Published in final edited form as:

Ann Otol Rhinol Laryngol. 2015 June ; 124(6): 484–489. doi:10.1177/0003489414566182.

Effect on Laryngeal Adductor Function of Vincristine Block of Posterior Cricoarytenoid Muscle 3-5 Months After Recurrent Laryngeal Nerve Injury

Randal C. Paniello, MD, PhD and Andrea Park, MD

Department of Otolaryngology – Head and Neck Surgery, Washington University, St. Louis, MO

Abstract

Objectives—It has been shown, in a canine model, that a single injection of vincristine into the PCA muscle at the time of recurrent laryngeal nerve (RLN) injury effectively blocks its reinnervation and results in improved adductor strength. But clinically, such injuries are usually diagnosed weeks or months after onset. Vincristine injection does not affect a muscle that is already innervated; thus, there is a limited time frame following RLN injury during which a vincristine injection could effectively improve ultimate laryngeal adductor functional recovery. A series of delayed injections were performed in a canine model and results assessed.

Study Design—Animal (canine) experiment.

Methods—The RLN was transected and repaired, and vincristine (0.4 mg) was injected into the PCA muscle at the time of injury (n=12), or at 3, 4, and 5 months later (n=8 each study group). Six months after RLN injury, laryngeal adductor function was measured. Results of vincristine injection without RLN injury (n=6), and longer-term (12 months) follow-up for time zero injections (n=4), are also reported.

Results—The animals injected at time zero had better adductor function than non-injected controls, as reported previously, and this result was further increased at 12 months. The 3-month delay gave results similar to the time zero group. The 5-month delay group showed no vincristine benefit, and the 4-month delay group gave an intermediate result. Vincristine to the PCA had no effect on adductor function when the RLN was left intact. Plasma levels showed 19% of injected vincristine reached systemic circulation, which was cleared within 69 hours.

Conclusions—Vincristine injection of the PCA muscle after RLN injury, which blocks this functional recovery. The window of opportunity to apply this treatment closes by four months after RLN injury in the canine model. Human RLN recovery follows a similar time course and can reasonably be expected to have a similar therapeutic window.

Corresponding author: Randal C. Paniello MD PhD, Department of Otolaryngology-Head and Neck Surgery, Washington University School of Medicine in Saint Louis, 660 S. Euclid Avenue, Box 8115, Saint Louis, Missouri, 63110, Phone: 314-362-7252, Fax: 314-367-7522, paniello@ent.wustl.edu.

Presented at the annual meeting of the American Bronchoesophagological Association, Orlando, FL, April 10, 2013.

This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Washington University School of Medicine.

Keywords

vocal fold; paralysis; synkinesis; injection; canine

INTRODUCTION

Unilateral vocal fold paralysis (UVFP) occurs most commonly following iatrogenic injury of the recurrent laryngeal nerve (RLN).{1} Natural recovery from RLN injuries may take 6-12 months, and most otolaryngologists wait at least 6 months before performing any sort of permanent surgical correction such as medialization thyroplasty, arytenoid adduction or reinnervation.{1,2} Following this “wait-and-see” approach, approximately 50% of patients will recover adequate laryngeal function and require no further intervention, some with true RLN recovery, but many with continued paralysis but good compensation from the uninvolved side.{3} Electromyographic studies show that most patients with UVFP have some measurable innervation, but it is either inadequate to provide functional adduction, or there are many adductor axons inappropriately reinnervating the abductor muscle (posterior cricoarytenoid, PCA), antagonizing the action of the adductor muscle group.{4} This innervation pattern is referred to as laryngeal synkinesis.{5}

Recently, a “new paradigm” was proposed in which the wait-and-see approach is replaced by a proactive, prevention approach to treating patients with new-onset UVFP.{6} The idea is that patients could undergo an injection of the PCA muscle with a drug that inhibits reinnervation, thereby preventing adductor-to-abductor synkinesis and allowing more effective spontaneous adductor recovery. Reduced PCA tension should, at minimum, allow a more medial position of the paralyzed vocal fold. Initially, experiments were conducted to identify an injectable agent that could effectively block reinnervation, ideally with a single intramuscular injection to the PCA muscle. Vincristine, a vinca alkaloid chemotherapy drug, was found to be effective for this purpose, first in a rat femoral nerve crush injury model,{7} and then in a rabbit facial nerve transection model.{8} McRae et al. independently confirmed that vincristine effectively blocked reinnervation in a rat RLN injury model.{9}

