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The Effect of N-Acetyl-Cysteine on Recovery of the Facial Nerve After Crush Injury

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Objective: Facial nerve dysfunction can vary in severity and recovery is dependent on the character of the injury. N-acetyl-cysteine prevents oxidative stress and cellular damage, and its use in the setting of nerve dysfunction from crush injury has not yet been established. In this study, rats with facial nerve crush injury will be treated with n-acetyl-cysteine or control and functional recovery and electrophysiologic outcome will be compared.

Study Design: Prospective, randomized animal study

Methods: Twenty-four Wistar rats underwent unilateral facial nerve crush injury. Rats were implanted with a subcutaneous osmotic pump filled with saline (n = 12) or n-acetyl-cysteine 50 mg/kg/day (n = 12). Functional and electromyographic recovery was recorded at two and four weeks postoperatively.

Results: When compared to untreated rats, n-acetyl-cysteine treated rats had a greater electromyography amplitude recovery at 2 weeks with regard to eye blink ($p=0.006$) but not vibrissae function. At four weeks, the electromyography amplitude recovery of the vibrissae function was greater in n-acetyl-cysteine treated rats ($P=0.001$), but the amplitude recovery difference in eye blink was only marginally significant between groups ($p=0.07$). The functional score was higher in n-acetyl-cysteine-treated rats than in untreated rats at all of the time points.

Conclusion: This study demonstrated that n-acetyl-cysteine facilitated facial nerve recovery with improved functional and electromyography outcomes in the setting of crush injury.

Key Words: Facial nerve, facial nerve trauma, n-acetyl-cysteine, nerve conduction.

Level of Evidence: NA

INTRODUCTION

Injury to the facial nerve is the most common cause of facial paralysis in children and the second most common cause in adults.[1–3] The most common mechanism of trauma is blunt injury to the temporal bone, usually from motor vehicle accidents, leading to stretch, crush, transection and loss of segment injuries. The resulting facial nerve injury has both serious functional and social consequences, making it imperative to continually improve upon treatment algorithms.

The extent of facial nerve injury, classified by Sunderland into five categories, corresponds to subsequent paths of recovery. In general, the most severe injury–axotomy of the nerve–follows a pattern of initial acute inflammation, up-regulation of reparative proteins, Wallerian degeneration of the distal nerve, axonal regrowth and joining of the distal and proximal segments and would require surgical intervention to promote recovery.[4] Less severe, crush injury to the facial nerve involves axonotmesis, injury to the axon and surrounding myelin, with no involvement of the epineurium. This subset of injury was shown to result in severe axonal injury with vacuolar degeneration and severe Wallerian degeneration, corresponding to a Sunderland's Degree III injury.[5]

The current treatment algorithm for facial nerve injury focuses on the differentiation between those likely to have spontaneous recovery and those less likely. Injuries associated with spontaneous recovery include incomplete paralysis, delayed onset paralysis, and blunt trauma to the extratemporal nerve. Medical treatments are appropriate for this category with a focus on minimizing edema and preventing viral infection. Injuries unlikely to have spontaneous recovery, those associated with immediate complete paralysis, should be treated with surgical intervention because of the assumed neuronal disruption. Surgical options include nerve decompression, direct end-to-end anastomosis and nerve graft repair.[1]

Crush injury to the facial nerve has a favorable prognosis; however, return to complete function can be prolonged and may be aided by an appropriate medical intervention. For example, crush injuries of rat facial nerves resulted in return of complete facial function after an average of 20 post-operative days with no therapy.[6] Although several medical therapies have been

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attempted in various facial nerve injury models, there is no accepted medical treatment for crush injuries. The current treatment options, aimed at reducing inflammation, have been gaining popularity.[7–9] Vakharia et al. (2011) used the rat model to compare atorvastatin, sildenafil and darbapoetin on crush injury recovery and showed that the darbapoetin treated rats had more significant recovery in the first two weeks; all other measurements were insignificant.[10] Furthermore Toros et al. (2013) showed that combination hyperbaric oxygen and corticosteroids improved the histopathological qualities in 1-week post-operative crush injured nerves.[2]

