Randomized Trial

Evaluation of the Effect of Duration on the Efficacy of Pulsed Radiofrequency in an Animal Model of Neuropathic Pain

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Background: Pulsed radiofrequency (PRF) is increasingly used in clinical practice, especially in neuropathic pain disorders. Although PRF is not new to clinical use, there are significant gaps in knowledge regarding its effectiveness.

Objectives: The current study was conducted to evaluate the effect of duration of application of PRF on its analgesic efficacy in improvement of neuropathic pain. Study Design: A randomized experimental trial.

Setting: An animal research facility at the College of Veterinary Medicine at Mansoura University in Egypt.

Methods: Chronic constriction of the sciatic nerve of 36 male Sprague-Dawley rats was performed to induce neuropathic pain. The rats were divided into 6 groups (6 rats each) in which PRF was applied for 2, 4, 6, or 8 minutes or not at all. In one group, RF cannula was applied without performing PRF intervention. The pain was assessed through observation of resting paw posture (RPP) at 3, 10, and 21 days. Nerve damage was assessed by histopathological evaluation of the sciatic nerve. Immunohistochemical localization of proinflammatory cytokines (interleukin 6 [IL-6] and tumor necrosis factor alpha [TNF-α]) was also done.

Results: RPP was improved in rats treated with PRF. This improvement was significant only in rats treated for 8 minutes. Increased duration for PRF application was associated with a significant decrease in IL-6 and TNF-α contents in all groups when compared with the control group. Histopathological evaluation of the constricted sciatic nerve revealed no statistical significance among the different study groups.

Limitations: The study was limited by the lack of measurement of other inflammatory markers that may help elucidate other relevant mechanisms.

Conclusions: Increased duration of PRF application resulted in better analgesic efficacy without any increase in tissue injury in an animal model of neuropathic pain. This effect may be attributed to decreased production of pro-inflammatory cytokines.

Key words: Pulsed radiofrequency, analgesic, rats, sciatic nerve, duration, neuropathic pain

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Neuropathic pain results from a lesion of the somatosensory system. It is mostly caused by lesions or diseases (such as diabetes, herpes, or trauma) in the peripheral nervous system (1). In spite of multiple pathophysiologies, the clinical manifestations of neuropathic pain remain relatively consistent.

Most of our knowledge of the pathophysiology of neuropathic pain is based on experimental stud-

ies using animal models of peripheral nerve injury. These studies confirmed the role of pro-inflammatory cytokines in the pathogenesis of neuropathic pain. Anticytokine agents are effective at treating inflammation and pain (2). Recent studies have improved our understanding of the pathogenesis of neuropathic pain and paved the road for the introduction of new therapeutic options for physicians, although curative treatment is still one of the challenges (3).

Pulsed radiofrequency (PRF) is a procedure in which intermittent application of high-voltage current is interrupted by "resting" periods that allows heat dissipation. This modification from the conventional (continuous) radiofrequency (CRF) maintains tissue temperature below 42° C and prevents possible tissue destruction (4). Podhajsky and colleagues (5) observed transient endoneurial edema caused by PRF compared to Wallerian degeneration caused by application of CRF.

Since PRF does not heat the tissue until thermocoagulation occurs, it has been suggested that PRF induces changes in the nerve cells through its electric field (6). PRF induces rapidly changing electric fields that may change the expression of the c-fos gene and thus interfere with pain transmission. Van Zundert et al (7) demonstrated that this PRF effect on c-fos expression lasts for 7 days after the application for 2 different durations (2 and 8 minutes), provided that the tissue heat is kept at 42° C. The prolonged expression of c-fos beyond that period was postulated to be the mechanism for long-term pain inhibition (8).

Clinically, PRF has been reported as a successful treatment of a variety of pain conditions, mostly in neuropathic pain (9). The use of PRF demonstrated few side effects compared to CRF, which was associated with a variety of limiting side effects such as neuritis-like reactions, motor blockade, and increased risk of deafferentation pain (10). These few side effects, transient neurological defects, plus the clinical pain-relieving efficacy in clinical practice increased its use in relieving neuropathic pain (11).

