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Vocal Fold Paralysis: Improved Adductor Recovery by Vincristine Blockade of Posterior Cricoarytenoid

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Abstract

OBJECTIVES/HYPOTHESIS—A new treatment for acute unilateral vocal fold paralysis was proposed, in which a drug is injected into the posterior cricoarytenoid muscle (PCA) shortly after nerve injury, before the degree of natural recovery is known, to prevent antagonistic synkinetic reinnervation. This concept was tested in a series of canine experiments using vincristine as the blocking agent.

STUDY DESIGN—Animal experiments.

METHODS—Laryngeal adductor function was measured at baseline and at 6 months following experimental recurrent laryngeal nerve (RLN) injuries, including complete transection, crush injury, and cautery. In the treatment animals, the PCA was injected with vincristine at the time of RLN injury.

RESULTS—Adductor function in the vincristine-treated hemilarynges was significantly improved compared with injury-matched noninjected controls (total n=43). Transection/repair controls recovered 56.1% of original adductor strength, vincristine-treated hemilarynges recovered to 73.1% (p=0.002). Cautery injuries also improved with vincristine block (60.7% vs 88.7%, p=0.031). Crush injuries recovered well even without vincristine (104.8% vs 111.2%, p=0.35).

CONCLUSIONS—These findings support a new paradigm of early, pre-emptive blockade of the antagonist muscle (PCA) to improve ultimate net adductor strength, which could potentially improve functional recovery in many UVFP patients and avoid the need for medialization procedures. Possible clinical aspects of this new approach are discussed.

Keywords

vocal fold; paralysis; synkinesis; injection; canine

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INTRODUCTION

Recovery of the recurrent laryngeal nerve (RLN) following traumatic injury (e.g., neck or chest surgeries{ 1 }) usually includes some degree of cross-innervation, in which disrupted adductor axons regrow into abductor channels, and vice-versa; this process has been well documented{2-10} and is referred to as laryngeal synkinesis. The resulting simultaneous co-contraction of the abductor muscle (posterior cricoarytenoid, PCA) antagonizes the action of the adductor muscles, and may be responsible in part for the typical paramedian position of the paralyzed vocal fold and the glottal gap during phonation.

Current management of acute unilateral vocal fold paralysis (UVFP) usually involves a wait-and-see approach, in which patients are asked to wait 6-12 months for possible RLN recovery before any *permanent* treatment is performed,{ 1 } but some early interventions have been developed. Many surgeons utilize injection laryngoplasty with absorbable agents to provide immediate symptomatic improvement during this waiting period.{11} Several recent studies (e.g., Friedman et al.,{12} Yung et al.,{13} Young et al.,{14} Alghonaim et al.,{15}) have shown that early injection medialization can reduce the need for subsequent permanent procedures, even in patients who do not regain vocal fold mobility. Systemic administration of nimodipine has recently been shown to enhance nonselective reinnervation in an animal model{16} and in a prospective clinical trial.{17} Rubin et al.{18} showed that gene therapy could improve recovery of the injured RLN in a rat model, but the approach has not been tested in patients.

However, an interim treatment to *prevent* laryngeal synkinesis, or to reduce its severity, has not been developed. After recovery, misdirected axons cannot be redirected; current treatment options include selective nerve section and botulinum toxin injections. An ideal early intervention would be easy to perform, safe, offer permanent improvement, and not impede spontaneous recovery. It also would not interfere with potential future treatment, should it become necessary.

