Acromioclavicular Disk as a Potential Source of Pain in AC Joint Injuries

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Background: Injuries of the acromioclavicular joint (ACJ) are common shoulder injuries that often lead to pain and dysfunction of the affected shoulder. Regardless of operative or nonoperative treatment, a relatively large number of patients remain symptomatic and experience pain. However, the specific source of persistent pain in the ACJ remains ambiguous.

Purpose: To investigate the presence of sensory nerve fibers or pain-generating neurotransmitters within the intra-articular disk of the ACJ to determine its potential role as an independent pain generator in ACJ disorders.

Study Design: Descriptive laboratory study.

Methods: Twelve paired ACJs from 6 fresh human cadavers (mean age, 56 years; range, 41-82 years) were harvested and freed from surrounding soft tissues, leaving only the ACJ capsule intact. The specimens were placed in 4.5% formaldehyde fixative for a minimum of 48 hours. Coronal plane sections were obtained and demineralized in EDTA for a week, embedded in paraffin for 12 hours, and dehydrated overnight. With a rotation microtome, 2-μm sections were cut and stained with hematoxylin and eosin to investigate tissue architecture and confirm the presence of a fibrocartilaginous intra-articular disk. The sections were immunohistochemically stained with antisera against S100, neuropeptide Y (NPY), and substance P (SP) to detect for neural tissue. Additionally, a nerve fiber count per 10 high-power fields representing an area of 0.2 mm^2 was conducted for S100 stains. All sections were examined for the presence of positive immunoreactivity to S100, NPY, and SP.

Results: The presence of a fibrocartilaginous intra-articular disk could be observed in all 12 examined ACJs. In all specimens, an immunoreactivity to S100, NPY, and SP could be observed within the superior peripheral region of the intra-articular disk. High-power field nerve counts of the S100 stains revealed a mean ± SD of 7.9 ± 2.28 nerves per 10 high-power fields (range, 4-12).

Conclusion: The documented immunoreactivity to S100, NPY, and SP indicates the presence of somatic and autonomic nerve fibers within the intra-articular disk of the ACJ.

Clinical Relevance: Confirming the presence of nerve fibers within the intra-articular disk of the ACJ suggests that the disk itself could be an independent source of pain after injury and thus a possible explanation for recalcitrant pain after treatment.

Keywords: acromioclavicular (AC) joint; AC joint dislocation; neural anatomy; intra-articular disk

Injuries of the acromioclavicular joint (ACJ) are common shoulder injuries, with an estimated incidence of up to 45 per 100,000 people, often leading to pain and dysfunction of the affected shoulder.[7,30]

Although low-grade injuries such as Rockwood I and II are typically treated nonoperatively, with a short period of sling immobilization and supportive care followed by early physical therapy and rehabilitation, Rockwood IV-VI injuries are commonly considered indication for surgical treatment.[11,12,18] The optimal treatment of Rockwood III injuries is still controversial and subject to ongoing debate. However, regardless of treatment, a large number of patients with ACJ injuries remain symptomatic, with pain being the predominant clinical symptom after failed treatment.[3,18,20] Up to 48% of patients with Rockwood I and II injuries are symptomatic and experience pain during heavy and overhead activities after nonoperative treatment, with up to 27% needing subsequent ACJ surgery.[3,8,21,23,34] Additionally, 6% to 27% of patients with Rockwood III injuries experience persistent pain and dysfunction, independent of treatment modality.[18,20,32] The source of this remaining pain is not yet clear, and the question arises: Are any structures other than those addressed with current treatment strategies a potential source of chronic pain?

