Nimodipine and Acceleration of Functional Recovery of the Facial Nerve After Crush Injury

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Objective: To establish whether nimodipine, a calcium channel blocker, accelerates or otherwise improves functional recovery of whisking after facial nerve crush injury in the rat.

Methods: Thirty rats underwent exposure of the left main trunk of the facial nerve followed by a standard crush injury and subsequent quantitative facial movement testing. Animals were randomized into an experimental group (n=15) and a control group (n=15). Four days prior to facial nerve manipulation, experimental animals underwent subcutaneous implantation of a nimodipine-secreting pellet. All animals were tested preoperatively and on postoperative days 2, 8 to 17, 20, 22, 24, and 31 using a validated, quantitative whisking kinematics apparatus. Whisks were analyzed for amplitude, velocity, and acceleration.

Results: Animals receiving nimodipine demonstrated significantly better whisking on 5 days (postoperative days 9, 11 to 13, and 20) compared with control animals (P<.001, P=0.003, P=.009, and P=.009, respectively; 1-tailed t test). Overall, the nimodipine-treated animals showed earlier recovery compared with the untreated animals.

Conclusions: We demonstrate that nimodipine improves recovery of whisking after facial nerve crush. This finding corroborates the semiquantitative findings of others, and provides complete whisking kinematic data on its effects. Given the low adverse effect profile of nimodipine, there may be clinical implications in its administration in patients experiencing facial nerve injury.

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Facial nerve injury carries clinically significant adverse social and functional consequences, including decreased ability to communicate using facial expression, synkineses, incomplete eye closure, external nasal valve collapse, and oral incompetence. Our laboratory is focused on the development and study of interventions designed to accelerate and improve recovery from these sequelae.

A variety of pharmacologic agents, including FK-506,1 Toki-shakuyaku-san (TJ-23),2 angiotensin II,3 and nitric oxide6,7 have been shown to improve the functional recovery of peripheral nerves after injury; however, none are in clinical use. Nimodipine, a calcium channel blocker, is a US Food and Drug Administration (FDA)-approved drug used to reduce the morbidity and mortality associated with delayed ischemic deficits in patients with subarachnoid hemorrhage. In addition to its activity in the central nervous system, nimodipine has shown promise in multiple rodent models as a possible pharmacologic treatment for peripheral nerve injury.6,8

Many researchers have used qualitative and semiquantitative methods to examine functional recovery of the facial nerve after injury1,10,11 and/or electrophysiologic and histomorphometric analysis to measure neural regeneration. However, electrophysiologic and histomorphometric characteristics do not necessarily correlate with functional recovery, making quantitative functional analysis the benchmark for successful reinnervation, demonstrating not only that the nerve has regenerated but also that the nerve has made appropriate end organ connections.12 We recently developed a quantitative system to measure the return of nerve function after injury in a rat model.13,14 This apparatus accurately measures the amplitude, velocity, and acceleration of whisks and provides a useful tool for precise observations of timing and completeness of facial nerve recovery after injury. We evaluated the effect of nimodipine on whisking kinematics after facial nerve crush injury, using a quantitative instrument to measure functional recovery. We hypothesized that treatment with nimodipine would accelerate and/or improve overall recovery from facial nerve crush compared with controls. If effective, nimodipine might provide a clinically useful treatment for facial nerve crush injuries.
HEAD FIXATION AND BEHAVIORAL ADAPTION

Thirty female Wistar-Hannover rats (Charles River Laboratories, Wilmington, Massachusetts), weighing 200 to 250 g, were handled daily for 2 weeks prior to surgery to condition them for behavior testing. Subsequently, the rats underwent surgical insertion of a light-weight titanium head implant that provided a set of 4 external attachment points for rigid head fixation, as previously described.13,14 One week after head-fixation device implantation, the rats were conditioned to a body restraint apparatus by brief daily placements into a snugly fitting sack. In the third week, a head restraint was added to the daily conditioning regimen. After the third week, the rats were sufficiently conditioned to undergo head and body restraint without struggling or showing signs of stress, and baseline testing was performed. All experimentation was conducted under protocols approved by the Massachusetts Eye and Ear Infirmary Animal Care and Use Committee and conducted in accordance with international standards on animal welfare as well as local and national regulations.