Technical factors which contribute to the diversity of the results of randomized controlled trials include the RF equipment to be used and the tip, length, and size of the needle to be used. Such factors resulted in different outcomes of PRF therapy trials (12).

Based on the previous knowledge, we hypothesized that the duration of application of PRF might affect the efficacy of its therapeutic use without increasing the tissue damage. This present study focused on the effect of duration of PRF exposure on the outcome of pain relief. Structural changes and local cytokines were measured in the affected nerve in an attempt to delineate the underlying mechanism of PRF-induced analgesia.

Methodss

Animals

Thirty-six male Sprague-Dawley rats (body weight 190–220 gm) were enrolled in this randomized, prospective experimental study. They were kept at a controlled temperature (20–24° C) under a light-dark cycle (12 hours light/12 hours dark) and were fed standard rat chow with free access to water for 2 weeks. The study was conducted at the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Mansoura University in Egypt. The study protocol was approved by the Medical Research Ethics Committee of Mansoura University, Egypt (code number R/113, 09/03/2015).

Sciatic Nerve Chronic Constriction Injury Model

Neuropathic pain was induced by chronic constriction injury (CCI) of the sciatic nerve as described by Austin et al (13). In summary, the rats were anesthetized by intraperitoneal injection of a combination of ketamine hydrochloride (50 mg/kg) and diazepam (5 mg/kg). A blunt dissection (3 mm long) was made in the skin of the right hind limb. The common sciatic nerve was exposed and freed. Three loose ligatures were tied with a double knot, 1 mm apart (Chromic Gut 4.0, Ethicon Inc., Somerville, NJ). The ligatures were then tightened until the muscles of the hind limb briefly twitched. The incision was closed. Ceftriaxone was injected subcutaneously once daily (3 days after the surgery) in a dose of 500 mg/kg.

Study Groups

The rats were divided into the following groups: group I (n = 6): CCI of the sciatic nerve was done with no PRF intervention; group II (n = 30): CCI of the sciatic nerve was done and then the rats were kept for 10 days. Group II was then subdivided into 5 subgroups (6 rats in each subgroup); group IIc: RF cannula was applied for 8 minutes without PRF intervention, group II_2: PRF was applied for 2 minutes, group II_4: PRF was applied for 4 minutes, group II_6: PRF was applied for 6 minutes, and group II_8: PRF was applied for 8 minutes. The rats of all groups II were kept for 21 days in individual cages where they were observed for behavioral tests then sacrificed for collection of tissue samples.

Application of PRF

The rats used in the CCI model were assigned to group II (n = 24). On the tenth postoperative day, a NeuroTherm NT500 Generator (Abbott St. Jude, Dubai, UAE) was used to apply PRF, with the maximum temperature of 42° C, through a radiofrequency cannula straight 22 gauge, 10 cm long, with 2 mm active-tip (SMK-S1002-22 (Abbott St. Jude, Dubai, UAE). The cannula was applied 1 mm proximal to the ligatures, and 1–2 mm was left between it and the exposed sciatic nerve to avoid direct mechanical stimulation (incision of the first surgery was reopened and a small portion of the nerve was exposed then the incision is sutured again). The RF cannula was applied for 2, 4, 6, or 8 minutes according to the assigned subgroup using a pulse duration of 20 ms (500 kHz) and a pulse rate of 2 Hz.

Behavioral Tests (Assessments of Resting Paw Posture) (14)

Observation of the resting paw posture (RPP) of the operated hind paw of the rats began on the tenth day after induction of chronic constriction injury (CCI) just before PRF technique. Then, it continued at days 0, 3, 10, and 21 following PRF technique. The assessment was carried out on the assigned day from 8:00 to 10:00 AM by an independent investigator. At recording, the animal was placed in a cage and allowed to accommodate for 15 minutes. Different positions of the rat hind paw were recorded for 2 minutes (120 seconds). As detailed by Farghaly et al (14) in 2014, the paw positions were plotted on a 5-point scale starting from 0 which represents the normal position of the hindlimb (no pain). Pain will force the limb to take abnormal positions starting by light pressing on the floor with ventroflexed toes (score one); only internal edge of hindlimb pressing on the floor (score 2); only heel pressing on the floor (score 3); the whole limb is elevated (score 4); animal licks hind paw, which represents severe pain (score 5).