In this study, an early intervention is proposed that meets these ideal criteria, in which the denervated posterior cricoarytenoid muscle is injected with vincristine. Vincristine prevents polymerization of microtubules by binding to dimeric tubulin. This results in disruption of fast axonal transport, which is heavily dependent on the microtubule system.{19} In preliminary studies, we found vincristine effectively blocked reinnervation in a rat posterior tibial nerve injury model{20} and a rabbit facial nerve injury model{21} the present study advances this work into the canine larynx. McRae et al. also found vincristine blocked reinnervation of the rat thyroarytenoid muscle complex.{22} We have previously used the canine larynx model to evaluate laryngeal adductor functional recovery following several types of RLN injuries.{23} In this study, we used the same model to test the use of vincristine to block the PCA muscle and determine its impact on the strength of vocal fold adduction. While this approach does not directly prevent cross-innervation, it does reduce reinnervation of the PCA from both adductor and abductor fibers, which was expected to diminish its antagonism and thereby improve adduction.

METHODS AND MATERIALS

43 hemilaryngeal preparations were performed on purpose-bred, conditioned, female mongrel dogs weighing 20-to 25 kg. The animals were maintained in a facility approved by the American Association for Accreditation of Laboratory Animal Care, and National Institutes of Health guidelines for animal care were followed strictly. All experiments were performed according to a protocol approved by the Institutional Animal Care and Use Committee of the Washington University School of Medicine.

Under general anesthesia, a permanent tracheostomy was performed following our previously described method.^{24} Each RLN was dissected and a Harvard electrode was applied. A pre-treatment baseline laryngeal adductor pressure (LAP) was measured by stimulating the RLN while measuring the pressure created by the vocal fold squeezing an endotracheal tube balloon attached to a pressure transducer.^{25} A custom laryngeal nerve stimulator (WR Medical electronics, Maplewood, MN) applied constant-current stimuli at frequencies from 20Hz to 100Hz at 10Hz increments in a random sequence, with the current adjusted to provide maximal LAP at 80 Hz

The experimental nerve intervention(s) (described below) were performed. In the vincristine study groups, 0.4mg vincristine (1mg/cc) was injected into the mid-PCA muscle under direct visualization. The wounds were closed and the animal was allowed to recover for six months. At the terminal procedure, the neck was re-explored, and the LAP measurements were recorded again, using the same endotracheal tube used initially. The LAP curves were normalized to the pretreatment measures for the same dog. The measures at 70-100 Hz were averaged to produce a single adductor plateau value for each experiment.

The following experimental groups were studied:

- A.** Complete transection model, with precise repair. The RLN main trunk was divided 4 cm inferior to the cricothyroid joint. For groups A1 and A2, an operating microscope was used to perform microneural re-anastomosis using 9-0 nylon sutures, with the cut ends oriented as closely as possible to their original orientation.
  - A1. Control series (no vincristine), n=8.
  - A2. Vincristine injection series, n=8.
  - A3. Non-repair control series; no anastomosis, no vincristine, n=5.
- B.** Nerve crush model. The RLN main trunk was crushed 4 cm inferior to the cricothyroid joint. To create the nerve crush, a small hemostat was applied to one click, held for 20 seconds, and released. An obvious depression at the crush site was evident in all cases.
  - B1. Control series (no vincristine), n=7.
  - B2. Vincristine injection series, n=6.
- C.** Nerve cautery model. The RLN main trunk was cauterized 4 cm inferior to the cricothyroid joint. To create the nerve cautery injury, a small bipolar electrocautery

tip was applied across the nerve (without completely squeezing the tips together), with the cautery set at 20 watts, and applied for 0.2 seconds. A discoloration at the cautery site, but not a complete burn, was evident in all cases.

C1. Control series (no vincristine), n=6.

C2. Vincristine injection series, n=3.

These nerve injury models were selected as examples of possible intraoperative RLN injuries, for example as might occur during thyroid surgery.

RESULTS

The pooled pre-treatment baseline LAP measures for all 43 experiments are shown in Figure 1. At low stimulation frequencies (20-30 Hz), the PCA is dominant and the LAP is low. As the frequency is increased, the TA becomes predominant and the LAP rises until it reaches a plateau at about 70-80 Hz. This pattern, reported previously,^{25} was seen in all experiments and the standard deviation (error bars) is small.