This approach was then tested in the canine larynx.{6} In a RLN transection and re-anastomosis model, stimulated laryngeal adduction pressure (LAP), which serves as a proxy measure for the strength of laryngeal adduction, was found to achieve 73.1 % of normal for larynges in which the PCA was injected with vincristine, compared with 56.1 % for non-injected controls.{6} This finding has generated interest in testing this approach in a clinical trial. However, in the canine experiment, the vincristine injection was performed synchronous with the nerve transection injury. In practice, it is rare that the otolaryngologist has the opportunity to intervene on the same day as the injury; patients with UVFP are typically referred weeks or months after its onset. The previous work also showed that a vincristine injection does not affect existing innervation.{7} Thus, there is likely a window of opportunity following nerve injury in which a vincristine blockade can be effective, before significant reinnervation occurs. The present study was performed to confirm this hypothesis and to determine the treatment window in the canine model.

METHODS AND MATERIALS

Twenty-seven purpose-bred, conditioned, female mongrel dogs weighing 20-25 kg were used in seven study groups. The animals were maintained in a facility approved by the American Association for Accreditation of Laboratory Animal Care (AAALAC), 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 (IACUC) of the Washington University School of Medicine.

Initial Procedure

Under general anesthesia, the neck was prepped with betadine solution and explored. A tracheotomy was performed as previously described{10} and the endotracheal tube repositioned into it. Each RLN was dissected and a bipolar Harvard electrode was placed around it, 4-6cm inferior to the cricothyroid joint, and connected to a custom laryngeal nerve stimulator. A second endotracheal tube was inserted into the tracheotomy and directed superiorly; the pilot line from the cuff was connected to a pressure transducer. Laryngeal adductor pressure (LAP) was measured by passing the inflated cuff (10cc) between the vocal folds while stimulating one RLN. A supramaximal stimulus level ensures that all RLN axons are depolarized; the LAP is the net squeeze on the cuff caused by the simultaneous co-contraction of all of the adductor/agonist muscles plus the abductor/antagonist PCA muscle.{11} The responses are frequency-dependent, and reach a maximum adduction plateau at stimuli of 70 Hz and above. LAP measurements were made at 10-Hz intervals up to 100 Hz. A second set of measurements was made with the stimulator connected to the contralateral electrode. The two sides are independent, allowing two experiments to be performed on each subject.

RLN Injury Model

For six of the seven study groups, a standard transection and repair model was performed. The RLN was transected 4cm inferior to the cricothyroid joint, and then immediately re-anastomosed end-to-end using an operating microscope and 9-0 nylon sutures. The orientation of the stumps was random (in a previous study, the same result was obtained with a precise realignment as with a 180° rotation of the stumps{12}). The stoma was matured and the wounds were closed.

Experimental Groups

Animals were randomly assigned to interventions as shown in Table 1. For animals receiving injections at time zero (groups B, F, and G), 0.4mg vincristine was injected directly into each PCA muscle prior to initial wound closure. For those receiving injections at a later time (groups C, D, and E), the superior aspect of the wound was opened under general anesthesia and the same vincristine injection procedure was followed. In a pilot experiment in three terminal dogs from other studies, in which PCA injections were attempted transorally using methylene blue, the dye was found to be deposited within the target muscle in only 4 of 6 PCAs; so an open injection procedure was chosen for this study to be certain of accurate placement.

Terminal Procedure

Six months after the initial surgical procedure (12 months for group F), the animals underwent general anesthesia. The RLNs were identified and dissected near the sternum, several cm inferior to the site of previous nerve transection and repair, and electrodes applied. The LAP data were collected using the same endotracheal tube that was used at the initial procedure. The larynges were harvested for histochemical analysis (not included in this report).

Data Analysis

At each frequency, LAP measurements were made with three passes of the endotracheal tube; the highest recorded pressure was used. The four adductor plateau values (at 70, 80, 90, and 100 Hz) were averaged for each animal. The final mean LAP value was expressed as a fraction of the initial (pretreatment) value. Some of the animals had no measurable LAP at the end of the experiment; these were excluded from the averages. Comparisons between groups were made using a two-tailed student's t-test; differences were considered significant if $p < 0.05$.

