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Pain Relief Through an Antinociceptive Effect After Radiofrequency Application

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Background: Many patients with chronic tendinosis have experienced early pain relief after application of bipolar radiofrequency treatment. Pathologic nerve ingrowth or nerve irritation in the tendon has been considered as a possible cause of the pain experienced with tendinosis.

Hypothesis: Bipolar radiofrequency treatment will ablate nerve fibers, resulting in pain relief.

Study Design: Controlled laboratory study.

Methods: Eighteen Sprague-Dawley rats were used in this study. Eight rats were treated with 2 points of bipolar radiofrequency applications applied to the hind paws with the Topaz microdebrider device, 6 sham rats had a needle applied to the hind paws, and there were 4 control rats. Tissues were processed for neural class III β-tubulin (TUJ-1) or calcitonin gene-related peptide (CGRP) immunohistochemistry by using the free-floating avidin-biotin complex technique. The numbers of TUJ1-immunoreactive and CGRP-immunoreactive nerve fibers in the epidermis were counted and compared with sham and control.

Results: The number of nerve fibers demonstrated by both the antibodies of TUJ1 and CGRP were significantly decreased (P = .0002-.002) during the first 2 weeks after bipolar radiofrequency treatment. Macroscopically, the foot pad showed 2 dimples on the surface after bipolar radiofrequency treatment. Although it still showed a scar after 7 days, after 14 days it looked no different than the untreated contralateral control foot pad and foot pad of the sham group.

Conclusion: Bipolar radiofrequency treatment induced acute degeneration and/or ablation of sensory nerve fibers.

Clinical Relevance: Degeneration or ablation of nerve fibers after bipolar radiofrequency treatment may explain the early postoperative pain relief after microtenotomy for tendinosis.

Keywords: bipolar radiofrequency; chronic tendinosis; microtenotomy; pain relief

Chronic tendinopathy, like medial and lateral epicondylitis or patellar and Achilles tendinopathy, remains a common problem for recreational and elite athletes.1,3,12 The characteristic pathologic changes of these tendinosis conditions have been shown to be collagen disorganization and fiber separation with increased mucoid ground substance.6,7 The majority of patients can be treated with combinations of conservative therapies including nonsteroidal anti-inflammatory medication, physical therapy, steroid injections, and orthoses.16,23 However, when conservative therapy fails, more invasive therapies are used.

Recently, extracorporeal shock wave therapy (ESWT) has been proven effective for pain relief and simulating healing of chronic tendinoses.15 Experimental forays into ultrasound-guided polidocinal injections to sclerose pathologic vascular ingrowth have also shown promise for relieving the chronic pain of tendinosis.3 If these conservative modalities fail, surgical intervention becomes the treatment of choice.

The surgeries for tendinopathy usually involve open or arthroscopic debridement of the damaged portion of the tendon with repair, lengthening, or pathologic release of the remaining healthy tendon.5,11,23 Recently, a microtenotomy using bipolar radiofrequency (bRF) was introduced to treat tendinopathies like chronic lateral epicondylitis. This technology has shown promising clinical results.21 Nearly 70% of the patients undergoing bRF microtenotomies...
noticed pain relief in the recovery room or on the first or second postoperative day. This observation suggested that bRF treatment provides early pain relief.

Alfredson et al,3 with a chronic Achilles tendinosis study, demonstrated the pathologic vascuoneural ingrowth in the tendon with protein gene product 9.5 immunoreactivity. Patients with lateral and medial epicondylitis were found to have calcitonin gene-related peptide (CGRP) immunoreactive (IR) nerve fibers in the tendon insertion.8 Messner et al,10 using a rat model for tendinosis, showed increased numbers of filaments and increased immunoreactivity to CGRP and substance P (SP).

Neuropeptides SP and CGRP are involved not only in transmitting nociceptive information to the spinal cord but also in peripheral effects including microvascular leakage and local edema formation.24 Pathologic nerve ingrowth in the tendon may be considered as a cause of pain in patients suffering from chronic tendinosis.8,8 We hypothesized that patients experience early pain relief from a microtenotomy with bRF by degeneration of the pathologic nerve fibers in tendon. The purpose of this study is to evaluate the effect of bRF application to peripheral pain nerve fibers using a rat model.