The 6-month LAP values for the RLN injury models are shown together in Figure 2 for comparison. It can be seen that the complete transection injury with repair and the cautery injury both recovered to about 60% of the pretreatment level at the higher frequencies. The crush injury, which is considered a reversible, Sunderland class I-II injury, essentially recovered to normal. Of the five dogs in the complete transection without repair group (A3), none showed evidence of any RLN recovery. There was no spontaneous vocal fold movement, and none could be stimulated with a nerve stimulator probe. Neck exploration showed no evidence of nerve re-growth between the cut ends of the RLN.

In Figure 3, the results from the vincristine injections are presented. For the transection/repair (A) and cautery injury (C) groups, vincristine blockade resulted in a significant increase in LAP compared with the non-injected controls ($p<0.05$). The average LAPs from 70-100 Hz are given in Table 1. In the crush injury (B) groups, the control group and the vincristine group both recovered to slightly higher LAP than normal, and the difference between these groups was not significant.

DISCUSSION

The vincristine study groups demonstrate clearly that blocking PCA reinnervation results in greater strength of adduction. This suggests a new, proactive paradigm in the treatment of new-onset vocal fold paralysis. Upon early diagnosis of vocal fold paralysis, a simple injection of the ipsilateral PCA muscle could lead to improved adductor recovery and perhaps avoid the need for future interventions such as laryngeal framework surgery. To be effective, the vincristine must be injected prior to any reinnervation, which commonly begins within 2-4 months of RLN injury; we previously found vincristine injection had no effect on muscles that were already innervated.^{20} For example, patients with recognized intraoperative RLN injuries could have the injection performed while under the same anesthetic.

Adoption of this preventive paradigm would mean that some patients would get injected who would have recovered anyway, which constitute about 40% of the potential patients. It is important to consider whether this would be a disservice to such patients. Four arguments suggest this approach is acceptable: 1) The vincristine blockade of PCA reinnervation is incomplete: in the vincristine study groups, the harvested PCA muscles did not show the same degree of atrophy as the complete transection control group (informal observation). Some muscle tone in the PCA is desirable, to help support the arytenoid on the cricoid and prevent it from prolapsing anteriorly. 2) Patients who experience significant recovery from UVFP most likely had partial injuries, suggesting some intact neurons remain. But the vincristine would not be expected to affect intact neuromuscular units, as discussed above. 3) Most patients who exhibit recovery from UVFP can adduct the involved focal fold, but cannot abduct it. This is likely due to the known tendency for the adductor muscles to recover earlier and more completely than the abductors, and/or to synkinetic reinnervation of the adductor muscles with abductor axons. Thus, blocking the PCA likely does not forfeit any abductor function for most patients. 4) If the contralateral PCA is functioning normally, there should be no airway concerns about blocking the PCA on the recovering side; this is no different than what occurs if there is no recovery or if the paralyzed vocal fold is surgically medialized. It is possible that future advances in prognostic laryngeal EMG will enable us to be more selective as to which patients would most benefit from an early vincristine injection.

This early intervention paradigm would probably most help the patients that are destined for “borderline” recovery of adductor function. Many patients with UVFP have partial recovery, but could benefit from just a bit more adductory strength; prevention of synkinetic PCA activity could offer just the little bit of extra adduction they need. It could also permit some patients to achieve adequate improvement with a small injection augmentation, and avoid the need for a full thyroplasty procedure.

For patients who are planning to undergo non-selective laryngeal reinnervation procedures, such as ansa cervicalis-to-RLN anastomosis, vincristine injection of the PCA could be a useful adjunct. The reinnervating ansa fibers will randomly distribute into both the adductors and the PCA; blocking PCA reinnervation can be reasonably expected to further augment the benefit of the adductor reinnervation. A similar result could be achieved by dividing the PCA branch of the RLN at the time of reinnervation; but injection is technically much easier, and does allow some residual muscle tone (as noted in argument 1, above).

