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Nerve Regeneration After Radiofrequency Application

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Background: Many patients with chronic tendinosis have experienced early pain relief after application of bipolar radiofrequency treatment. It is hypothesized that the mechanism of action may be the acute degeneration and/or ablation of sensory nerve fibers.

Hypothesis: After ablation or degeneration by bipolar radiofrequency, nerve fibers will have the ability to regenerate with time.

Study Design: Controlled laboratory study.

Methods: Eighteen Sprague-Dawley rats were used in this study. These rats were divided into 3 groups (30, 60, and 90 days after bipolar radiofrequency). These rats were treated with 2 points of bipolar radiofrequency applications to the left hindpaw with the Topaz microdebrider device. Right hindpaws were used as the contralateral control. Tissues were processed for neural class III β-tubulin or calcitonin gene-related peptide immunohistochemistry by using the free-floating avidin biotin complex technique. The numbers of neural class III β-tubulin-immunoreactive and calcitonin gene-related peptide-immunoreactive nerve fibers in the epidermis were counted and compared with those in the contralateral control.

Results: Although the numbers of nerve fibers demonstrated by both the antibodies of neural class III β-tubulin and calcitonin gene-related peptide were significantly decreased (P < .0001) until 60 days after bipolar radiofrequency treatment, regeneration of the epidermal nerve fibers occurred 90 days after treatment.

Conclusion: Bipolar radiofrequency treatment induced degeneration of sensory nerve fibers immediately after treatment, but by 90 days posttreatment, there was evidence of complete regeneration.

Clinical Relevance: Early degeneration followed by later regeneration of nerve fibers after bipolar radiofrequency treatment may explain long-term postoperative pain relief after microtenotomy for tendinosis.

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

Chronic tendinopathy, like medial and lateral epicondylitis and patellar and Achilles tendinopathy, is a well-known painful orthopaedic disease. The characteristic pathologic characteristics of all these tendinosis conditions are linked to microrupture of the tendon,4,5 granulation tissue,9 and degenerative changes.13,15 Nonsteroidal anti-inflammatory medication, physical therapy, steroid injections, and orthoses are symptomatic treatment for these and various other orthopaedic conditions, while arthroscopic or open surgery is reserved for patients with insufficient response to less invasive procedures.

Neovessels and accompanying free nerve endings have been observed in tendinosis tissue using Protein Gene Product 9.5 immunohistochemistry.2 Furthermore, the tendon insertion, which is supplied with substance P (SP) and calcitonin gene-related peptide (CGRP) innervation, has been implicated in the origins of tennis elbow.11 The rat model of Achilles tendinosis showed an increased number of nerve filaments and increased immunoreactivity for SP and CGRP.12 Neuropeptides SP and CGRP are not only involved in transmitting nociceptive information to the spinal cord, but also in peripheral effects, including microvascular leakage and local edema formation.21 Pathological nerve ingrowth may have been involved in the origin of tendinosis.11 On the
other hand, CGRP is important for formation of new vessels during wound healing. Furthermore, CGRP is vasoactive in normal rabbit medial collateral ligaments, suggesting that it may participate in the maintenance of ligament homeostasis and the promotion of soft tissue healing.

Recently extracorporeal shock wave therapy (ESWT)\(^{17}\) and bipolar radiofrequency (bRF) microtenotomy\(^{20}\) have been used for the treatment of chronic tendinosis. Clinically, the majority of patients with chronic lateral epicondylitis received at least some pain relief within the first 7 to 10 days, and it lasted at least 2 years after bRF microtenotomy. The pathomechanism of pain relief after ESWT\(^{15}\) and bRF microtenotomy\(^{18}\) is thought to be acute degeneration of epidermal nerve fibers in the early period. A previous study showed that bRF treatment induced acute degeneration or ablation of the sensory nerve fibers for at least 2 weeks.\(^{18}\) Although regeneration of the epidermal nerve fibers was observed 2 weeks after ESWT,\(^{14}\) regeneration after bRF is still unknown. We hypothesized that microtenotomy with bRF-induced degeneration of the nerve fibers would be followed by regeneration. Regeneration of nerve fibers would be necessary to resume normal tendon condition that leads to long-term effect by bRF, similar to the process of wound healing.\(^{19}\) The purpose of this study was to evaluate the regeneration potential of peripheral nerve fibers after bRF application using a rat model.

**MATERIALS AND METHODS**

Following Institutional Animal Care and Use Committee and our institution’s Animal Subjects Committee approval, we used 18 male Sprague-Dawley rats (weight, 250-300 g) divided into 3 groups (30, 60, and 90 days after bRF). They were anesthetized with isoflurane (Vedco Inc, St Joseph, Mo) and sodium pentobarbital (Abbott Labs, Chicago, Ill) and treated aseptically throughout bRF applications. Two separate points of bRF were equally applied (at a distance of 1-3 mm from each other for 500 ms) to the middle 2 footpads of the left hindpaw of the 18 rats. The TOPAZ Microdebrider device (ArthroCare, Sunnyvale, Calif) connected to a System 2000 generator at a setting of 4 V-RMS (voltage-root metered squared) 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). After treatment, the rats were allowed activity as tolerated.

