roselló-Sastre 2000), only patients with the following criteria: (1) PFM on CT; (2) persistent pain and/or instability causing significant disability in daily life despite appropriate non-operative treatment for a minimum of 6 months; (3) no previous knee surgery; and (4) no associated intra-articular pathology (such as synovial plica, meniscal tears, ACL/PCL tears, osteochondritis dissecans or osteoarthrosis), confirmed arthroscopically.

In the immunohistochemical study, a fragment of the lateral retinaculum of 2 cc was immediately frozen in liquid nitrogen. This study was performed on 8 μm sections, using as primary antibody polyclonal anti-neural growth factor (Santa Cruz Biotech. Inc., California, 1/500). After 2 hours of incubation, the LSAB kit (DAKO Diagnostics, S.A., Barcelona, Spain) was used as secondary antibody and the reaction was shown with a supersensitive diaminobenzidine (DABplus, DAKO Diagnostics, S.A., Barcelona, Spain) counterstaining the nuclei with hematoxylin. As positive controls, we used normal skin and gut, both tissues rich in neural fibers.

In the immunoblot study, a fragment of 1 cc of the specimens was deeply frozen with liquid nitrogen and mechanically pulverized. The frozen powder was melted in reducing Laemmli sample buffer with 8 M urea at 0.2 mg/μL It was then further homogenized with an ultraturrax (polytron), heated for 5 min at 100ºC, and centrifuged at 10,000 g for 15 min at room temperature. The supernatant (5 μL) was then analyzed with SDS-PAGE in a 15% acrylamide gel using Laemmli (1970) conditions. When the course was completed the proteins were transferred to Immobilon P (Millipore), following standard procedures (Burnette 1981), and blotted using anti-human NGF antibodies (sc-548, 1/100) or the same antibodies in the presence of the corresponding antigen (sc-548P) from Santa Cruz Biotechnologie (California). Antibody binding was detected with anti-rabbit IgG conjugated to peroxidase (A-9169) from Sigma (Madrid, Spain).

Results

Immunohistochemistry

We found NGF expression in all 4 cases with pain as the main symptom. NGF had a granular pattern in the axons and in the cytoplasm of Schwann cells of the thick nerve fibers and in the muscular wall of the arterial vessels (Figure 1). Less NGF immunoreactivity or even absence of NGF reactivity was detected in the 3 cases with instability as the main symptom.

Immunoblot finding

Our immunohistochemical findings were confirmed by the immunoblot study. NGF was higher in patients with knee pain as the main symptom than in those with instability as the main complaint. The immunoblot analysis showed a thick band at the site of the long form precursor of NGF

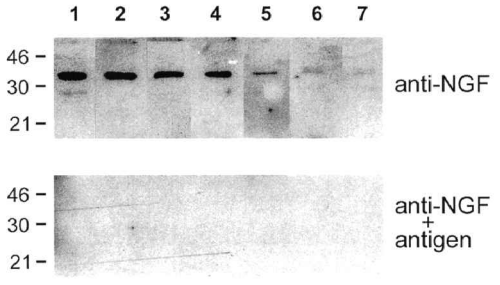

Figure 2. Immunoblotting detection of NGF showing a thick band at the level of the NGF precursor in patients with pain (cases 1–4) and an absence or a very thin band in the patients with instability as the main symptom (cases 5–7). The numbers at the left indicate molecular mass in kD.

(35 kD) in patients with pain, while an absence or a very thin band was seen in the patients with instability as the main symptom (Figure 2).

Discussion

In a previous paper, we used the presence of NGF and SP in the lateral retinaculum of patients with isolated symptomatic PFM by immunohistochemical techniques to study the pathogenesis of anterior knee pain in active young people (Sanchis-Alfonso and Roselló-Sastre 2000). Immunohistochemical techniques, however, merely showed large amounts of these neuropeptides, hindering assessment of subtle differences between cases. Molecular biology techniques, such as immunoblot, are far more sensitive. Therefore, we supplemented our previous analyses with immunohistochemistry to identify and locate NGF, and immunoblotting to detect even minimal expression of this and were able to confirm our earlier findings (Sanchis-Alfonso and Roselló-Sastre 2000). We could then also quantify NGF in PFM.

Nerve proliferation in PFM chiefly depends on nociceptive sensory SP positive nerves in the lateral retinaculum (Sanchis-Alfonso and Roselló-Sastre 2000). In this study, we found that PFM patients with pain show higher levels of NGF than those with instability as the main complaint. This NGF is chiefly found in the vessel walls and large neural structures, and as an active precursor of 35 kD, which means that the nerve fibers of these lateral retinaculae must still be in a proliferative phase.

NGF synthesis can be induced by ischemia (Lee et al. 1996, Abe et al. 1997, Woolf et al. 1997). It has been shown that NGF hastens neural proliferation in vessel walls (Isaacson and Crutcher 1995, Kawaja 1998), and it is just this pattern of hyperinnervation that is seen in the lateral retinaculum of patients with painful PFM (Sanchis-Alfonso et al. 1998, Sanchis-Alfonso and Roselló-Sastre 2000). In this respect, we suggest that ischemia may be the main problem in painful PFM (Sanchis-Alfonso and Roselló-Sastre 1998) due to vascular torsion, secondary to patellar malalignment. This condition, unlike a lax lateral retinaculum in knees with patellar instability, consists of medial traction over a retracted lateral retinaculum (Sanchis-Alfonso 1995). Episodes of ischemia may then be induced which trigger NGF release. When NGF is present in the tissues, it causes hyperinnervation, SP-release and pain, and attraction of mastocytes (Malcangio et al. 1997, Sanchis-Alfonso and Roselló-Sastre 2000), leading to the ischemia-hyperinnervation-pain circle.

We suggest that two pathobiological mechanisms may lead to symptomatic PFM: (1) pain as the main symptom, with detectable levels of NGF that cause hyperinnervation and stimulate of SP release, and (2) instability as the main symptom, with lower levels of local NGF release, less neural proliferation and less nociceptive stimulation.

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