Vincristine is known to cause neurotoxicity when given systemically in chemotherapy regimens, raising concern about side effects from this potential intervention. We did not observe any local effects to the mucosa or the surrounding soft tissue in any of the injected dogs, nor was there any evidence of systemic neurotoxicity. When vincristine is used for chemotherapy, it is typically given in daily doses of 2-4mg; the single intramuscular dose of 0.4mg used for these studies is only 10-20% of this, and the drug probably stays primarily within the muscle, rather than circulating systemically. If this approach is used in patients, it would be reasonable to check a plasma level in the first few patients to determine whether there is any significant systemic uptake.

The canine larynx is a good neuromuscular analog to the human larynx, in size as well as anatomy, but any findings using this model, such as those in this study, need to be verified in human subjects. For example, in humans it might be necessary to perform more than one injection at some defined interval (e.g., 4-6 weeks) to achieve the same level of blockade as found in this canine study. A randomized clinical trial would be the best way to determine the usefulness of early vincristine injection as a new paradigm for managing patients with vocal fold paralysis.

The measure of evoked adductor strength used in this study, the “laryngeal adductor pressure” (LAP), is determined by placing the inflated cuff of an endotracheal tube between the vocal folds and applying a supramaximal stimulus to the RLN. Nerve stimulation thresholds are highly dependent on the local tissue milieu, and the same nerve will have different stimulation parameters on different days; thus, only supramaximal stimulation can be reliably repeated.

Stimulation of the RLN causes simultaneous maximal stimulation of both adductor and abductor fibers, a non-physiologic construct that never occurs naturally but makes a reliable experimental tool.^{25} We have found the measure to be highly dependable.^{23} It is not known, however, how much the added adductor strength gained from PCA blockade would translate into improved clinical outcomes in patients; this can only be determined by a clinical trial.

Vincristine injection of the PCA could be performed in combination with other early interventions for UVFP. It would not be difficult to perform a vincristine injection into the PCA at the same time as injection laryngoplasty,^{11-15} either in the operating room or as an in-office procedure,^{26-28} and perhaps offer synergistic benefit. Similarly, nimodipine therapy is expected to increase axon growth non-selectively;^{16-17} vincristine injection into the PCA could be used to reduce the effects of synkinetic reinnervation. The relative merits of each of these interventions would best be compared in a clinical trial with multiple arms.

CONCLUSION

A new paradigm of early management of vocal fold paralysis patients was proposed, using vincristine injection to the posterior cricoarytenoid muscle to block its reinnervation and thereby improve eventual adductor functional recovery. Crush, cautery, and transection models of RLN injury showed this injection significantly enhanced recovery of evoked adductor strength. This paradigm should be further investigated with a randomized clinical trial.

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Figure 1.

Pre-treatment baseline LAP measurements for all animals included in study. Error bars are one standard deviation.

Figure 2. 6-month LAP measurements for four RLN injury models compared with pre-treatment baseline.

Figure 3.

Effect of vincristine injections on 6-month LAP results. A, complete transection with repair model; B, Crush model; C, Cautery model.

Table 1

Experimental schema and 6-month postop LAP values for 43 experiments (see text). a groups had RLN injury alone; b- groups received vincristine injection to PCA muscle; the c group was the unrepaired complete transection model. p-values vs. non-injected control a groups using Student's t-test.

Group	Model	n	Injection	LAP 70-100	±SD	p
1a	Transect/repair	8	None	0.561	0.110	
1b	Transect/repair	8	Vinc	0.731	0.064	0.002
1c	Transect/no repair	5	None	0.000	0.000	<0.001
2a	Crush	7	None	1.048	0.096	
2b	Crush	6	Vinc	1.112	0.133	0.35
3a	Cautery	6	None	0.607	0.151	
3b	Cautery	3	Vinc	0.887	0.124	0.031
	Total	43				