**MATERIALS AND METHODS**

After Institutional Animal Care and Use Committee and University of California–San Diego Animal Subjects Committee approval, 18 male Sprague-Dawley rats (weight, 250-300 g) were divided into 3 groups (control, 4 rats; bRF group, 8 rats; and sham group, 6 rats). They were anesthetized with isofluorane (VEDCO Inc, St Joseph, Mo) and sodium pentobarbital (Abbott Labs, North Chicago, Ill) and treated aseptically throughout bRF applications. In the sham group, 2 incisions were made on the left hind paw middle two foot pads using a 22-gauge needle at a distance of 1 mm from each other. In the bRF group, 2 points of bRF applications (at a distance of 1 mm from each other) for 500 milliseconds were applied to the left hind paw middle two foot pads of the rats using the TOPAZ Microdebrider device (ArthroCare, Sunnyvale, Calif). The TOPAZ Microdebrider device connected to a System 2000 generator at setting 4 was used to perform the bRF application. In this process, bRF energy is used to excite the electrolytes in normal saline, and the energized particles have sufficient energy to break molecular bonds, ablating soft tissue at low temperatures (40-70°C).17,25-27 After treatment, the rats were allowed activity as tolerated.

The control rats (n = 4), sham rats after 7 days (n = 3) and 14 days (n = 3), and rats treated by bRF after 7 days (n = 4) and 14 days (n = 4) were anesthetized with ketamine (80 mg/kg intraperitoneally) and isofluorane and perfused transcardially with 0.9% saline, followed by 500 mL 4% paraformaldehyde in a phosphate buffer (0.1 M pH 7.4). The foot pads of both rat hind paws were resected (left for study group, right for contralateral control group). After being stored in 0.01 M phosphate-buffered saline (PBS) containing 20% sucrose for 20 hours at 4°C, the specimens were sectioned at a 30 µm thickness on a cryostat. Twenty sections were collected in PBS. Half of them were processed for neural cell III β-tubulin (TUJ-1) immunohistochemistry, and the other half were processed for calcitonin gene-related peptide (CGRP) immunohistochemistry14 by using the free floating avidin-biotin complex technique. They were incubated for 20 hours at 4°C with mouse antibody for TUJ-1 (1:500; Convance, Berkeley, Calif) or rabbit antibody for CGRP (1:500; ImmunoStar Inc, Hudson, Wis) diluted with a blocking solution in 0.01 M PBS containing 0.3% Triton X-100, 5% skim milk (Becton, Dickinson and Company, Sparks, MD), and 0.05% bovine serum albumin (Sigma-Aldrich, St. Louis, Mo). After thorough washing, sections were incubated for 90 minutes in an Alexa Fluor 488 labeled goat anti-mouse IgG (1:100; Molecular Probes, Eugene, Ore) or Alexa Fluor 488 labeled anti-rabbit IgG (1:100; Molecular Probes).

The sections were then viewed with fluorescent light microscopy by 1 blinded observer (NT), and nerve fibers that passed through the basement membrane of the epidermis were counted. Branching occurred within the epidermis, but these additional branches were not counted. The numbers of TUJ1-IR and CGRP-IR fibers were counted for each section per 16.3 x 10⁻³ mm² of epidermis in a foot pad. After we counted all sections in all time periods, the numbers of TUJ1-IR and CGRP-IR fibers in the 10 sections of each rat were summed and then averaged in the each group.

Statistical analysis of results was performed using Statcel (Statsoft, Tokorozawa, Japan). Results were presented as mean ± standard deviation (SD) in each group. The mean numbers of nerve fibers between the sham group and the bRF study group were matched with their respective contralateral control group and were analyzed using a paired t test. The mean numbers of nerve fibers between the sham group and the bRF study group on day 7 and day 14 were compared by nonpaired Student t test. Significance was defined as P < .05.

**RESULTS**

Macroscopically, the RF-treated foot pad had a dimple on the surface just after bRF application (Figure 1A). At 7 days postoperative day, the RF-treated foot pad had a dimple on the surface, and the dimple had a smooth surface ( Figure 1A). A, left middle foot pads at 14 days after bRF treatment; notice the two dimples on the surface (arrows). B, left middle foot pads at 14 days after bRF treatment. C, right (contralateral) middle foot pads at 14 days after no bRF treatment. No differences between Figure 1B and 1C are seen.
days we noted a scar, but at 14 days no macroscopic difference was detected between the bRF foot and the contralateral control foot (Figure 1B, 1C). The rats were observed ambulating with a normal gait immediately after the bRF applications. The rats of the sham group had a little bleeding after the incision; however, there was no difference macroscopically between the sham foot and contralateral control sham foot at 7 and 14 days.

