Topical pregabalin and diclofenac for the treatment of neuropathic orofacial pain in rats

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Objective. The aim of this study was to evaluate the effect of topical treatment with pregabalin and diclofenac on neuropathic orofacial pain induced by infraorbital nerve injury in the rat.

Study Design. Sixty-four Sprague-Dawley rats underwent infraorbital nerve injury. Seven days after surgery, pain was verified and the rats randomly assigned to topical or systemic treatment with pregabalin or diclofenac, or to no treatment. Pain intensity and motor coordination were assessed at baseline, after surgery, and daily after treatment for 4 consecutive days. Medication plasma levels were assessed at the end of the study.

Results. Topical treatment with 10% pregabalin or 5% diclofenac reduced the pain significantly. A significant decrease in motor coordination was found in the systemic pregabalin. The medications’ plasma levels were significantly higher in the systemic treatment compared with the topical.


Neuropathic pain is defined as pain that arises from injury, disease, or dysfunction of the peripheral or central nervous system.1, 2 This condition affects 2%–3% of the general population.3, 4 Neuropathic pain can impair the quality of life and affect activities of daily living. It can have physiologic implications, affect mood, reduce occupational performance, and generate a major health care costs.5, 6 In the orofacial region, pain can result from neuronal injury associated with facial trauma, fractures, inflammation related to injury or infection, systemic metabolic disturbances, neoplasia, or even trauma secondary to various dental procedures, such as endodontic therapy.7

Pharmacologic therapy is the first line of treatment for neuropathic pain. Medication categories include mainly antidepressants and antiepileptics. The efficacy of these medications varies from patient to patient depending on factors such as location of the pain, age of the patient, and/or any comorbid systemic disease.8–10 Systemic pharmacotherapy is often accompanied by unpleasant side effects, including sedation, dizziness, and weight gain. Medications used for neuropathic pain may also be contraindicated for the medically compromised or elderly patient.11 Topical application of medications constitutes a unique method of local drug delivery that is less likely to induce systemic side effects or interact with other medications. Topically applied medications are believed to treat mainly a specific peripheral target with minimal systemic effect.12–15 Local application should require simpler dose titration processes compared with the complicated titration commonly necessary for systemic therapies.16

In the present study, the effect of topical pregabalin and diclofenac was evaluated in a rat model of neuropathic orofacial pain (NOP). Pregabalin is a medication commonly used to manage neuropathic pain. It is an anticonvulsant drug also used as adjunctive therapy for seizures and which has also been found to be effective for generalized anxiety disorders.16 Recent reports have also shown that pregabalin is effective in treating chronic pain associated with fibromyalgia.17 Pregabalin has a reduced potential for abuse and a limited dependence liability if misused. Side effects include dizziness, drowsiness, visual disturbances, ataxia, lethargy, and memory impairment. Pregabalin binds to the α2δ subunits of calcium channels within the central nervous system, reducing calcium influx into the nerve termi-
Pregabalin is a nonsteroidal antiinflammatory drug (NSAID). Its indication is to reduce inflammation; it has analgesic (mainly for musculoskeletal and postoperative pain) and antipyretic utilities. The exact mechanism of action is not entirely understood; however, as a nonsteroidal cyclooxygenase inhibitor, it prevents the synthesis of prostaglandin. There is some evidence that diclofenac may have an effect on lipoxygenase (reducing leukotriene formation) as well as phospholipase A2 function. Its major side effect is the reduced activity of goblet cells lining the stomach, diminishing their protective effect on the stomach lining and predisposing the patient for the development of ulcerations caused by gastric acid and for gastrointestinal bleeding.14,16

MATERIALS AND METHODS

All procedures and experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Medicine and Dentistry of New Jersey (UMDNJ; no. 09084E1212).

Sixty-four adult male Sprague-Dawley rats, weighing 200-250 g at the time of surgery, were used for this study. All rats were ordered from a single lab and were housed in UMDNJ animal facility under veterinary supervision. The rats were fed with standard rodent chow and reverse-osmotically treated water ad libitum. They were maintained on a 12-hour day-night cycle.