Pharmacological Measures

In two additional animals, vincristine was given as described above and blood samples were drawn at various time intervals over the next three days. Maximum levels were determined on the day of sacrifice by delivering the same total vincristine dose intravenously and drawing a blood sample 30 minutes later. The plasma was separated by centrifuge, then frozen and sent to an analytic pharmacology lab for determination of vincristine levels.

RESULTS

Four animals expired 1-3 months after their initial procedure: one from a mucus plug occluding the tracheostoma, one from a severe pulmonary infection, and two from unknown causes. There were no other complications. Twenty three animals completed the 46 planned experiments as shown in Table 1.

Results for all study groups are given in Table 2. For Group A (transection/repair control, no vincristine), the mean LAP for the 8 experiments in this study was 54.9% of pretreatment baseline, consistent with the result reported previously.^{12} Group B (vincristine injection at time zero) LAP results were also similar to the previous study with a mean value of 70.9%. These previous results were added to the new data from the present study to create larger comparison groups and strengthen the statistics, as delineated in Table 2. These pooled results were used for subsequent comparisons as well as for the plot in Figure 1.

Group C, (vincristine injection after a three month delay) showed the same improvement in adductor function as Group B with no delay. It is reasonable to surmise that other delay times less than 3 months would give similar results. However, group E (5 month delay) did not show any vincristine benefit, and group D (4 month delay) had an intermediate result that was not statistically different from the no-vincristine control group (A). Thus, the data

support vincristine blockade of the PCA as an early intervention that may improve ultimate adductor function after RLN injury if given within three months of injury.

Group F dogs received vincristine injections at time zero and then were allowed 12 months of healing prior to data collection, in order to assess a longer-term result. This group exhibited remarkably complete recovery of adductor strength, with LAP measures that were even higher than pre-treatment values. This degree of recovery cannot be accounted for by PCA blockade alone, but instead suggests there must have been adductor reinnervation contributions from other nearby nerves (that are somehow activated when the RLN is stimulated). A suitable non-vincristine 12 month control group is needed to better explore this possibility.

Group G was studied to confirm the previous finding in the rat model that vincristine injection does not affect muscles that are already innervated. As seen in Figure 1, the LAP in these four experiments was essentially unchanged from pretreatment baseline by the injection. It also confirms that the LAP methodology is repeatable and reliable.

Mean vincristine levels are shown in Figure 2. It can be seen that the peak plasma level following intramuscular PCA injection is 19% of the maximum level determined by direct intravenous injection, suggesting that 81% of the vincristine remained within the muscle. The levels followed a typical logarithmic decay with an excellent fit ($R^2 = 0.985$), with the animal clearing 7% of the circulating vincristine per hour (the exponent in the line of best fit).

DISCUSSION

This study demonstrates that an early (3 months post-injury) vincristine injection to the PCA muscle can effectively block synkinetic reinnervation of that antagonistic muscle, potentially leading to more complete or stronger adductor recovery. Current interventions for treating UVFP focus on moving the vocal fold to a more medial position; blocking reinnervation of the antagonist PCA muscle by vincristine injection is a simple approach that may achieve this without the need for more invasive procedures such as thyroplasty.

This study did not formally assess vocal fold position, but laryngoscopy at the terminal procedure did suggest a more medial resting position, and many of the dogs could actively close the vocal folds completely despite the bilateral RLN transection injuries and generate a loud bark. The stimulated LAP was chosen as a proxy for adductor function because it can be repeated over time without regard to subtle changes in the healing milieu that might affect other measures. For example, it is observed during these experiments that sanguinous collections around the electrode can alter the stimulation threshold of the RLN. The use of supramaximal stimulation overcomes this potential problem; the stimulating current can simply be increased as needed to stay supramaximal. The stimulated laryngeal adduction is a non-physiologic construct; in nature, the adductor muscles and PCA muscle likely never simultaneously co-contract maximally. But this experimental design enables a reliable repeat measure of the function of interest. It is unknown how the measured changes in LAP will

translate into clinical benefit, i.e., whether a 10-20% increase in adductor pressure will provide meaningful benefit to patients. This can only be determined with a clinical trial.