Histopathological Evaluation of Sciatic Nerve (15)

After the rats were sacrificed (21 days after the onset of PRF application), about 3 cm of the sciatic nerve (distal to the site of ligation) was removed. The isolated sciatic nerve tissues were stored in 10% formaldehyde solution for 48 hours then embedded in paraffin and sliced with a microtome into 5µm sections for histopathological analyses. Hematoxylin and eosin staining was performed and the samples were evaluated under the 10 × 40 light microscopic fields by an expert pathologist blinded to the study groups. Histopathological changes were scored according to the following scale: 0 = no inflammation, 1 = small focal mild edema, 2 = moderate edema and inflammation, and 3 = extensive edema and marked inflammation.

Immunohistochemical Analysis (16)

Immunohistochemical analysis for tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) was done to assess the effect of PRF on the inflammatory cytokines. Paraformaldehyde-fixed paraffin-embedded sciatic nerve sections (5 mm thickness) were incubated with antibodies to TNF-α (MP6-XT22, Novus Biologicals, Littleton, CO) and IL-6 (20F3, Thermo Fisher Scientific, Waltham, MA). The IL-6 and TNF-α immune-reactive cells were stained using 3,3′-diaminobenzidine (DAB) for cytokines localization. The cells were using a microscope connected to the camera where images were captured. The percentage of IL-6 and TNF-α positive nuclei was counted in at least 10 fields at magnification 200× per sample by 2 blinded independent observers.

Statistical Analysis

Parametric data (number of fibers immunopositive for IL-6 and TNF-α) were presented as mean ± standard deviation, and analysis of variance (ANOVA) was used to compare between more than 2 groups of parametric data followed by post-hoc Tukey for multiple comparisons when $P \leq 0.05$. Nonparametric data (behavioral tests and histopathological evaluation) were reported as median, and interquartile range and Kruskal-Wallis test were used to compare between more than 2 groups of numerical (nonparametric) data followed by pairwise comparisons when $P \leq 0.05$. Statistical analysis was performed using SPSS Version 18.0 (SPSS Inc., Chicago, IL).

Results

RPP

Since the observation of the posture started before application of PRF in the assigned groups, basal levels of the posture in all groups show the hind paw in an inverted position and only heel pressed to floor (Table 1). There was no statistically significant difference among the groups. Three days later, the posture of the hind limb (pain score) started to improve in the groups treated with PRF. However, this improvement that indicates partial pain relief was statistically significant only in group II_8 when compared with the non-treated rats (group

Table 1. *Median and interquartile range of RPP behavioral test of CCI rats 10 days after induction of CCI on days 0, 3, 10, and 21 after PRF application. PRF was not applied (group I) or RF cannula was applied for 8 minutes with no PRF technique (group IIc); PRF was applied for 2 minutes (group II_2), 4 minutes (group II_4), 6 minutes (group II_6), or 8 minutes (group II_8); comparison in between the groups.*

		Group I	Group IIc	Group II_2	Group II_4	Group II_6	Group II_8	*P*-Value
Day 0	Median	3.0	3.0	3.0	3.0	3.0	3.0	----
	IQR	3.0–3.0	3.0–3.0	3.0–3.0	3.0–3.0	2.0–3.0	3.0–3.0	
Day 3	Median	3.0	3.0	1.0	1.0	2.0	1.0 axd	0.002
	IQR	2.0–3.0	2.0–3.0	0.0–2.0	1.0–3.0	1.0–3.0	0.0–1.0	
Day 11	Median	2.0	2.0	2.0	1.0	1.0	0.0 b	0.016
	IQR	0.0–2.0	0.0–2.0	2.0–3.0	0.0–1.0	0.0–3.0	0.0–1.0	
Day 21	Median	2.0	2.0	1.0	0.0	1.0	1.0	-----
	IQR	2.0–2.0	2.0–2.0	1.0–3.0	0.0–2.0	0.0–2.0	0.0–2.0	

Test used: Kruskal Wallis test followed by pairwise comparisons.
a = Significant when compared with group I.
x = Significant when compared with group IIc.
b = Significant when compared with group II_2.
c = Significant when compared with group II_4.
d = Significant when compared with group II_6.
IQR = interquartile range