One potential source of persistent pain after ACJ dislocations is the intra-articular disk.[10,33] However, not only are disruptions of the intra-articular disk difficult to verify with current routine imaging techniques, but little is known regarding its mechanism as a potential source of

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pain within the ACJ.[10,15] Besides the ACJ, many joints in the human body—such as the uncovertebral joints, the temporomandibular joint, the wrist joint, and the knee joint—have fibrocartilaginous disklike structures within their intra-articular compartments.[13] The main role of these intra-articular fibrocartilaginous disks is to enhance the congruency of the articulating surfaces; additionally, they act as a bumper to distribute contact pressures within the joint.[8,13] The acromioclavicular articulation has an intra-articular fibrocartilaginous disk, which has been shown to be a complete disk (very rare) or is meniscoid-like.[9,29] The fibrocartilaginous disklike structures of the uncovertebral joints, the temporomandibular joint, the wrist joint, and the knee joint all have sensory innervation; thus, it has been reported in the literature that these structures are a potential source of pain within those joints.[1,2,5,24,27]

Therefore, the aim of the present study was to investigate the presence of sensory nerve fibers or pain-generating neurotransmitters within the intra-articular disk of the ACJ through the use of immunostaining with specific antisera to determine its potential role as a pain generator in the ACJ. We hypothesized that positive stainings would be observed in all intra-articular disk specimens.

METHODS

This study was approved by the institutional ethics committee before investigation (2198/2015). For the purpose of this study, 12 paired ACJs were examined from 6 fresh human cadavers (mean age, 56 years; range, 41-82 years). Exclusion criteria included a history of ACJ pathology, ACJ pain, symptomatic ACJ arthritis, cancer, or inflammatory systemic disease.

Within a 3-month study period, 30 autopsies were carried out at the Department of Pathology (Medical University of Vienna). Six cadavers could be included in the study, whereas 24 had to be excluded owing to $\geq$1 of the aforementioned exclusion criteria. A pathologist (S.L.) harvested all ACJs with adjacent parts of the clavicle and the acromion within the first 24 hours postmortem. After careful dissection of the surrounding soft tissues, leaving only the ACJ capsule intact, the specimens were placed in 4.5% formaldehyde fixative for a minimum of 48 hours. Macron sections were then cut across the coronal plane with a separating grinding system (Sanova Inc). The obtained sections were demineralized in EDTA for a week, embedded in paraffin for 12 hours, and dehydrated overnight (Tissue Teck VIP; Sakura). With a rotation microtome (Microm Inc), 2-μm sections were then cut.

Hematoxylin and eosin stains were used to score tissue architecture and to confirm the presence of the fibrocartilage tissue of the intra-articular disk. The sections were then stained in an immunohistochemical stainer (Ventana Inc) with antisera against the following: S100 (1:500 dilution; Immunostar), which is known to be a protein specific to nerve fibers that is used to detect for free nerve endings[27]; neuropeptide Y (NPY; 1:100 dilution [Immunostar]), a noradrenergic sympathetic postganglionic nerve fiber marker that is used to detect for autonomic nerve fibers; and substance P (SP; 1:100 dilution [Immunostar]), a neurotransmitter specific for nociceptive fibers of the somatic nervous system.[17,22,28] The S100 staining protocol provided higher specificity for nerve tissue without confounding background staining. Hence, a nerve count per 10 high-power fields representing an area of 0.2 mm^2 was additionally conducted for this protocol. All stained sections were analyzed with an Olympus BH2 microscope, and images were taken with an Olympus C-35AD-4 camera.

Given the nature of the present study, no quantitative statistical analyses were performed. The mean number of nerves per 10 high-power fields was calculated. All sections were examined by 2 experienced pathologists (C.B., S.L.). Descriptive statistics including means, standard deviations, and ranges were presented where appropriate. All statistical analyses were performed using SPSS Statistics for Macintosh Version 22.0 (IBM Corp).

RESULTS

Light microscopic evaluation of the hematoxylin and eosin stains confirmed the presence of the fibrocartilage tissue of the intra-articular disk in all 12 examined ACJs (Figure 1).