After 3 days of habituation to the laboratory environment, baseline levels for pain-related behavior and motor coordination were measured. The baseline measurements were taken several times until the data were consistent and repeatable. Under general anesthesia with a solution of ketamine and xylazine administered intraperitoneally (IP), a left infraorbital nerve neuropathy was induced by chronic constriction injury. Seven days after surgery, the development of pain was verified with the use of von Frey filaments and a blunted acupuncture needle prick. Twenty percent or more difference from baseline in one of the tests indicated development of neuropathic pain.

The rats were then randomly assigned to one of the following groups (n = 8 for each group): no treatment, topical application of vehicle without medication, systemic (IP) administration of pregabalin (300 mg/kg), topical application of 5% pregabalin, topical application of 10% pregabalin, systemic (IP) administration of diclofenac (1 mg/kg), and topical application of 5% pregabalin and 5% diclofenac combination (Figure 1).

The concentration of the topical medication selected (10%) was the maximum that could be incorporated in the nonactive vehicle of the compound pluronic lecithin organogel and anhydrous gel base. The concentrations of the systemic medication were those proven to be effective in pain reduction in previous studies in our laboratory. Topical medications were applied to the infraorbital nerve territory in the vibrissae area (~1 cm²) for 1 minute 3 times per day (8:00 a.m., 12:00 noon, and 4:00 p.m.) with the use of a cotton tip applicator. Systemic medications were administered intraperitoneally once daily. The rats were treated for 4 consecutive days and tested daily for pain and motor coordination 120 minutes after the application of the first topical or the systemic administration. On the fourth day the rats were killed 4 hours after the final topical application or IP injection. Blood samples were collected with the use of cardiac puncture under terminal anesthesia before death to determine plasma levels of the medications.
Chronic constriction injury surgery (CCI)
After verification of the anesthesia (ketamine-xylazine rodent cocktail: 60 and 7.5 mg/kg respectively), the area of the face to be tested (the infraorbital nerve territory) was carefully shaved with special care not to touch or damage the rat’s vibrissae. The same area was tested later for the presence of neuropathic pain.

The area of surgery was antisepticized with povidone-iodine and an isopropyl alcohol wipe. After lubricating the rats’ eyes, the rats were placed on a warming pad to maintain a constant body temperature during surgery. A single thoroughly trained investigator performed all of the surgeries.

Infraorbital nerve chronic constriction injury was executed according to Imamura et al.19 An incision ∼1 cm long was made along the gingivobuccal margin. The incision began just proximal to the first molar. Approximately 0.5 cm of the infraorbital nerve was freed from adherent tissues, and 2 chromic gut ligatures were loosely tied around it (2 mm apart). The incision was closed at 3 points with the use of 4-0 silk suture.

Behavioral assays (pain assessment)
Two pain behavioral assays were performed: tactile allodynia and mechanical hyperalgesia.

Tactile allodynia was measured by the withdrawal response to calibrated von Frey fibers of varying thicknesses delivering calibrated amounts of force. A decrease in the detection threshold indicated an increase in pain.20 The von Frey series of filaments (North Coast Medical, Morgan Hill, CA) included monofilaments sorted by ranks expressing the log10 of the force applied (in mg) × 10. Filaments used were labeled from 2.36 to 4.31, applying a force of 0.02-2 g, respectively. Each fiber was calibrated daily on a weight scale to assure delivery of its labeled amount of force. The test was performed daily at the same time of the day by the same investigator, who was blinded to the treatment group.

Before sensory testing, the rats were habituated for 5 minutes in a small clear plastic cage. The operator’s hand entered the cage slowly with a von Frey filament. No restraints were used to test the rat’s face. The monofilaments were tested in the order of increasing stiffness; each was applied 5 times at intervals of 1-4 seconds to slightly different loci of the infraorbital nerve innervation. The first filament to evoke at least 1 withdrawal response was recorded and designated the tactile detection threshold; its labeled force was registered. A lower threshold of withdrawals indicated increasing tactile allodynia.