Table 2. *Median and interquartile range of RPP behavioral test of CCI rats 10 days after induction of CCI on days 0, 3, 10, and 21 after PRF application. PRF was not applied (group I) or RF cannula was applied for 8 minutes with no PRF technique (group IIc); PRF was applied for 2 minutes (group II2), 4 minutes (group II4), 6 minutes (group II6), or 8 minutes (group II8); comparison within the same group.*

	Day 0		Day 3		Day 11		Day 21		*P*-Value
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	
Group I	3	3.0–3.0	3	2.0–3.0	2	0.0–2.0	2	2.0–2.0	----
Group IIc	3	3.0–3.0	3	2.0–3.0	2	0.0–2.0	2	2.0–2.0	----
Group II_2	3	3.0–3.0	1 a	0.0–2.0	2	2.0–3.0	1	1.0–3.0	0.01
Group II_4	3	3.0–3.0	1	1.0–3.0	1 a	0.0–1.0	0	0.0–2.0	0.005
Group II_6	3	2.0–3.0	2	1.0–3.0	1	0.0–3.0	1	0.0–2.0	----
Group II_8	3	3.0–3.0	1 a	0.0–1.0	0 a	0.0–1.0	1	0.0–2.0	0.001

Test used: Friedman test followed by pairwise comparisons.
a = Significant when compared with day 0 in different groups.
b = Significant when compared with day 3 in different groups.
c = Significant when compared with day 11 in different groups.
IQR = interquartile range

I) and rats treated for 6 minutes (group II_6) ($P = 0.002$). No statistical significance was detected among other groups. Eleven days after PRF application, improvement of pain scale (RPP) continued in all of the groups. The rats in group II8 showed significant improvement when compared with those in group II_2 ($P = 0.016$).

When followed by the groups (Table 2), RPP (pain score) improved in all of the groups by time. This improvement was significant in group II_2 on day 3, group II_4 on day 11, and group II_8 on days 3 and 11 ($P = 0.01$, 0.005, and 0.001, respectively). The changes in group II_6 were not significant at any of the time intervals.

Immunohistochemical Localization of IL-6 and TNF-α in the Fibers of the Sciatic Nerve

Table 3 shows that IL-6 immunopositive neurons were decreasing as a function of time. Increased duration for PRF application is associated with decreased IL-6 content, which was significant when compared with all groups with less duration. Accordingly, the least IL-6 level was detected in group II_8, which was

Table 3. *Mean and standard deviation of nerve fibers immunopositive for IL-6 and TNF-α of CCI rats after scarification. PRF was not applied (group I) or RF cannula was applied for 8 minutes with no PRF technique (group IIc); PRF was applied for 2 minutes (group II_2), 4 minutes (group II_4), 6 minutes (group II_6), or 8 minutes (group II_8); comparison in between the groups.*

	Group I	Group IIc	Group II_2	Group II_4	Group II_6	Group II_8	*P*-Value
IL-6	126.14 ± 11.17	124.36 ± 9.6	90.71 ± 3.95 ^{ax}	58.86 ± 6.01 ^{axb}	32.43 ± 4.50 ^{axbc}	19.00 ± 2.16 ^{axbcd}	< 0.0001
TNF-α	59.29 ± 2.50	57.68 ± 1.8	29.57 ± 3.10 ^{ax}	8.29 ± 2.56 ^{axb}	25.00 ± 2.16 ^{axbc}	4.86 ± 2.41 ^{axbd}	< 0.0001

Test used: ANOVA test followed by post-hoc Tukey.
a = Significant when compared with group I.
x = Significant when compared with group IIc.
b = Significant when compared with group II2.
c = Significant when compared with group II4.
d = Significant when compared with group II6.