Rats treated by bRF after 30 (n = 6), 60 (n = 6), and 90 days (n = 6) were anesthetized with ketamine (80 mg/kg intraperitoneal) and isoflurane and perfused transcardially with 0.9% saline, followed by 500 mL 4% paraformaldehyde in a phosphate buffer (0.1 M, pH 7.4). The footpads of both rat hindpaws were resected (left for study group, right for the contralateral control group). After being stored in 0.01 M phosphate buffer (0.1 M, pH 7.4), 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 class III β-tubulin (TUJ-1) for immunochemistry, and the other half were processed for CGRP immunohistochemistry\(^{1}\) by using the free-floating avidin biotin complex (ABC) technique. They were incubated for 20 hours at 4°C with mouse antibody for TUJ-1 (1:500; Convance, Berkshire, 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 observer in our laboratory (N.O.), who was blinded to the experimental group and time period of each sample, 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-immunoreactive (IR) and CGRP-IR fibers were counted for each section per 16.3 × 10-1 mm\(^2\) of epidermis in a footpad. After counting 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 each group.

Statistical analysis of results was performed using StatView 5.0 (Statsoft, Tokorozawa, Japan). Results were presented as mean ± standard deviation (SD) in each group. The mean numbers of nerve fibers between the bRF study groups were matched with their respective contralateral control group and were analyzed using a paired \(t\) test. The mean numbers of nerve fibers between the bRF study group on day 30, day 60, and day 90 were compared using a nonpaired Student \(t\) test. Significance was defined as \(P < .05\).

**RESULTS**

Although the RF-treated footpad had a dimple on the surface just after bRF application, no macroscopic difference was detected between the bRF foot and the contralateral control foot at 30, 60, and 90 days after bRF. The rats were observed ambulating with a normal gait immediately after the bRF applications.

Figure 1A shows the nerve fibers in the epidermis of the footpad (the area in which the bRF was applied), with immunoreactivity of the TUJ-1 in the contralateral control sample. The contralateral control epidermis is richly innervated by TUJ1-IR nerve fibers.

After bRF application, nerve fibers were rarely seen in the epidermis at day 30 (Figure 1B) and day 60 (Figure 1C). There was a significant difference between the contralateral control and bRF group in the numbers of TUJ1-IR–stained nerve fibers (mean number of nerve fibers ± SD) at day 30 (172.9 ± 20.4 vs 91.6 ± 20.5, \(P < .0001\)) and at day 60 (169.9 ± 15.2 vs 119.1 ± 20.5, \(P < .0001\)). There was no significant difference between the contralateral control and bRF group in the numbers of TUJ1-IR–stained nerve fibers at day 90 (169.2 ± 20.5 vs 164.7 ± 24.0, \(P = .6557\)) (Figure 1D; Figure 2).
The reaction of CGRP-IR nerve fibers after RF application was similar to that of TUJ1-IR nerve fibers. Figure 3A demonstrates the immunofluorescent micrographs showing CGRP-IR nerve fibers (white arrows) innervating the middle footpad of the contralateral control. Figure 3B shows few fibers seen in the epidermis at 30 days after bRF treatment. Figure 3C shows slightly more fibers seen in the epidermis than at 30 days. Figure 3D shows no differences between Figures 3A and 3D seen.

Figure 3. Immunofluorescent light micrographs showing CGRP-IR nerve fibers. (A) Micrograph showing nerve fibers innervating the epidermis of the contralateral control footpad (white arrows) shows the richly innervated left middle footpad of the rat. (B) Left middle footpads at 30 days after bRF treatment. A micrograph shows few nerve fibers in the epidermis. (C) Left middle footpads at 60 days after bRF treatment. A micrograph shows slightly more nerve fibers in the epidermis than at 30 days. (D) Left middle footpads at 90 days after bRF treatment. No differences between Figures 3A and 3D are seen.

The reaction of CGRP-IR nerve fibers after RF application was similar to that of TUJ1-IR nerve fibers. Figure 3A demonstrates the immunofluorescent micrographs showing...

**DISCUSSION**

In the present study, we demonstrated that regeneration of the epidermal nerve fibers occurred gradually from 30 days to 90 days. Finally it became obvious that there was no statistical difference in the number of nerve fibers with...
dons, leading to healing of the tendon.\textsuperscript{20} Although the
genic markers demonstrated that bRF microtenotomy can
evidence of neovessel formation and an increase in angio-
ations significantly decrease the number of TUJ1-IR nerve

tions and to confirm the expected same mechanism.