Mechanical hyperalgesia was assessed by measurement of the withdrawal reaction to a blunted acupuncture needle prick (draw score). An increase in the withdrawal activity indicated an increase in pain.20

The rat was placed in a perforated cage and the tip of a 0.2-mm-diameter blunted acupuncture needle was pushed against the face affected by the neuropathic injury until the needle bent slightly (dimpling the skin without penetration). This produced a mean force of 10.5 g as measured on a laboratory scale. The acupuncture needle was calibrated daily on a weight scale to assure that the exerted mean force was 10.5 g and examined under magnification to assure consistent blunting. Scoring of the rat’s response to this stimulus (draw score) was performed on an ordinal scale based on a similar scoring system used by Benoliel et al.: 0 = no response; 1 = detection; 2 = detection and withdrawal; 3 = detection, withdrawal, and escape or attacking movements; 4 = as in response 3 but with prolonged ipsilateral facial grooming. The test was performed daily at the same time of the day by the same investigator, who was blinded to the treatment group.21

Assessment of motor coordination
Rotarod test was used to evaluate the side effects of the drugs affecting the rats’ motor coordination. In this test, the rat was placed on a rotating rod. The speed of rotation was gradually increased and the rodent’s ability to remain on the rotating rod was recorded. The rats were tested before and 120 minutes after administration of medications. The speed used was 4-40 rpm and the drop height was <30 cm. The purpose of the rotarod test was to assess the rodents’ sensory motor coordination as a guide to assess a medication’s sedative effect.24-26 The rats were tested at baseline before any surgical procedure and at the 8th, 9th, and 10th days after the procedure at the same time of the day by the same investigator, who was blinded to the treatment group.

Concentration of medications in plasma
The plasma concentration of diclofenac and pregabalin was determined by a high-performance liquid chromatography (HPLC) method as described by Torres-Lopez et al.27,28 Blood samples were taken from the animals (2 separate samples from each rat) before killing them and placed into Eppendorf tubes. Afterward, the samples were acidified and extracted by agitation in a vortex. After centrifugation, organic layers were transferred into a clean conical glass tube and injected into the chromatographic system. The results demonstrated the retention time of the organic layer in the chromatographic system and were compared with standard retention times. The test was performed by the Clinical Laboratory of the University Hospital at UMDNJ.

Data analysis
Data were tabulated and analyzed with the use of Statview software version 5.0 (SAS Inst., San Francisco,
CA). Alpha (2 tailed) for significance in all analyses were set at 0.05. For tactile allodynia, mechanical hyperalgesia, and motor coordination (Rotarod) time points of relevance were analyzed with repeated-measurements analysis of variance (ANOVA) and post hoc Bonferroni test when indicated. Plasma concentrations of medications were analyzed with the use of factorial ANOVA followed by Bonferroni test when indicated. Eight rats were included in each group.

**RESULTS**

**Tactile allodynia**

Results are expressed as pain threshold measurements (force log\textsubscript{10}) with von Frey filament stimulation of the area innervated by the infraorbital nerve. After infraorbital nerve chronic constriction injury, a decrease in pain threshold compared with baseline was demonstrated in all of the groups on the ipsilateral side 1 week after the procedure (Figure 2). The 4-day treatment was initiated on the 7th day after surgery, and pain was assessed on the 8th, 9th, and 10th days. Pain threshold significantly increased (indicating less pain) in the treatment groups compared with the group that received no treatment and the group receiving only application of topical vehicle. Specifically, the pain thresholds increased in the systemic pregabalin group on days 8, 9, and 10, in the systemic diclofenac group on days 9 and 10, in the topical 10% pregabalin group on days 8 and 9, in the topical 5% diclofenac group on day 9, and in the group receiving a combination of pregabalin and diclofenac on day 9. Topical pregabalin at 5% did not reduce the pain. A significant increase compared with the no-treatment and vehicle groups ($P < .05$) was observed in the groups receiving systemic pregabalin (days 8, 9, and 10), systemic diclofenac (days 8, 9, and 10), topical 10% pregabalin (days 8, 9, and 10), topical 5% diclofenac (day 10), and combination of topical medications (day 10) compared with the groups receiving no treatment or topical vehicle.

