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Increased Substance P in Subacromial Bursa and Shoulder Pain in Rotator Cuff Diseases

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Summary: The subacromial bursa is recognized as a site associated with the shoulder pain caused by rotator cuff disease in the middle-aged and elderly. Substance P is contained in primary afferent nerves, and its quantity increases during chronic pain. The amount of substance P in the subacromial bursa of patients with rotator cuff disease was examined. Radioimmunoassay and immunohistochemistry were employed to quantify and localize substance P. The preoperative pain level was measured with a visual analogue scale with 0 as no pain, 5 as moderate, and 10 as severe. Thirty-seven patients that had undergone operation were divided into two groups: one composed of 19 patients with subacromial bursitis and a partial-thickness tear of the rotator cuff (nonperforated cuff) and the other composed of 18 patients with a full-thickness tear (perforated cuff). Subacromial bursae obtained from seven fresh cadavers with no shoulder pain before death were used as controls. The visual analogue scale showed significantly greater pain in the group with the nonperforated rotator cuff than in the group with the perforated cuff. Consistent with these results, the amount of substance P in the subacromial bursa was significantly greater in the former group than in the latter. Nerve fibers immunoreactive to substance P were localized around the vessels, with a larger number of fibers in the group with the nonperforated rotator cuff. Therefore, an increased amount of substance P in the subacromial bursa appears to correlate with the pain caused by rotator cuff disease.

Rotator cuff disease frequently causes severe shoulder pain in the middle-aged and elderly. The subacromial bursa has been recognized as a site and source of pain in patients with rotator cuff disease (22,27-29). Clinically, the injection of corticosteroids, with or without local anesthetic, into the bursa effectively relieves shoulder pain caused by this disease (4).

Substance P is involved in the transmission of pain that is activated by the stimulation of Aδ/C fibers in the dorsal horn (32). In addition, substance P is contained in small sensory neurons in the peripheral tissue and can be released from the spinal cord by noxious stimuli (12); its release from the sensory neurons mediates neurogenic inflammation (16,24). Systemic treatment with capsaicin depletes the substance P in the primary sensory neurons, decreasing the sensitivity toward chemical, mechanical, and thermal nociceptive stimuli (11,13,14). Lembeck et al. reported that the amount of substance P increased in primary afferent nerves during chronic pain (18).

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The degree of shoulder pain varies in patients with rotator cuff disease. Gschwend et al. reported that patients with subacromial bursitis or partial-thickness tears usually feel more pain than those with full-thickness tears (10). In this study, we hypothesized that the amount of substance P in the bursa reflects the degree of shoulder pain that occurs with rotator cuff disease. Sensitive and specific radioimmunoassay was utilized to measure the amount of substance P in the bursa of patients with this disease. Immunohistochemistry was also employed to localize substance P.

MATERIALS AND METHODS

Thirty-seven patients (average age: 56 years; range: 45-72 years) with rotator cuff disease were candidates for this study. They were divided into two groups: 19 with a partial-thickness tear and subacromial bursitis (macroscopically ulcerated and frayed, respectively [nonperforated rotator cuff]) and 18 with a full-thickness tear (perforated cuff). The full-thickness tear was larger than 1 cm. The average duration of pain was 10.5 months in the group with the nonperforated cuff and 12.5 months in the group with the perforated cuff. The bursae around the greater tuberosity (impingement area) were harvested for specimens during the operation. Immediately, half of each specimen was stored at −80°C for radioimmunoassay, and the other half was immersed in Zammboni solution for immunohistochemistry. All specimens were obtained with informed consent. For control specimens, subacromial bursae were obtained from seven fresh cadavers (average age: 60 years;