We concluded that bRF treatment induced degeneration and
regeneration of the nerve fibers in the rat sole. These
results may explain the rapid pain relief as well as one of
the healing responses after bRF treatment in tendinosis.

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immunoreactivity for CGRP-IR nerve fibers may result in
changing the hypovascularity condition such as occurs in
tendinosis to be healed with the increase responses of the
vascularity. Although this study did not clarify the confu-
sion in the literature about the vascularity,\textsuperscript{2,16} we demon-
strated that the CGRP-IR nerve fibers regenerate by 90
days and resumed normal appearance. We postulated that
the regeneration of the CGRP-IR nerve fibers accompanied
by the increase of vascularity is important for the healing
process in tendinosis that may in turn be partially caused by
hypovascularity.

Clinically, the majority of patients with chronic lateral epi-
condylitis received at least some pain relief within the first 7
to 10 days that lasted at least 2 years after bRF microteno-
tomy.\textsuperscript{19} Although degeneration of sensory nerve fibers would
lead to early pain relief,\textsuperscript{18} our study explained that the nerve
fibers regenerated, along with the healing process, and with
potential of no return of the pathological condition.

One of the limitations of this study is using rat skin instead
of pathological tendon. There are no suitable animal models
for assessing effectiveness of bRF treatment of tendinosis. We
analyzed the abundant nerve fibers in the rat sole for a
response to bRF. Extracorporeal shock wave therapy study has
already showed the validity of using this model.\textsuperscript{14} We
assume that the effects of bRF would be the same for normal
epidermal nerves and the pathologic nerves found in tendi-
nosis. The long-lasting effectiveness of bRF in clinical cases\textsuperscript{19}
would correlate with the regeneration of the nerve fibers the
same as in the contralateral control. Further study is neces-
sary to determine the effects of bRF on pathological condi-
tions and to confirm the expected same mechanism.

We concluded that bRF treatment induced degeneration and
regeneration of the nerve fibers in the rat sole. These
results may explain the rapid pain relief as well as one of
the healing responses after bRF treatment in tendinosis.

Figure 4. Line graph showing the mean value ± standard devi-
ation of CGRP-IR nerve fibers at 7, 14, 30, 60, and 90 days after
bRF treatment. Although there were significant differences in
the number of nerve fibers between the contralateral group and
bRF-treated group at 30 and 60 days (P < .0001), no significant
differences were observed between the contralateral group and
90 days post-bRF (P = .9331). There was significant difference in
the number of nerve fibers among day 30, day 60, and day
90 time periods (day 30 vs day 60, P = .0013; day 30 vs day 90,
P < .0001; day 60 vs day 90, P < .0001). *P < .05. The values of
7 and 14 days were previously described.\textsuperscript{18}

Neovascularity has been proposed to be one of the fac-
tors in the origin of tendinosis. Hypovascularity induces
not only necrosis and fibrillation in the tendon\textsuperscript{1} but also
induces the rupture of the tendon. Lack of vascularity com-
promises the nutrition required by tendon cells, making it
more difficult for those cells to synthesize extracellular
matrix necessary for repair and remodeling for fatigue-
damaged tendon.\textsuperscript{20} Regarding the effect of bRF, histologic
evidence of neovessel formation and an increase in angi-
genic markers demonstrated that bRF microtenotomy can
increase tissue vascularity when properly applied to ten-
dons, leading to healing of the tendon.\textsuperscript{20} Although the
results revealed macroscopic and microscopic changes in
the early term, bRF-treated tendons resumed a normal
appearance by 90 days.\textsuperscript{20}

Neural class III β-tubulin antibody, which recognizes class
III β-tubulin exclusively, reveals the axons and their
terminations, as well as significant injury-related alter-
ations.\textsuperscript{3} In this study, we demonstrated that bRF applica-
tions significantly decrease the number of TUJ1-IR nerve
fibers by 60 days, which gradually increase by 90 days.
This increase after decrease in numbers of nerve fibers was
also found after ESWT to the rat skin but resumed rapidly
at 2 weeks.\textsuperscript{14}

Calcitonin gene-related peptide has previously been
reported also to promote angiogenesis and tissue regenera-
tion,\textsuperscript{3,10} partly by stimulating proliferation of endothelial
cells and fibroblasts.\textsuperscript{3,10} The effect of CGRP on endothelial
cells has a potential effect in angiogenesis, including for-
mation of new vessels in wound healing.\textsuperscript{10} The increase in

the contralateral control groups. Regeneration of TUJ1-IR
and CGRP-IR nerve fibers seemed to be similar to each other.

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![Graph showing the mean value ± standard deviation of CGRP-IR nerve fibers at 7, 14, 30, 60, and 90 days after bRF treatment.](https://example.com/image.png)

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