**Mechanical hyperalgesia**

Recorded data were determined by the draw score test according to the withdrawal reaction showed by the animals when a blunted acupuncture needle was applied on the affected side. Seven days after the chronic constriction injury of the infraorbital nerve, an increase in the draw score (hyperresponse) was demonstrated in all of the groups on the ipsilateral side (Figure 3). The 4-day treatment was initiated on the 7th day after surgery and pain was assessed on the 8th, 9th, and 10th...
days. A significant reduction in the draw scores compared with the group that received no treatment and the group receiving only application of topical vehicle were found in the group receiving systemic pregabalin on days 8 and 9, topical 10% pregabalin on days 8, 9, and 10, topical 5% diclofenac on days 8 and 9, and combination of topical pregabalin and diclofenac on day 8 (P < .05). Systemic diclofenac and topical pregabalin at 5% did not reduce the draw score significantly.

Motor coordination
Results are expressed in seconds spent by the rat exercising on a rotarod (Figure 4). No differences were found between the groups at baseline before the surgical procedure. The groups not receiving any medication, the topical vehicle group, and the systemic pregabalin group showed a significantly shorter time spent on the Rotarod compared with the baseline. The motor coordination of all groups receiving medication showed some improvement (not approaching statistically significance) in the time spent on the rotarod compared with the vehicle and no-treatment groups. Only the groups receiving systemic diclofenac and topical pregabalin at 10% spent significantly longer time on the rotarod compared with the time spent on the device before the treatment.

Concentration of pregabalin and diclofenac in plasma
Concentration of pregabalin in the plasma after systemic administration (24.69 ± 3.54 μg/mL) was significantly higher compared with the levels found after topical 5% pregabalin (9.000 ± 0.424 μg/mL; P = .009), topical 10% pregabalin (7.30 ± 2.70 μg/mL; P = .006), and topical pregabalin and diclofenac combined (0.30 ± 0.10 μg/mL; P = .002) groups. Significant differences were not found in the pregabalin plasma levels was found between the 5% topical pregabalin, 10% topical pregabalin and the pregabalin diclofenac combined topical groups (Figure 5).

Concentration of diclofenac in the plasma after systemic administration (4.35 ± 0.25 μg/mL) was significantly higher compared with the levels found after topical 5% diclofenac (0.43 ± 0.13 μg/mL; P = .0005) and topical pregabalin and diclofenac combined (0.12 ± 0.03 μg/mL; P = .0004). Significant differences were not
found between the topical 5% diclofenac and topical pregabalin and diclofenac combined groups (Figure 5).

**DISCUSSION**

In line with previous reports, the present study demonstrated that chronic constriction injury of the infraorbital nerve in rats can induce significant and measurable neuropathic orofacial pain. The most significant finding of the study is that topical medications can efficiently reduce this pain and can be as effective as systemic medications commonly used for neuropathic pain.

The systemic medications used in this study, pregabalin and diclofenac, both served as positive controls. As expected, pregabalin was effective in reducing tactile allodynia and mechanical hyperalgesia, whereas diclofenac was effective in reducing only tactile allodynia. This may be related to the strong antiinflammatory effect of diclofenac, because research shows that the inflammatory process affects large myelinated nerve fibers more significantly than other fibers. Because tactile allodynia primarily tests large myelinated fibers, this medication-selective effect was expected.

Topical pregabalin at 10% was as effective as systemic pregabalin in reducing neuropathic pain, but with significantly reduced effect on motor coordination as measured by Rotarod test. Moreover, the tactile detection threshold after the treatment was higher than the baseline levels, suggesting local anesthetic effect.

Topical administration of medication has the potential to act on specific peripheral targets with minimal or very limited systemic absorption and reduced systemic effects. A recent clinical study suggested that topical medications may be useful in the treatment of NOP. However, that was a retrospective study, the medications levels in the plasma were not measured, and various medications were used. The present study design aimed to address those shortcomings by evaluating selective medication effects prospectively and by measuring the tested medication plasma levels. As expected, systemic pregabalin administration induced a plasma level that was significantly higher than that induced by the topical route. Both 5% and 10% topical pregabalin preparations induced similar plasma levels which were less than one-third of the level found in the plasma after systemic administration. Yet the 5% topical pregabalin was not as effective in reducing pain as the 10% preparation was. This strongly suggests that the efficacy of the 10% pregabalin in pain reduction
was related largely to its local effect and not to the relatively low level in the plasma. However, a possible combined peripheral and central effect can not be ruled out and should be explored in further studies.