TREATMENT AND SURGERY

Animals were randomized into experimental (n = 15) and control groups (n = 15). Four days prior to facial nerve injury, the experimental animals were implanted with a subcutaneous pellet of nimodipine15 (n = 15) (40-mg, 21-day sustained-release pellet; Innovative Research of America, Sarasota, Florida). Forty-milligram pellets have been shown to provide a sustained plasma concentration of 15 ng/mL in the rat and have been solely used in other studies. In humans, a therapeutic dose of nimodipine is 7 mL/mg for the treatment of subarachnoid hemorrhage. Thus, our dose was calculated as a safe dose that maximized the plasma concentration in rats in an attempt to achieve a therapeutic effect.16,17 Rats were anesthetized with an intramuscular injection of ketamine hydrochloride, 50 mg/kg (Fort Dodge Animal Health, Fort Dodge, Iowa), and dexadetomidine hydrochloride, 0.25 mg/kg (Orion Corp, Espoo, Finland), mixed in normal saline. The left infra-auricular area was shaved and sterilely prepared. Left facial nerve exposure involved a preauricular incision, reflection of the parotid gland, and visual identification of the main trunk of the facial nerve as it emerged anteriorly to the posterior belly of the digastric muscle. The common trunk was electrically stimulated with a nerve stimulator (Montgomery Nerve Stimulator; Boston Medical Products, Westford, Massachusetts) at a setting of 1 mV to verify complete hemifacial movement. The nerve was then crushed for 30 seconds using a jeweler’s microforceps, and the crush injury was repeated for an additional 30 seconds in the same location.18 The loss of electrical conductivity was verified by stimulating the proximal nerve at a setting of 2 mV and observing an absence of facial movement. The wound was closed, and the animal was allowed to recover on a warming pad and were monitored postoperatively for signs of discomfort, including changes in grooming, social interaction, and for maintenance of normal body weight.

FUNCTIONAL RECOVERY TESTING

Baseline whisking testing was performed preoperatively and on postoperative day 2. Daily postoperative testing of the animals began on day 8 and continued through day 17, with additional testing on days 20, 22, 24, and 31. Whisking recovery was measured using our previously described testing apparatus.13,14 Briefly, on the day of testing, animals were placed in the body restraint device, their right and left C-1 whiskers were marked using poly-imide tubes (Small Parts Inc, Miramar, Florida), and placed into the monitoring apparatus. The horizontal movement of the marked C-1 whiskers was independently tracked using commercial laser micrometers (MetraLight, Santa Mateo, California) and a data acquisition computer.14 A computer-controlled air valve was used to deliver 10-second sustained flows of scented air toward the snout to elicit whisking behavior at 2 random time points during each 5-minute data recording session per animal.

STATISTICAL ANALYSIS

The 3 largest amplitude whiskers were detected and analyzed in an automated fashion for each rat on each day of recording using software adapted from Bermejo et al.19,20 The data were normalized within each animal across the 2 sides of the face by dividing the amplitude on the injured side by the amplitude on the uninjured side, giving the relative recovery of function. This is based on prior kinematic analysis by Bermejo et al19,20 and Gao et al21 that confirmed symmetric whisking in head-fixed animals. In addition, this will account for the behavioral changes in whisking effort seen with repeated testing. A group average for relative recovery of amplitude was calculated for each testing day. Independent 2-sample, 1-tailed t tests were then performed for postoperative days 8 to 17, 20, 22, 24, and 31 between the experimental group and the control group. The same data analysis was performed for the 3 whiskers with the largest velocity and the 3 whiskers with the greatest acceleration for postoperative days 10 to 14, the anticipated window of accelerated recovery.

All animals in both groups exhibited normal cage behavior throughout the study. They had normal weight gain and did not exhibit aggressive behavior. There were no postoperative wound infections after the head mount, pellet implantation, or facial nerve crush procedures. One animal in the control group did not become conditioned appropriately to the testing apparatus and was therefore not tested and not included in the study. All other animals tolerated testing throughout the duration of the study. No increased morbidity was noted in the drug treatment group.