N-acetyl-cysteine (NAC) is a glutathione substrate that can readily be taken up by neurons. Glutathione in neurons acts as a free radical scavenger, protects the cytochrome oxidase system in mitochondria, and improves neuronal regeneration. It is hypothesized that NAC may thus improve neuronal survival by blocking entry into cell cycle, improving free radical surveillance, and preserving mitochondrial function.[4,11,12] NAC has been studied in nerve transection models and has been shown to reduce neuronal and glial cell death, preserve mitochondrial architecture and have greater regeneration of neurons over corticosteroids;[11,12] however, NAC has not yet been studied in facial nerve recovery after crush injury specifically. In this study we use a rat model of facial nerve crush injury to measure both functional and electromyography (EMG) recovery of the facial nerve with the use of NAC.

METHODS

Animals. Male Wistar rats obtained from the Charles River Laboratories (Charleston, South Carolina) were used for experimentation. They were exposed to a 12-hour light dark cycle and were fed a standard rodent diet with unlimited access to water. The Institutional Animal Care and Use Committee approved all animal care and procedures (IACUC protocol 13-237).

Twenty-four rats were each given six-digit identification numbers, and using these numbers were then sequentially randomized into two groups: control (no therapy) and systemic NAC. Following treatment, the randomization technique was concealed and animals were identified using only their six digit numbers.

Surgical Technique. Right facial nerve crushing was performed under anesthesia with ketamine (40 mg/kg) and xylazine (5 mg/kg) injected into the peritoneum. Sterile technique was used and included interscapular and postauricular hair removal and Betadine skin prep. A 1.5–2cm postauricular incision was made and the base of the facial nerve was identified as it emerged anterior to the posterior belly of the digastric and posterior to the parotid gland. Electrical stimulation was applied to the main trunk of the nerve to verify complete facial movement and record the maximal amplitude of conduction in the upper and lower divisions of the nerve (Fig. 1). The nerve was then crushed for one minute using a standard micro-hemostat held to three clicks. After removing the hemostat, the nerve was electrically stimulated proximally to the injury to verify complete paralysis. The incision was closed in a single layer and interrupted nylon sutures were placed over the area of nerve injury to ensure accurate site identification for future testing.

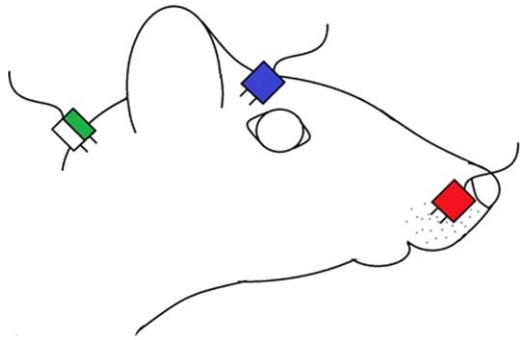

Fig. 1. Standard placement of the bipolar needle electrodes in the orbicularis oculi (blue), the vibrissae pad (red) and grounding electrodes (green/white).

A horizontal interscapular incision was then made and a one-inch pocket created by subcutaneous dissection. A 2 mL Azlet osmotic pump (DURECT Corporation, Cupertino, CA) was loaded with NAC (50 mg/kg/day) or normal saline (control) and placed into the pocket. The incision was closed in a single layer. The pump eludes at a rate of 2 ul/hr and over approximately four weeks.

Functional Testing. All animals were observed to have bilateral full and equal facial movement before surgery. The animals were observed over a four-week period and underwent testing at 14 and 28 days postoperatively. There were two blinded raters that scored each rat independently through video analysis. Functional testing included direct observation of eye blinking, vibrissae orientation and vibrissae movement. Eye blink reflex was obtained by placing one drop of saline from a pipet onto the eye. No interventions were needed to observe vibrissae movement and orientation. An established grading system (Table I) for functional recovery was used, with larger numbers indicating greater recovery.[4] Functional scores were independently given for both eye blink and vibrissae recovery by experimenters through direct observation and video analysis.