The combined pregabalin and diclofenac preparation induced only minimal increase in the medications’ plasma levels but had some effect of pain reduction. The diclofenac plasma level was not different from the level achieved when 5% diclofenac was administrated alone. The pregabalin plasma level, however, was lower than the level achieved after 5% pregabalin application alone. It is possible that the pregabalin-diclofenac combination affects the level of pregabalin penetration through the skin, yet the mechanism is not clear and future research should address this issue.

The few clinical studies performed suggest that topical medications require a minimum of 2 weeks for significant pain relief. In the present study, topical application of the tested medications significantly reversed pain thresholds—overt responses to von Frey filaments and pinprick stimuli—and exhibited their effectiveness as soon as 1 day after the initial administration. Rodents’ response to systemic pregabalin is faster than in humans, so it is possible that the response to topical medication is faster as well. The rapid onset demonstrated in this study is encouraging.

As mentioned earlier, among the various topical medications assessed in this study, the most effective was topical pregabalin at 10%. Topical pregabalin at 5% had some effect as well but did not achieve complete pain remission. None of the published clinical studies assessed pregabalin as a topical treatment, and the mechanism of its topical effect is puzzling, mainly because pregabalin and other anticonvulsants are known to reduce neuronal excitability in the central nervous system. The increase in pregabalin plasma level after topical application was limited and probably not sufficient to decrease the pain. Pregabalin is thought to interact with the α2δ subunit of voltage dependent calcium channels, reducing neuronal activity and neurotransmitter release.

Future studies will need to address this issue; however, in theory similar effect can take place in peripheral hyperactive neurons as well.

The Rotarod test was used to evaluate the medications’ side effects; however, of interest was that the untreated control group and the group receiving only the vehicle, showed decreased motor coordination (shorter duration on the Rotarod). The rats were not tested on day 7 before any treatment, however, relying on the no-treatment group as a negative control, it is possible to assume that animals experiencing discomfort and/or pain during the required activity may have had difficulty tolerating this exercise. It may be similar to humans experiencing chronic pain who avoid exercise. This may explain the decreased motor coordination observed in the groups receiving less effective treatment (topical 5% pregabalin, topical 5% diclofenac, and combination of both topical medications).

As anticipated, a significant decrease in motor coordination was demonstrated in the systemic pregabalin group. On the other hand, rats treated with topical pregabalin at 10% demonstrated significantly improved motor coordination compared with all other groups. This may be related to the low level of the medication in the plasma together with significant pain reduction. The robust peripheral effect of pregabalin is promising; however, the clinical relevance will be determined only by clinical double-blind prospective trial with objective outcome assessment.

Topical diclofenac at 10% concentration, used in a pilot study, induced skin rash; therefore, only 5% concentration was used in the present study. The 5% preparation reduced tactile allodynia but had no effect on hyperalgesia scores, probably via the medication’s anti-inflammatory effect.

Before clinical trials, future studies should define the most effective and safe dose to use by testing a range of doses of pregabalin and other medications with similar mechanisms of action. The present study assessed the treatment side effect only by evaluating posttreatment motor coordination, however other factors, such as body weight and open field test (level and scope of motion), should be considered as well.

CONCLUSION

This study demonstrates that neuropathic orofacial pain, induced by nerve damage in rats, is reduced with topical application of pregabalin or diclofenac. This suggests that a topical approach has the potential to provide pain relief for orofacial neuropathies. Among the topical medications used in this study, the most effective in pain relief was topical pregabalin at 10%. This medication also demonstrated a minimal side effect profile. The peripheral mechanism of pregabalin is not clear, and further research should address this question. Future studies using various medications, range of doses, and combinations of treatments (systemic and peripheral, etc.) for neuropathic pain will increase knowledge and potentially improve treatment.

REFERENCES


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