Immunoreactivity was observed in all examined stains of all 12 ACJs: S100, suggesting sensory innervation (Figure 2); SP, suggesting the presence of nociceptive fibers of the somatic nervous system (Figure 3); and NPY, suggesting the presence of autonomic nerve fibers (Figure 4).

Immunoreactivity was localized in the superior capsular region (close to the capsular attachment) of the intra-articular disk in all specimens. In the distal region of the fibrocartilage tissue of the intra-articular disk, no immunoreactivity could be identified in any of the examined stains (Figure 5).

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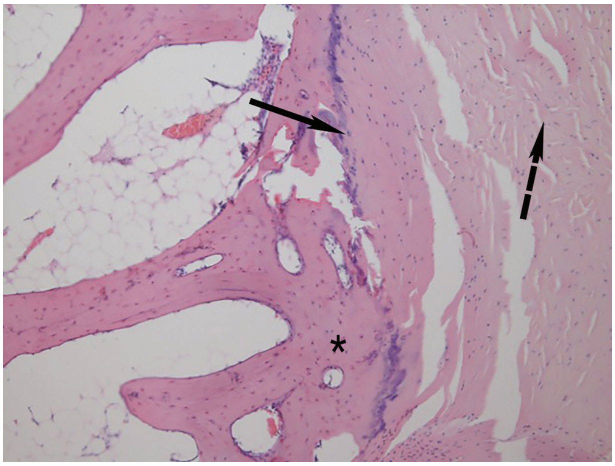

Figure 1. Hematoxylin and eosin staining. Asterisk, bone; arrow, hyaline cartilage; arrow with striped line, fibrocartilage tissue of the intra-articular disk.

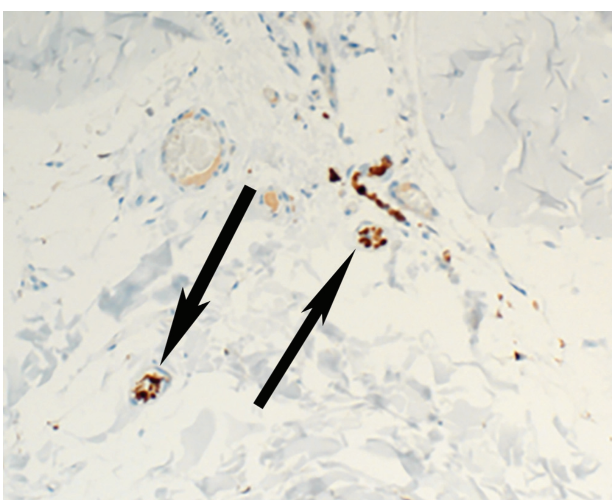

Figure 2. S100 immunostaining; arrows show free nerve endings with immunoreactivity of S100, suggesting sensory innervation.

Nerve counts of the S100 stains revealed a mean ± SD of 7.9 ± 2.28 nerves per 10 high-power fields (range, 4-12).

DISCUSSION

The present study reports on nerve innervation of the intra-articular disk of the ACJ using immunohistochemical methods. The most important finding was that the presence of somatic and autonomic nerve fibers could be confirmed in the intra-articular disk of the ACJ, which therefore could be a pain generator in ACJ injuries.[5,19,22,27]

The presence of S100-positive nerve endings within the fibrocartilage disk of all examined 12 ACJs suggests sensory innervation of this intra-articular structure. Our finding that the majority of nerve fibers was located in the superior peripheral region, close to the capsular attachment, suggests that innervation may arise from the capsule. This is similar to published innervation patterns of other fibrocartilage disks within the human body, such as the meniscus or the triangular fibrocartilage complex of the wrist joint.[2,22,27]