The hypothesis that there is a therapeutic window of opportunity was confirmed by these data. The measured benefit of vincristine injection was the same if the drug was given at the time of nerve injury as it was if given three months later, but not four or more months later. This window is likely a function of the site of injury. The lesion in this study was created 4cm inferior to the cricothyroid joint; lesions that are more proximal, such as in the thoracic cavity, can be expected to require more time for the axons to grow over the longer distance to reach the laryngeal muscles, and the window of opportunity would be expected to stay open a little longer, perhaps to four months. The canine model is a good model for the expected time frame of recovery in human subjects with RLN injuries, and these findings are likely to translate well to human trials. It would be reasonable to use a three month window as the limit for the first human studies of this “new paradigm.”

There are occasional situations when the otolaryngologist does have a chance to treat a patient on the same day as an RLN injury. Sometimes the RLN is sacrificed in thyroid cancer surgery; sometimes it is transected intentionally in order to perform an end-to-end anastomosis for a laryngeal reinnervation procedure. In these situations, a vincristine injection could be performed as a part of the surgical therapy, and the potential benefit demonstrated by the “time zero” injection groups in this study would apply.

A vincristine blockade of the PCA muscle would also prevent non-synkinetic reinnervation of the PCA (although the vincristine block is incomplete). If this approach is applied clinically, it must be used with the knowledge that some of the “normal” reinnervation of the PCA muscle that might have otherwise occurred will be relinquished. An incomplete block is likely preferable, as some PCA tone is desirable to stabilize the arytenoid on the cricoid. Antero-inferior displacement of the arytenoid was not seen in any of the animals in this study. Vincristine is associated with certain side effects when used for chemotherapy; however, the usual human adult systemic dose of $1.4\text{mg}/\text{m}^2$ (2.6mg for a 70kg patient) daily is substantially more than the single intramuscular dose of 0.4mg used in this study (the recommended limit in dogs is $0.75\text{mg}/\text{m}^2$, or 0.6mg for the dogs in this study). If patients undergo this treatment they will have to be monitored for toxicities, but the risk seems low.

In order to evaluate this intervention in a clinical trial, some additional steps will be needed: 1) completion of histologic study of harvested PCA muscles and overlying mucosa, to demonstrate lack of local reaction to intramuscular injection (grossly, there appears to be no reaction); 2) approval from FDA to use intramuscular vincristine in a human trial (currently it is approved for intravenous use only); 3) appropriate IRB approval with safety monitoring committee. These steps have been initiated. It would be preferable to administer these injections to patients transorally via laryngoscopy, which may require general anesthesia for location certainty.

CONCLUSION

In the canine model, a single vincristine injection to the PCA muscle shortly after RLN transection and reanastomosis leads to improved adductor function at 6 months post-injury. The injection needs to be given within three months of the nerve trauma; vincristine injection into an innervated muscle has no significant effect. This approach may offer a simple means to prevent some synkinesis and to reduce the number of unilateral vocal fold paralysis patients that ultimately need more involved rehabilitation procedures such as thyroplasty.

ACKNOWLEDGEMENT

This research was supported in part by the Analytical Pharmacology Core of the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins (NIH grants P30 CA006973 and UL1 TR 001079) and the Shared Instrument Grant (1S10RR026824-01). Grant Number UL1 TR 001079 is from the National Center for Advancing Translational Sciences (NCATS), a component of the National Institutes of Health (NIH) and NIH Roadmap for Medical Research, and its contents are solely the responsibility of the authors and do not necessarily represent the official view of NCATS or NIH. We thank Michelle Rudek-Renaut, Pharm.D., Ph.D., and her staff for the vincristine level determinations.

This work was supported by NIH grant R01DC010884.