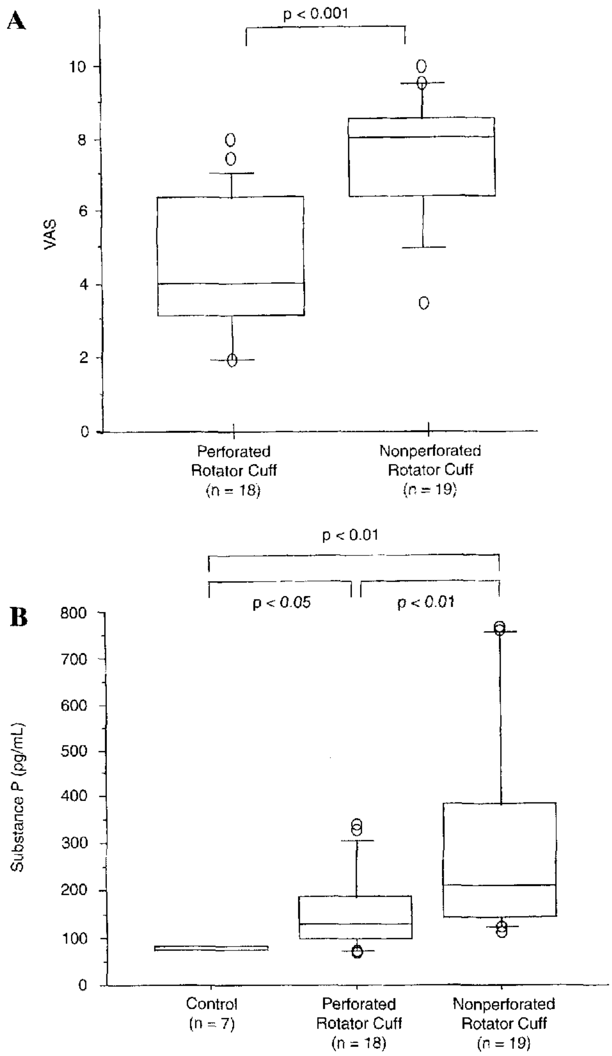

FIG. 1. A: Graph comparing the preoperative pain on the visual analogue scale (VAS) between the group with the nonperforated rotator cuff and the group with the perforated cuff. Median values are shown as a separate line through the box or as the bottom line of the box. The box includes the first-third quartiles. Small circles denote outliers. **B:** Graph comparing substance P content in the subacromial bursa of the experimental and control groups. Median values are shown as a separate line through the box or as the bottom line of the box. The box includes the first-third quartiles. Small circles denote outliers.

range: 50-78 years), none of whom had shoulder pain before death.

The level of shoulder pain was evaluated in each patient before the operation with a visual analogue scale as previously described (5,15,26,30). The degree of pain was graded from 0 to 10 according to a subjective symptom scale with 0 as no pain, 5 as moderate, and 10 as severe. Patients' symptoms were recorded by hospital staff that were blinded to this study. Patients for whom sufficient data were not produced were excluded from the study.

Each frozen tissue sample was weighed before extraction. The tissues were cut into small pieces and boiled for 10 minutes in 10 volumes of 2 *M* acetic acid in 4% EDTA, homogenized in a Polytron homogenizer (Kinematica, Lucerne, Switzerland) for 15 seconds, sonicated for 30 seconds, and centrifuged at 3,000 g for 15 minutes. The supernatants were lyophilized and resuspended in equal amounts of radioimmunoassay buffer. These samples were kept at −40°C until analysis. Substance P-like immunoreactivity was assessed with use of an antiserum raised in a rabbit against conjugated synthetic substance P (Peptide Institute, Osaka, Japan). The antiserum reacts with substance P and its sulfoxide but does not react with other tachykinins. Human substance P was used as the standard, and [^{125}I]tyrosin human substance P that was purified by high performance liquid chromatography was used as the radioligand. The assay was performed as previously described (1-3).