On postoperative testing, the experimental and control animals demonstrated symmetric whisking with a relative amplitude of 1.00 (SE, 0.023) and 1.08 (SE, 0.025), respectively. All animals had absence of whisking function on postoperative day 2 with a relative amplitude in the experimental group of 0.09 (SE, 0.011) and a relative amplitude in the control group of 0.04 (SE, 0.013). Animals were noted to have return of whisking function starting on postoperative day 9. Return of function followed a sigmoid curve, with rapid recovery of function between postoperative days 11 and 17. The mean amplitude, velocity, and acceleration were calculated for each group. The nimodipine-treated group showed a statistically significant improvement in amplitude on days 9, 11 to 13, and 20 compared with the controls (P < .001, P = .003, P = .009, P = .009, and P = .009, respectively; 1-tailed t test). A plateau of recovery was achieved between postoperative days 17 and 31, as expected. At postoperative day 31, both groups continued to demonstrate a statistically significant difference in amplitude between the operated and nonoperated sides (P = .009; 1-tailed t test) (Figure 1). The nimodipine-treated group also showed significantly better whisking velocity on days 11 to 13 and acceleration on days...
11 to 14 compared with the controls (\( P = .007, P = .006, \) and \( P = .01 \), respectively for velocity and \( P = .007, P = .006, P = .01, \) and \( P = .03 \) on respective days for acceleration; 1-tailed \( t \) test) (Figure 2).

## COMMENT

In this study, we administered nimodipine to facial nerve-crushed rodents, under the hypothesis that it would improve functional recovery. This hypothesis was based on literature showing that nimodipine, a calcium channel antagonist, improves the electrophysiologic recovery of the recurrent laryngeal nerve and the functional recovery of the sciatic nerve after peripheral nerve crush.\(^7\)\(^,\)\(^22\) This study corroborates the previously reported benefits of nimodipine in peripheral nerve recovery and provides quantitative functional data that demonstrate a statistically significant improvement in the relative recovery of whisking amplitude, velocity, and acceleration during the period of rapid recovery after facial nerve crush injury.

Nimodipine has demonstrated a functional benefit after both nerve crush and nerve transection or repair; however, to our knowledge, functional analysis after extracranial facial nerve crush injury has not been previously studied. After sciatic nerve crush injury, nimodipine leads to earlier onset of functional recovery in a dose-dependent manner.\(^22\) This hastening of recovery by approximately 1 to 2 days after nimodipine treatment in the sciatic nerve is similar to our present findings in the facial nerve. In the rat laryngeal nerve, improved neuromuscular function has also been reported after nimodipine administration.\(^7\) In a case report, nimodipine was thought to improve vocal cord function after transection and repair of the recurrent laryngeal nerve.\(^23\) Nimodipine has also been shown in an intracranial facial nerve transection and repair model to decrease neuronal cell death,\(^9\) and in a peripheral nerve transection and repair model to increase axonal sprouting and decrease the polyneuronal innervation of target muscles;\(^6\) however, functional analysis was not performed. In an intracranial facial nerve crush model, nimodipine did not attenuate the modest (13%) facial motor nucleus cell loss caused by axonotmesis but did accelerate the onset of axonal growth and functional recovery.\(^3\) Visual assessment of whisking after intracranial facial nerve crush identified the initiation of movement as occurring approximately 6 days sooner for nimodipine-treated rats than for controls. This more pronounced hastening of whisking recovery than found in the present report may be due to differences between intracranial vs extracranial nerve crush locations in regeneration length from the point of injury and cellular milieu at the point of injury.