Nerve Conduction Testing. NCS were performed at two and four weeks postoperatively using a Neural Integrity Monitor (Medtronic, Jacksonville, FL). For each NCS, a standard bipolar needle electrode was placed in both the vibrissae and orbicularis oculi muscle pads and a grounding electrode was placed in the back. A monopolar stimulating electrode was placed at the site of the facial nerve exit from the stylomastoid foramen. A stimulating pulse was applied to generate a compound muscle action potential and a supra-maximal stimulus was determined. Wave amplitudes for the orbicularis oculi and vibrissae muscle beds were generated by the EMG machine and recorded. The peak amplitude of the orbicularis oculi and orbicularis oris was measured at supra-maximal stimulus. Two reviewers were blinded to the treatment group and scored the rats independently. Each postoperative test was compared to the initial

TABLE I.
Facial Movement Scoring Scale

Eye Blinking	Vibrissae Movement
1. Absence of eye blinking/closure	1. Absence of movement and posterior position of vibrissae
2. Presence of orbicular muscle contraction, without blinking reflex	2. Slight shivering and posterior position
3. 50% of eye closure through blinking reflex	3. Greater shivering and posterior position
4. 75% eye closure	4. Normal movement with posterior position
5. Complete eye closure and blinking reflex	5. Symmetrical movement and anterior position
[4]	

amplitude and these measurements were used as a baseline to which subsequent recordings could be compared. All post-operative amplitudes were compared against pre-operative measurements and the percentage recovery of the original amplitude was recorded at the aforementioned time points.

Statistical analysis was performed in conjunction with a dedicated biostatistician and the null hypothesis was tested using the computer software program Statistical Package for the Social Sciences (SPSS). The differences between the groups were analyzed using the standard T-test. The data were expressed as a mean ± standard deviation (SD). The level of statistical significance was set at $P < 0.05$.

RESULTS

All crush injuries resulted in loss of electrical conductivity as well as functional loss according to the NCS and functional analysis. Refer to Figures 2 and 3 for the results of the NCS at each time point. At two weeks the treatment group demonstrated a mean eye blink amplitude recovery of 76% while that of the control group was 58% ($P = 0.006$) however the difference in vibrissae function was not statistically significant at this time point ($P = 0.2$). At four weeks a significant difference was found in the amplitude recovery of the vibrissae function at 88% in the treatment group compared to 70% in the control group ($P = 0.001$), however the eye blink function was only marginally significant ($P = 0.07$).

The functional score of the NAC-treatment group was significantly better when compared to the untreated animals at each time point (Table II). The NAC-treatment group demonstrated improved recovery when compared to controls.

Four of the 24 rats did not complete the study. One rat from the control group expired from complications related to anesthesia, while a second developed a wound infection and was euthanized. Two rats in the treatment group experienced extrusion of the osmotic pump prior to the end of the study and therefore the dose of medication was not consistent with the study parameters and they were excluded.

DISCUSSION

Facial nerve dysfunction can vary in severity and is dependent on the character of the injury to the nerve. While re-establishment of direct neural integrity through re-anastomosis is the first-line therapy for transection injury, there is no universally accepted treatment for crush injury to the nerve. Several medical therapies have been tested in various conditions with facial nerve dysfunction and while early administration of steroids has shown improved functional outcomes in certain conditions of facial nerve dysfunction, such as Bell's Palsy,

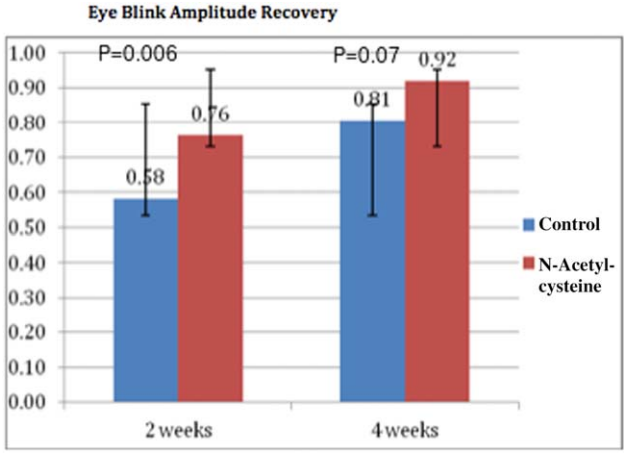

Fig. 2. Eye Blink Amplitude Recovery was expressed as the percentage of the baseline pre-operative amplitude recorded at each post-operative test. Error bars represent standard deviation from the mean.