The bursal tissue was immersed in Zammboni solution at 4°C for 48 hours. Fifteen-µm-thick cryostat sections were placed on slides coated with silane as an adhesive. Endogenous peroxidase was blocked by incubation in methyl alcohol with 0.1% hydrogen peroxide at room temperature for 15 minutes and then was treated with 1.5% normal goat serum (in phosphate buffered saline, pH 7.4) for 20 minutes to block nonspecific binding. The slides were placed in a humidity chamber, and the sections were treated with a polyclonal rabbit primary antibody (Amersham, Buckinghamshire, England) at 4°C overnight and a peroxidase-labeled polyclonal antibody against a rabbit secondary antibody (Amersham) at room temperature for 30 minutes. The peroxidase label was developed in 0.2% 3,3′-diaminobenzidine tetrahydrochloride and 0.6% diaminobenzidine in 50 m*M* Tris (pH 7.6) with 0.003% hydrogen peroxide. All incubation steps were preceded by three 5-minute washes in 0.01 *M* phosphate buffered saline. The sections were dehydrated in ethanol and placed under cover slips.

Mann-Whitney nonparametric analysis of variance was used to test for differences between the two groups. P values less than 0.05 were considered significant.

RESULTS

Relationship between Pain and Substance P-like Immunoreactivity on a Visual Analogue Scale

The distributions of the visual analogue scale in the two groups are shown in Fig. 1A. Pain in the group with the nonperforated rotator cuff was significantly greater than that in the group with the perforated cuff ($p < 0.001$).

The concentrations of substance P-like immunoreactivity were measured by radioimmunoassay. The concentrations in the group with the nonperforated cuff were significantly greater than those in both the group with the perforated cuff and the controls. The concentrations in both groups were significantly

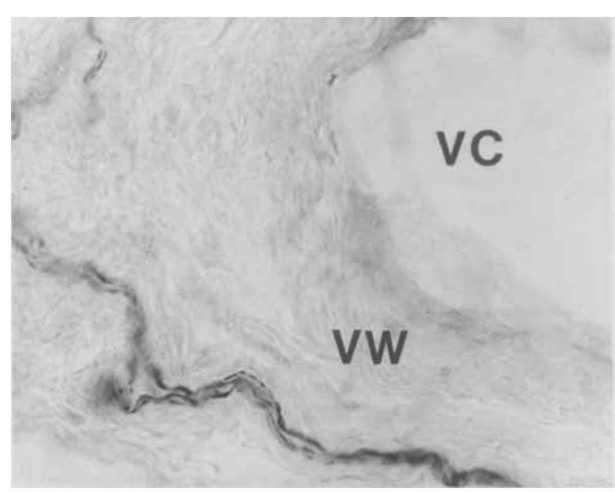

FIG. 2. Photomicrograph of substance P-like immunoreactive nerve fibers around a blood vessel. VC = vessel cavity and VW = vessel wall. Magnification: ×600.

greater than those in the controls. These results corresponded with those of the visual analogue scale (Fig. 1B).

Localization of Substance P-like Immunoreactivity

To localize substance P-like immunoreactivity, immunohistochemistry was employed. Substance P-like immunoreactivity was observed inside and immediately beneath the synovial lining and to a lesser extent in the stroma. Abundant substance P-like immunoreactivity was noted around the vessels (Fig. 2). An increase in the number of immunoreactive nerve fibers was observed in the synovial tissue of both groups, and the group with the nonperforated rotator cuff had the larger number of fibers. Fewer immunoreactive nerve fibers were detected in the bursal tissue of the controls.

DISCUSSION

Previous immunohistochemical studies mentioned the presence of neuropeptide-immunoreactive nerve fibers and gave only a rough estimate of quantitative changes in neuropeptides (16,20,27,29). Radioimmunoassay offers a means of quantitating neuropeptides with greater accuracy (1-3). In this study, we measured substance P in the synovium of the subacromial bursa with a sensitive and specific radioimmunoassay.