Investigators\(^5\)\(^,\)\(^16\)\(^,\)\(^24\) have previously shown in rats that subcutaneous pellet administration of nimodipine is safe, enhances spatial learning, and decreases the age-related decline in performance on behavioral tasks. These studies also proved the effectiveness of subcutaneous nimodipine pellets in providing dose-dependent levels of nimodipine in both plasma and the brain.\(^5\)\(^,\)\(^16\) Because subcutaneous pellets obviate daily drug injections, they reduce the neurophysiological trauma that can be detrimental to behavioral testing\(^15\)\(^) and eliminate the intake and bioavailability issues associated with oral dosing regimens.\(^17\)

Nimodipine acts by blocking L-type voltage-gated calcium channels; however, the precise mechanism of action

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**Figure 1.** Relative recovery of whisking amplitude over time. Relative recovery was calculated by dividing the value on the injured side by the value on the uninjured side (1 = complete recovery). Curves represent the calculated average relative amplitude of the 3 largest amplitude whiskers for each animal on each day of testing. The nimodipine-treated group showed a statistically significant improvement on postoperative days 9, 11 to 13, and 20 compared with controls (\( P < .05; 1 \text{-tailed } t \text{ test} \)). Error bars indicate 2-tailed SE.

**Figure 2.** The relative recovery of facial function for postoperative days 10 to 14, the days of rapid recovery of function. Relative recovery was calculated by dividing the value on the injured side by the value on the uninjured side (1 = complete recovery). The nimodipine-treated group showed a statistically significant improvement in relative amplitude on days 11 to 13 compared with the controls (\( P < .05; 1 \text{-tailed } t \text{ test} \) (A)). The nimodipine-treated group also showed significantly better relative whisking velocity on days 11 to 13 (B) and relative acceleration on days 11 to 14 (C) compared with the controls (\( P < .05; 1 \text{-tailed } t \text{ test} \)). Error bars indicate 2-tailed SE.
by which nimodipine exerts its neuroprotective effects is still unknown. It has been theorized that it may enhance the supply of oxygen and nutrients to the injured region. Additionally, it was noted that on withdrawal therapy, an effect thought to be secondary to improved microcirculation, was shown to be secondary to improved microcirculation. In addition, it may exert a positive affect on the calcium accumulation of calcium, which leads to cell death. Nimodipine is an FDA-approved, orally available drug with a low adverse effect profile. It is the only available therapy to treat subarachnoid hemorrhage-associated vasospasm and has been proven to reduce the morbidity and mortality associated with delayed ischemic deficits. Recently, vasoactive treatment, utilizing the prophylactic use of nimodipine in combination with hydroxyethylstarch, was shown to improve hearing preservation after acoustic neuroma surgery, an effect thought to be secondary to improved microcirculation. In addition, it was noted that on withdrawal of hydroxyethylstarch and nimodipine, patients with acoustic neuroma developed a delayed-onset facial paralysis. Thus, future work could be aimed at comparing facial nerve outcomes in patients with acoustic neuroma who were and were not treated with nimodipine for hearing preservation. Aside from nimodipine, there is no other pharmacologic treatment shown to improve facial nerve function after injury in an animal model that is safe for routine clinical use. Herein, we demonstrate a statistically significant functional improvement after facial nerve crush injury. Given that nimodipine is an FDA-approved drug with a low adverse effect profile, this represents a critical step in bringing us closer to a clinical treatment for patients after peripheral nerve crush.

In conclusion, the present study demonstrates accelerated functional recovery associated with nimodipine treatment after facial nerve crush injury. These results are consistent with prior findings of enhanced peripheral nerve recovery in rats and further indicate that nimodipine treatment may have clinical utility for patients after facial nerve injury including after acoustic neuroma surgery.

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Author Contributions: Drs Lindsay and Hadlock had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Lindsay, Heaton, Smits, Edwards, and Hadlock. Acquisition of data: Lindsay, Smits, and Edwards. Analysis and interpretation of data: Lindsay, Heaton, Smits, and Edwards. Drafting of the manuscript: Lindsay. Critical revision of the manuscript for important intellectual content: Heaton, Smits, Edwards, and Hadlock. Statistical analysis: Lindsay, Heaton, Smits, and Edwards. Obtained funding: Hadlock. Administrative, technical, and material support: Hadlock. Study supervision: Hadlock.

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