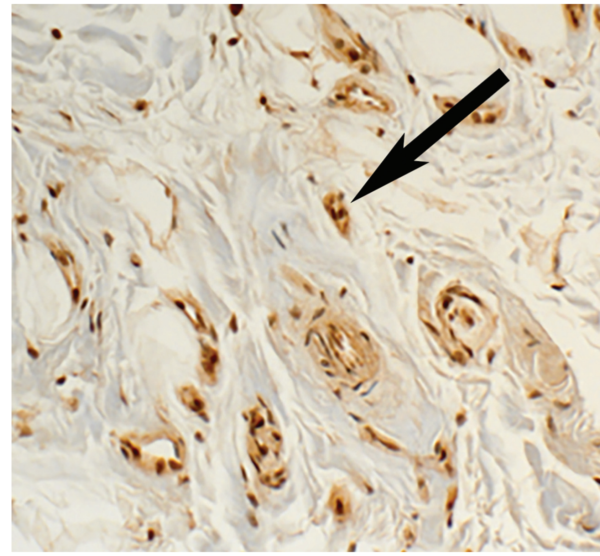

Figure 3. Substance P immunostaining; arrow shows free nerve ending with immunoreactivity of Substance P, suggesting the presence of nociceptive fibers of the somatic nervous system.

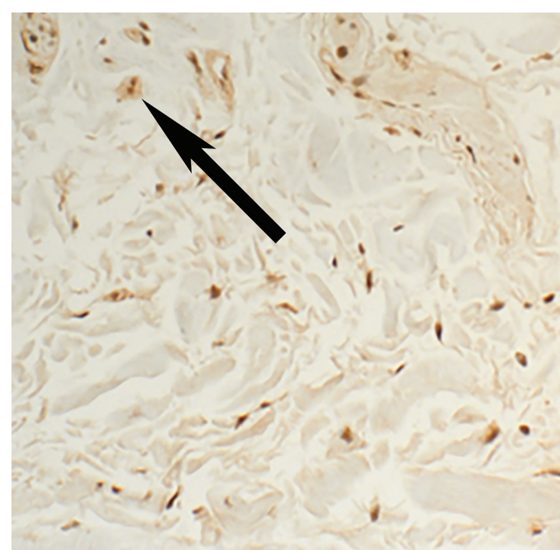

Figure 4. Neuropeptide Y immunostaining; arrow shows free nerve ending with immunoreactivity of Neuropeptide Y, suggesting the presence of autonomic nerve fibers.

For a tissue to be a potential source of pain, it must be innervated by pain fibers, making the presence of free nerve endings a prerequisite. However, as not all free nerve endings are nociceptors, a more specific differentiation of pain fibers can be achieved through the

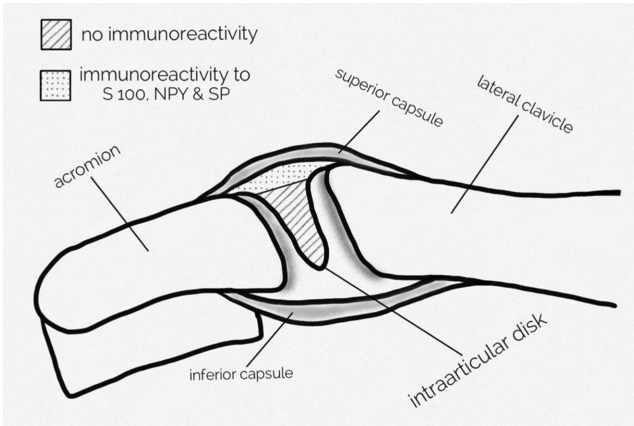

Figure 5. Schematic map of the acromioclavicular joint showing areas of immunoreactivity to S100, neuropeptide Y (NPY), and substance P (SP).

identification and demonstration of neuropeptides known to be strongly associated with pain, such as SP.[16] Therefore, to investigate the possible presence of nociceptive fibers from the somatic nervous system, immunohistochemical staining with antisera against SP was carried out. SP belongs to a family of closely related peptides known as tachykinins and is a neurotransmitter specific for nociceptive fibers.[16,17,22] The presence of SP within peripheral nerve fibers especially seems to have several functions related to nociception, inflammation, and vasoactivity.[4,16] Peripheral SP-containing nerve endings are sensitized by chemical inflammatory mediators, such as histamine, prostaglandin E2, and bradykinin.[26] As a consequence, SP is released from those peripheral nerve endings, which stimulates an inflammatory reaction.[14] Furthermore, SP can be found in the central nervous system, evoking pain sensation.[14] Thus, the identification of SP-positive nerve endings is highly suggestive of pain-mediating nerve endings in the intra-articular disk of the ACJ.