REFERENCES

1. Benninger MS, Crumley RL, Ford CN, et al. Evaluation and treatment of the unilateral paralyzed vocal fold. *Otolaryngol. Head Neck Surg.* 1994; 111:497–508. [PubMed: 7936686]
2. Paniello RC. Laryngeal reinnervation. *Otolaryngol. Clin. North Amer.* 2004; 37:161–181. [PubMed: 15062692]
3. Misono S, Merati AL. Evidence-based practice: evaluation and management of unilateral vocal fold paralysis. *Otolaryngol. Clin. North Amer.* 2012; 45:1083–1108. [PubMed: 22980687]
4. Maronian N, Waugh P, Robinson L, Hillel A. Electromyographic findings in recurrent laryngeal nerve reinnervation. *Ann. Otol. Rhinol. Laryngol.* 2004; 113:341–8. [PubMed: 15174759]
5. Crumley RL. Laryngeal synkinesis: its significance to the laryngologist. *Ann. Otol. Rhinol. Laryngol.* 1989; 98:87–92. [PubMed: 2916830]
6. Paniello RC. Vocal fold paralysis: improved adductor recovery by vincristine blockade of posterior cricoarytenoid. in press.
7. Paydarfar JA, Paniello RC. Functional study of four neurotoxins as inhibitors of post-traumatic nerve regeneration. *Laryngoscope.* 2001; 111:844–850. [PubMed: 11359163]
8. Yian CH, Paniello RC, Spector JG. Inhibition of motor nerve regeneration in a rabbit facial nerve model. *Laryngoscope.* 2001; 111:786–791. [PubMed: 11359156]
9. McRae BR, Kincaid JC, Illing EA, Hiatt KK, Hawkins JF, Halum SL. Local neurotoxins for prevention of laryngeal synkinesis after recurrent laryngeal nerve injury. *Ann. Otol. Rhinol. Laryngol.* 2009; 118:887–93. [PubMed: 20112524]
10. Dahm JD, Paniello RC. Tracheostomy for long-term laryngeal experimentation. *Otolaryngol. Head Neck Surg.* 1998; 118:376–380. Paniello, R.C. and West, S.E.: Laryngeal adductory pressure as a measure of post-reinnervation synkinesis. *Laryngoscope* 109:447–451, 2000. [PubMed: 9527120]
11. Paniello RC, West SE. Laryngeal adductory pressure as a measure of post-reinnervation synkinesis. *Ann. Otol. Rhinol. Laryngol.* 2000; 109:447–451. [PubMed: 10823472]
12. Paniello RC, Rich JT, Debnath I. Laryngeal adductor function in experimental models of recurrent laryngeal nerve injury. in press.

Figure 1.

Mean LAP values for 7 study groups at endpoint (6 months for all groups except F), shown as the percentage of pre-treatment baseline measurement. For groups B-F, statistically significant difference from group A is indicated by a *. Group G is compared with the baseline value. NS, not significant.

Fig. 2.
Mean plasma levels from two dogs following vincristine injection into both PCA muscles.
Top, linear scale; bottom, log scale.

Table 1

Experiments groups & details. DOS, date of surgery.

Group	Description	N	RLN Surg	Vinc inj	Final data
A	control - no vinc	8	+	none	6 mo
B	vinc time zero	4	+	DOS	6 mo
C	delayed vinc	8	+	3 mo	6 mo
D	delayed vinc	8	+	4 mo	6 mo
E	delayed vinc	8	+	5 mo	6 mo
F	long term	4	+	DOS	12 mo
G	control - no surg	6	–	DOS	6 mo
		46			

Table 2

Mean laryngeal adductor pressures (LAP).

Group	Description	notes	Completed N	Mean LAP	±SD	p (vs A)
A	control - no vinc	<i>a</i>	8	0.549	0.145	
A	control – no vinc	<i>b</i>	8	0.561	0.110	
A	control – no vinc	<i>c</i>	16	0.555	0.125	
B	vinc time zero	<i>a</i>	4	0.709	0.202	
B	vinc time zero	<i>b</i>	8	0.731	0.064	
B	vinc time zero	<i>c</i>	12	0.724	0.103	<i>0.0003</i>
C	vinc 3 mo delay	<i>a</i>	5	0.747	0.191	<i>0.047</i>
D	Vinc 4 mo delay	<i>a</i>	6	0.656	0.203	0.157
E	Vinc 5 mo delay	<i>a</i>	7	0.586	0.278	0.393
F	long term	<i>a</i>	4	1.110	0.247	<i>0.039</i>
G	control - no surg	<i>a</i>	6	0.956	0.176	<i>0.0007</i>

p- values from student's t-test comparing study group with combined result from Group A.

a data from this study;*b* data from prior study{6};*c* combined data from this study and prior study.