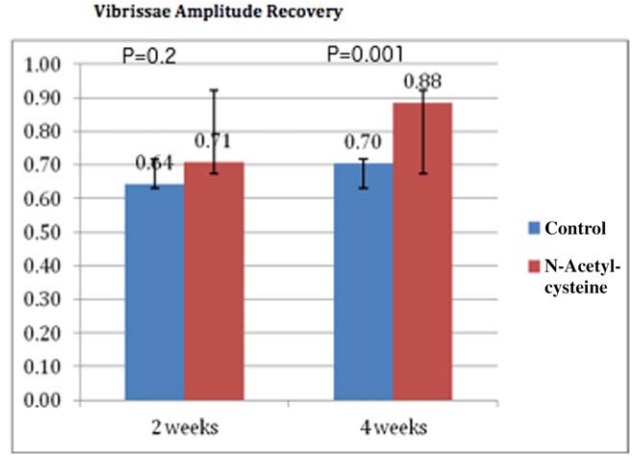

Fig. 3. Vibrissae Amplitude Recovery was expressed as the percentage of the baseline pre-operative amplitude recorded at teach post-operative test. Error bars represent standard deviation from mean.

TABLE II.
Facial Nerve Functional Score

	Eye Blink				Vibrissae Function			
	2 weeks		4 weeks		2 weeks		4 weeks	
	Mean (SD)	P Value	Mean (SD)	P Value	Mean (SD)	P Value	Mean (SD)	P Value
Control	2.16 (0.39)		4.00 (0.60)		2.08 (0.51)		4.16 (0.57)	
L-NAC	2.83 (0.39)	0.0003	4.66 (0.49)	0.006	3.25 (0.62)	0.0002	4.83 (0.38)	0.006

L-NAC = N-acetylcysteine; SD = standard deviation.

our understanding of the application of other neuroprotective agents in this setting is limited.[13–15]

NAC is a well-known antioxidant that prevents oxidative stress and cellular damage with potential neuroprotective applications. It was shown to significantly increase nerve regeneration in a nerve transection model; however, its use in the setting of nerve dysfunction from crush injuries has not been established. Our study demonstrated a significant improvement in the functional and EMG outcomes of facial nerve function after crush injury. Notably, a significant difference was demonstrated at the two-week post-operative evaluation indicating that systemic treatment with this agent might facilitate a more rapid recovery as well.

There are several mechanisms by which NAC can facilitate facial nerve recovery after traumatic injury. It has been shown to augment circulation in healing areas by increasing vascularity and permeability, as well as decreasing free radical discharge and promoting cellular healing.[16,17] Following nerve injury, the presence of myelin debris has been shown to prevent remyelinization and requires removal by macrophages prior to regeneration. Karlidag et al. demonstrated that NAC leads to an improved reduction in myelin debris following nerve injury and showed a statistically significant difference compared to steroids with best neural regeneration in a transection model. NAC has been studied in the setting of spinal cord injury as well, demonstrating decreased ischemia-reperfusion injury with decreased motor dysfunction at a dose of 50 mg/kg.[18] In addition, it has been shown to prevent brain damage in the setting of traumatic brain injury by regulating mitochondrial dysfunction.[19]

While our study demonstrated a benefit of systemic NAC in the recovery of facial nerve function following crush injury, there are several questions that need to be elucidated. We used a dose of 50 mg/kg/day administered over a one-month period as had been described in previous studies evaluating its efficacy in facial nerve recovery following transection, but there is no consensus on the standard dose or time frame that would yield the optimal benefits in the setting of facial nerve crush.[12] Histopathology analysis is merited as well to determine if the same benefits demonstrated at a cellular level in prior studies can be re-demonstrated in the setting of a crush injury.

CONCLUSION

This study demonstrated that NAC facilitated facial nerve recovery with improved functional and EMG outcomes in the setting of crush injury. This was present at two weeks indicating that it may facilitate a more rapid recovery as well. Based on the findings of this study, further investigation is warranted to elucidate the histopathology findings with the use of NAC in the setting of crush injury and to evaluate its efficacy compared to and in conjunction with other neuro-protective treatment modalities.

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