Table 4. *Median and interquartile range of histopathological score of sciatic nerve fibers of CCI rats after scarification. PRF was not applied (group I) or RF cannula was applied for 8 minutes with no PRF technique (group IIc); PRF was applied for 2 minutes (group II_2), 4 minutes (group II_4), 6 minutes (group II_6), or 8 minutes (group II_8); comparison in between the groups.*

		Group I	Group IIc	Group II_2	Group II_4	Group II_6	Group II_8	*P*-Value
HE Score	Median	3.00	3.00	1.00	2.00	1.00	1.00	0.2178
	Percentile 25	2.00	2.00	1.00	1.00	0.50	1.00	
	Percentile 75	3.00	3.00	2.50	2.50	2.50	2.00	

Test used: Kruskal Wallis test.

significant when compared with all other groups ($P < 0.0001$). A similar change was detected when TNF-α was immunohistochemically localized. One slight difference was that the comparison of TNF- α immunopositive neurons between group II_8 and group II_4 was insignificant.

Histopathological Evaluation of Sciatic Nerve

The median histopathological score of the non-treated rats (group I) was 3, indicating extensive edema and marked inflammation. Lower values were observed after PRF in all other groups, but nerves were still in damage. However, no statistical difference was observed among the different groups.

Discussion

Efficacy and safety of PRF require more clinical evidence. This research was conducted to study the effect of duration of PRF application on the efficacy in the alleviation of pain as indicated by the changes in the RPP. The less the posture score was, the more efficacious PRF therapy was. Intraneuronal cytokines (IL-6 and TNF-α) were measured to settle a link to the possible pathophysiology of neuropathic pain.

We hypothesized that increasing the duration of application of PRF will result in more effective pain relief without significant structural damage, and this may be due to the reduction of the level of inflammatory mediators.

The results of our study support our hypothesis. The baseline levels of RPP were high and nearly the same at the beginning of the experiment for all groups. The PRF-treated groups showed improved pain scores. Increased duration of application of PRF improved the pain scale score more (rats treated with PRF for 8 minutes when compared with those treated for 6 minutes and 2 minutes, 3 days and 11 days after treatment, respectively).

This is the first trial comparing the analgesic effectiveness of various durations of PRF. Most studies have applied PRF for 120 seconds (17). Some authors applied PRF for a longer duration. In 2008, Akkoc et al (18) reported the successful application of RPF at 42° C for 240 seconds in a case of regional pain syndrome following spinal disc herniation surgery. In a recent study, Thapa et al (19) reported good response when 4 cycles of PRF (42° C, each lasting 120 seconds, i.e., for a total of 8 minutes) were applied on the mandibular root of the trigeminal nerve in a resistant case of right facial pain. All of these studies were randomized clinical trials with no comparisons between different durations.

Our results show that CCI increased intraneuronal IL-6, which decreased significantly by PRF application.