For control specimens, we obtained subacromial bursae from fresh cadavers with no shoulder pain at the time of death. No apparent injury in the rotator cuff was found. The control bursae were harvested within 6 hours of death. We had previously used fresh cadaveric specimens (obtained within 6 hours of death) for immunohistochemical study of cytokines and thus confirmed their validity (9). Prior to this experiment, preliminary immunohistochemistry for substance P showed firm positive immunoreactivity in fresh cadaveric spinal dorsal horns (obtained within 6 hours of death), further substantiating the validity of the cadaveric specimens used in the present study. Consequently, the possibility of degradation of the epitope due to the use of cadaveric specimens would be less conceivable.

There has been little interest in the relationship between rotator cuff tears and shoulder pain. Gschwend et al. reported that patients with subacromial bursitis or partial-thickness tears usually feel more pain than those with full-thickness tears (10). Our preoperative evaluation of shoulder pain concurred with their findings; the degree of pain in the group with the nonperforated cuff was significantly greater than that in the group with the perforated cuff. Furthermore, the amount of substance P in the subacromial bursa of the former group was significantly greater than that in the subacromial bursa of the latter, which corresponded with the results of the visual analogue scale. Therefore, an increase in the amount of substance P in the bursa was associated with an increase in the level of pain caused by rotator cuff disease.

Whether the increase in the amount of substance P was due to the residing nerve fibers or to an increase in the number of nerve fibers is unclear. However, the latter seems more probable in view of immunohistochemical findings that show a larger number of substance-P immunoreactive nerve fibers in the group with the nonperforated rotator cuff than in the group with the perforated cuff.

The differences in the amount of substance P between the two groups may be due to the amount of the subacromial bursal tissue in the vicinity of the impingement area. The tissue was more abundant in the patients with the nonperforated cuff than in those with the perforated cuff. We obtained the subacromial bursa as near the greater tuberosity as possible, which was not exactly at the site of the greater tuberosity but lay medial, anterior, or posterior to the tear in the group with the perforated cuff. It is believed that impingement wears out the bursa of the greater tuberosity as the cuff tear progresses. In other words, natural bursectomy due to impingement can have a denervation effect, which would explain the different degrees of pain and amounts of substance P in the two groups in our study. Alternatively, substance P released from the nerve fibers in the subacromial bursa in the group with the perforated cuff can escape from the bursa to the glenohumeral joint, which would eventually decrease its inflammation-induced effect at the bursa. This may be due in part to the differences in pain between the two groups.

In our study, substance-P immunoreactive fibers were located predominantly near vessels; this suggested a role in the local regulation of blood circulation, especially in inflammation (31). Intraarticular infusion of substance P has been shown to increase the severity of arthritis, which in turn could be blocked by infusion of a specific substance-P antagonist (6). Several studies have indicated that inflammation is associated with an increased amount of substance P (7,8,13,19,20-24). It is evident that subacromial bursitis takes place in patients with rotator cuff disease (25). Although the relationship between the degrees of bursitis and substance P was not examined in the present study, increased amounts of substance P in the subacromial bursa may determine the severity of bursitis caused by rotator cuff disease. Studies on these mechanisms are ongoing.

The subacromial bursa is anatomically vulnerable to the undersurface of the acromion and coracoacromial ligaments. In patients with rotator cuff disease, subacromial impingement may persist, producing secondary bursitis at the site (22). A noxious mechanical stimulus can also induce an increase in the amount of substance P in afferent nerves (17). Thus, both mechanical and chemical factors (i.e., impingement and bursitis) generate shoulder pain with increased amounts of substance P in the subacromial bursa of patients with rotator cuff disease. In the present study, we confirmed the different degrees of pain that occur with partial-thickness and full-thickness tears, which concurred with the differences in the amounts of substance P in the subacromial bursae. In conclusion, we believe that the subacromial bursa is the site associated with the shoulder pain caused by rotator cuff disease and that targeting the subacromial bursa for treatment leads to successful pain relief in patients with the disease.

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