The observation of immunoreactivity to NPY suggests sympathetic innervation to the disk and supports the idea of the intra-articular disk as a potential source of pain. NPY is a noradrenergic sympathetic postganglionic nerve fiber marker that has been identified not only in paravertebral sympathetic ganglions but in peripheral nerves.[31,36] NPY has also been identified in a number of spinal structures—for example, the intervertebral disk in patients with chronic low back pain associated with herniated nucleus pulposus[25] and degenerative disk disease.[6] Related to these findings, investigators have linked the sympathetic nervous system to select nonsegmental spinal pain patterns.[31]

The presence of SP- as well as NPY-positive nerve fibers within the intra-articular disk suggests that some of the pain experienced in ACJ pathologies might arise from the disk itself, which could very well have clinical relevance. In acute ACJ injuries, treatment options mainly focus on the degree of ligament instability.[10] However, these injuries may lead to not only disruptions of the stabilizing ligaments and the joint capsule but also damages to intra-articular structures, such as the cartilage or the intra-articular disk.[23,33] As our current surgical treatment strategies mainly focus on regaining joint stability and are directed toward ligament instability, missing any such additional intra-articular disorders is a potential risk for recalcitrant pain. Furthermore, magnetic resonance imaging studies have thus far not established standard protocols that allow us to reliably detect intra-articular disk pathologies.

In contrast to surgical stabilization methods, nonoperative treatment of acute ACJ lesions of lower grade (eg, Rockwood I and II) consists of a variable time of sling placement with pain medication. In higher-grade injuries, patient education, physical therapy, and activity modification after a prolonged sling immobilization are additionally part of the nonoperative treatment regimen.[11] However, regardless of the type of treatment, a relevant number of patients with Rockwood III injuries remain in a chronic painful state.[3,21,23] More interesting, patients with even low-grade injuries, such as Rockwood I and II, which are widely considered to be eligible for nonoperative treatment, were symptomatic and experienced pain during heavy and overhead activities after nonoperative treatment, with some patients needing subsequent ACJ surgery.[3,8,21,23,34,35] The development of ACJ arthrosis after ACJ injuries is one possible explanation for these findings.[11] However, the question arises: What structures other than those in our current treatment strategies (eg, a ruptured intra-articular disk) might be a source of chronic pain? With this knowledge, arthroscopic disk removal, for example, could be considered a viable treatment option in patients with lower-grade injuries who experience persistent pain but whose nonoperative treatment failed. Hence, the clinical relevance of sensory innervation within the intra-articular disk of the ACJ should be investigated further.

Limitations

As the presence of the described histochemical substances and nerves has been evaluated in a limited number of specimens with a mean age of 56 years, the findings of this study might not be universally applicable to the general population. However, no age-related histochemical changes, at least in spinal structures, have yet been reported in the literature.[5] Second, the used method of proof is still an indirect one. Nevertheless, even in the absence of any known painful joint condition in the evaluated specimens, we were able to observe those histochemical substances, and it can be assumed that in individuals presenting with a painful ACJ, such substances are increased. Third, given the explorative nature of the study, no quantitative statistical analyses were applicable.

CONCLUSION

The documented immunoreactivity to S100, NPY, and SP indicates the presence of nerve fibers from the somatic and autonomic nervous system within the intra-articular disk of the ACJ. These findings suggest that the intra-articular disk of the ACJ itself could be a pain generator in ACJ injuries.

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