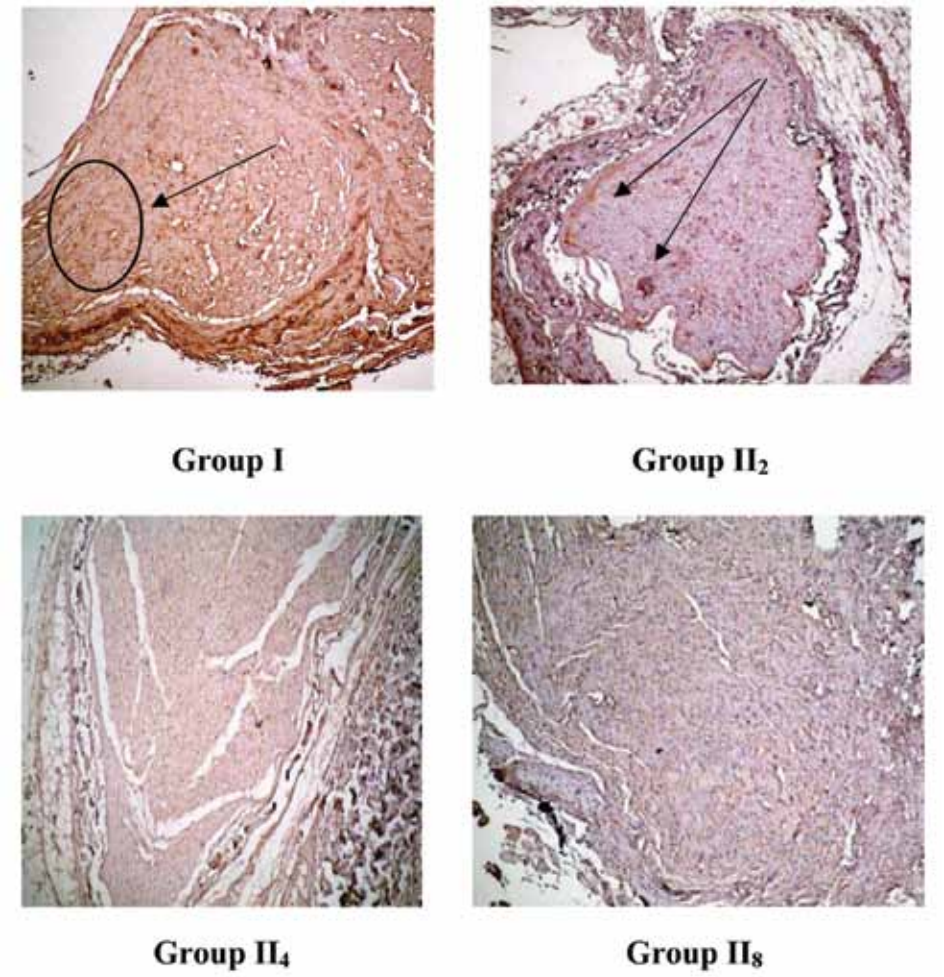

Fig. 1. *Immunopositive nerve fibers for TNF-α of CCI rats after scarification.*

This reduction in IL-6 was more pronounced with the duration of application. Rats treated with PRF for 8 minutes showed the lowest IL-6, which was significant when compared with all of the other groups (Fig. 1). On the other hand, TNF-α showed similar change, except for in group II_6. These results were inconsistent with those of most authors. Vallejo et al (20) showed that 4 genes were modulated upon spared nerve injury (SNI) induction, in which there was significant up-regulation of TNF-α ($P = 0.006$) and IL-6 ($P = 0.013$). Following SNI lesions, PRF treatment significantly down-regulated TNF-α ($P = 0.032$) and IL-6 ($P = 0.035$), with gene expression levels returning down to control levels.

The unexpected persistence of pain and increase of TNF-α in group II_6 may be explained by the simultaneous occurrence of another condition that increased this cytokine. Bradley (21) discussed the causes of increased TNF-α. A possible cause in our case is infection; particularly, rats of the same group were experimented on the same day. Although increased, the TNF-α level in group II6 was still significantly below the control level.

These results demonstrate that PRF can reduce the expression of pro-inflammatory cytokines in adjacent tissues either directly or indirectly. As TNF-α is one of the major players in the development and maintenance of neuropathic pain, the decreased expression of TNF-α, and other cytokines modulated by it, indicates that PRF potentially alleviates neuropathic pain states by attenuating neuroinflammation at the molecular level (22).

Our results show that longer durations of PRF caused more damage to the sciatic nerve fibers, which was insignificant when compared with other groups as calculated by median and range histopathological scores. This loss may be explained by the assumption that tissue changes may be caused by different mechanisms other than increased cytokines or that PRF of longer durations caused tissue damage or both. Lindenlauba and colleagues (23) found that application of anti-TNF-α antibodies in an animal model of CCI improved the pain as exhibited by behavioral tests but with no effect on nerve regeneration. Cosman Jr. and Cosman Sr. (24) demonstrated the occurrence of heat spikes around the PRF needle, which was dependent on tissue impedance and the pulse width. Their research did not extend to investigate ablative effects of these heat spikes. Edrine and colleagues (25) illustrated microscopic damage in cell membranes, mitochondria, and microtubules of the PRF-treated nerves. Finally, CCI-induced pain is a chronic neuropathic pain that may need extra time given to the nerve to record regeneration. Thus, the study was limited by the short duration of recording signs.

This study was also limited by the small number of rats in the experimental group, the short duration of observation, the lack of performing other behavioral tests to test pain sensation, and the lack of measurement of other inflammatory or anti-inflammatory markers (e.g., IL-10). The use of an animal model was a step for extrapolation of the results on humans. Future molecular studies are recommended before such action occurs.

Conclusion

In conclusion, increased duration of PRF application resulted in better analgesic efficacy without significant increase in tissue damage in an animal model of neuropathic pain. This effect may be attributed to decreased production of pro-inflammatory cytokines.

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