Figure 2 shows the nerve fibers in the epidermis of the foot pad (the area in which the bRF was applied), with immunoreactivity of the TUJ1 in the control sample (Figure 2A) and contralateral control sample (Figure 2B). The control and contralateral control epidermis are richly innervated by TUJ1-IR nerve fibers in both samples described above. There is no statistical difference between the control and contralateral control samples on the
number of nerve fibers identified by the antibodies of both TUJ1 (mean number of nerve fibers ± SD, 172 ± 5.7 vs 177 ± 10.2) and CGRP (87 ± 1.4 vs 89.2 ± 18.2, P > .05). The sham group showed no significant difference between contralateral control of the sham rats and sham with respect to the number of nerve fibers identified for TUJ1 (mean number of nerve fibers ± SD, at 7 days 170.3 ± 15.6 vs 167.0 ± 18.6, P = .73; at 14 days, 180.5 ± 4.7 vs 174.0 ± 18.8, P = .49) (Figure 2C) or CGRP (at 7 days, 86.4 ± 10.7 vs 88.1 ± 9.5, P = .74; at 14 days, 91.9 ± 12.8 vs 92.9 ± 15.3, P = .87) (Figure 2D). Fibers in the epidermis of the foot pad (where the needle was applied) for the TUJ1 antibody and CGRP antibody are shown in Figure 2E and 2F.

After bRF application, nerve fibers were rarely seen in the epidermis at day 7 (Figure 3A) and day 14 (Figure 3B). There was a significant difference between contralateral control and bRF group on the numbers of TUJ1-IR-stained nerve fibers (mean number of nerve fibers ± SD) at day 7 (177 ± 5.7 vs 78.2 ± 15.8, P = .001) and at day 14 (174.8 ± 10.7 vs 75.3 ± 21.7, P = .0002) (Figure 4).

The reaction of CGRP-IR nerve fibers after RF application was similar to that of TUJ1-IR nerve fibers. Figure 5 demonstrates the immunofluorescent micrographs showing CGRP-IR nerve fibers (white arrows) innervating the middle foot pad of the rat. Figure 5A shows the few nerve fibers innervating the epidermis at 7 days after bRF treatment. Scar tissue, however, was observed in the keratinous layer at 7 days (open arrow). Figure 5B shows very few nerve fibers at day 14, but does show normalization of the keratin layer (open arrow). There were significantly more CGRP-IR nerve fibers present in the contralateral control epidermis when compared with bRF-treated nerve fibers at day 7 (89 ± 18.2 vs 39 ± 12.2, P = .002) and at day 14 (113 ± 12.8 vs 53.5 ± 10.8, P = .002) (Figure 6).

DISCUSSION

After conservative treatments fail, several surgical options are available for chronic tendinopathy. Specifically for
lateral epicondylitis, surgeries include excision of diseased tissue, repair of involved tendons, and decortication of the lateral epicondyle by way of open, percutaneous, and arthroscopic means.²³ Open debridement of the degenerative tissue in the tendon and repair relieved pain for 2.6 months postoperatively.¹³ Six weeks after open lateral release of the common extensor origin, 40% of patients still showed moderate or severe pain.²² Percutaneous lateral release of the common extensor origin afforded pain relief an average 9 weeks after surgery,⁵ and 93% of patients experienced the pain relief as early as 2 weeks after arthroscopic capsular release.¹⁴ Novel bRF techniques have shown significant pain relief within the first 2 weeks.²¹

TUJ1 antibody is characterized and highly reactive to neuron specific class III β-tubulin, making it suitable for the anatomical marker on peripheral and central neurons.⁴ In this study we demonstrated that bRF applications significantly decrease the number of TUJ1-IR nerve fibers in the early postoperative period. This decrease in number of nerve fibers was also found after ESWT to the rat sole.¹⁴,¹⁸,¹⁹ Ablation of these nerve fibers probably plays a role in the early decrease in pain after bRF applications. CGRP-containing axons mediate neurogenic inflammation, inducing tissue edema.²⁴ The decrease in immunoreactivity for CGRP-IR nerve fibers, or loss of these nerve fibers, may relieve pain by lowering the potential for local edema and microvascular leakage.

The lack of an animal model for tendinosis makes the scientific evaluation of bRF difficult. Although biopsied tendon from patients with tendinosis showed pathologic nerve fibers, healthy tendon tissue has too few nerve fibers to assess the degenerative effect of bRF treatment. We analyzed the abundant nerve fibers in the rat sole for a response to bRF. This model has proven useful for similar investigations with ESWT.¹⁸,¹⁹ We assume that the effects of bRF would be the same for normal epidermal nerves and the pathologic nerves found in tendinosis. The immediate pain relief seen in the clinical setting correlates with the degeneration of pathologic nerve fibers.

We chose to follow the experimental animals for 7 and 14 days to try to explain our acute and early clinical findings. Further study is necessary and ongoing to determine the long-term effects that bRF has on nerve fibers and if they possibly recover or regenerate. A similar study has shown regeneration of nerve endings for a couple of weeks after ESWT.¹⁴ Anecdotally, we have not seen recurrence of painful pathologic tendinosis in the clinical setting.

We concluded that bRF treatment induced the degeneration of the nerve fibers in the rat sole. These results may explain the pain relief in acute phase after RF treatment in tendinosis.

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