Toxicity Trial of Canine Posterior Cricoarytenoid Intramuscular Vincristine Injections

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INTRODUCTION

Vincristine is a vinca alkaloid chemotherapeutic agent that has been in clinical use for over 50 years to treat a variety of malignancies. In the early days of its use, some cases of extravasation of intravenous vincristine injection were found to cause sloughing and necrosis of overlying tissue, causing it to be labeled a desiccant and to be approved by the Food and Drug Administration (FDA) for intravenous use only.

In 2001, we reported a study in rats in which a single intramuscular injection of vincristine effectively blocked reinnervation of the target muscle.1 We proposed that this strategy could be used to prevent unwanted reinnervation that may occur following a motor nerve injury and its random spontaneous recovery, based on its ability to bind tubulin and prevent microtubule formation that is needed for nerve ingrowth. Follow-up studies showed that this strategy was successful in a rabbit facial nerve injury model2 and in a canine recurrent laryngeal nerve (RLN) injury model.3,4 Of note, local toxicity from the intramuscular (IM) vincristine injections was not seen in any of the rats, rabbits, or dogs used in these studies. Halum et al. and McRae et al. also reported success using IM vincristine in a rat larynx model without toxicity at low doses.5,6

A number of case reports have been published in which vincristine was inadvertently delivered as IM injections to patients, without untoward side effects.7–11 Before embarking on a clinical trial using IM vincristine in human larynges, we performed a formal toxicity trial in the canine larynx model to confirm the safety of vincristine injections into the posterior cricoarytenoid (PCA) muscle.

MATERIALS AND METHODS

Sixteen female purpose-bred mongrel hounds, average weight 19.9 kg (range, 18–24 kg), were obtained and housed in...
a facility approved by the American Association for Accreditation of Laboratory Animal Care. The study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the National Institutes of Health 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 of Washington University School of Medicine. The study design followed FDA guidelines for a formal toxicity trial\(^{12}\) for a new route of administration and a new indication for a previously approved drug, in consultation with FDA officials.

**Test Doses**

Two vincristine doses and two time points were planned, resulting in four study groups of four dogs each. The lower dose selected was 0.4 mg, which is the same dose that was used in the previous canine studies.\(^3\,^4\) The maximum injectable IM dose was determined by first injecting methylene blue into the PCA muscle of a series of eight fresh canine cadaver larynges. It was found that the methylene blue began to extrude from the PCA muscle at a volume of 0.7 to 0.8 mL in all cases (Fig. 1). Because the vincristine was prepackaged as a 1 mg/mL solution, the concentration could not be increased, so 0.6 mg was selected as the higher test dose. The two time points were 24 hours and 14 days, as recommended by the FDA. One of the 24-hour dogs could not be sacrificed as scheduled and was switched to the 14-day group.

**Initial Procedure**

Under general anesthesia, direct suspension laryngoscopy was performed, exposing the posterior larynx. The side for vincristine injection was randomly chosen, and the planned dose was injected IM into the PCA muscle. The opposite side was injected with the same volume of normal saline solution. The time of the vincristine injection was recorded.

A series of blood samples were collected to determine the pharmacokinetics of the IM vincristine. All dogs had samples drawn at 15 minutes postinjection, then in the afternoon of day 0 and the morning of day 1, with the blood draw times carefully documented. The 14-day dogs had additional samples drawn the afternoon of day 1 and the morning of day 2. On the day of sacrifice, the 14-day dogs were given the same dose again, intravenously, and a blood sample was drawn 15 minutes later; this was considered the maximum blood level for that dog. Each sample was spun down by centrifuge to separate the plasma, which was frozen and stored until all samples were collected. Samples were then shipped to the analytic pharmacology lab at Johns Hopkins for determination of vincristine levels.

**Terminal Procedure**

At 24 hours or 14 days, the dogs were anesthetized and direct laryngoscopy was performed, with photodocumentation of the posterior laryngeal mucosa (injection site) obtained. The neck was opened and the recurrent laryngeal nerves were...
exposed. Harvard electrodes were applied and connected to a constant-current nerve stimulator. Stimulation of each RLN resulted in movement of the vocal fold, which varied by stimulus intensity and frequency; these movements were documented by videoendoscopy through the laryngoscope. Strength of adduction was measured using laryngeal adductor pressure as previously described. Briefly, the squeezing force exerted on an endotracheal balloon passed between the vocal folds was measured with a force transducer and compared with historic controls.

After data collection was complete, dogs were sacrificed by lethal injection, and the larynx was harvested and placed in formalin for histologic processing. The region of the PCA muscle with some underlying cricoid cartilage and overlying posterior laryngeal mucosa was sectioned from both sides (Fig. 2). Sections were stained with hematoxylin and eosin and examined by our pathologist (R.C.), who was blinded to the assigned study groups.

RESULTS

All dogs survived the experiment with no apparent complications. On endoscopy, there was no mucosal ulceration or other gross change seen in any of the dogs at either time point (Fig. 3). At the time of the exam, we were unable to distinguish which side had received the vincristine and which side had received the saline injection.

When the recurrent laryngeal nerves were stimulated, normal vocal fold movement was observed on both sides in all 16 dogs. Mean laryngeal adduction pressures for the vincristine side were within 610% of the saline side for all four groups and within 65% for three of the groups. This is within the normal range of variability for these measurements.

The histologic findings are shown in Table I. A moderate-to-marked degree of acute inflammation was only seen in the high-dose, 24-hour group and not in the lower-dose or 14-day group (Fig. 4). There were no abnormalities seen in the cricoid cartilage. All of the saline-injected sides had normal histologic findings in all tissue layers.

The plasma levels taken 15 minutes after the IM injections averaged 0.93 ng/mL for the 0.4-mg injection groups and 1.62 ng/mL for the 0.6-mg groups; the overall average was 1.28 ng/mL. For the nine dogs that had intravenous injections given at 14 days, the levels were 10.95 ng/mL for the 0.4-mg dogs and 13.54 ng/mL for

<p>| TABLE I. Histopathologic Findings on Vincristine-Injected Side (Left or Right as Indicated) in 16 Study Animals. |</p>
<table>
<thead>
<tr>
<th>No.</th>
<th>Mucosa</th>
<th>Muscle</th>
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<tr>
<td>24 hours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1L</td>
<td>Min ACI</td>
<td>Mild Al</td>
</tr>
<tr>
<td>2R</td>
<td>Mild ACI</td>
<td>Min CI</td>
</tr>
<tr>
<td>10L</td>
<td>Ni</td>
<td>Ni</td>
</tr>
<tr>
<td>11R</td>
<td>Mild CI</td>
<td>Ni</td>
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<tr>
<td>14 days</td>
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</tr>
<tr>
<td>3L</td>
<td>Min CI</td>
<td>Ni</td>
</tr>
<tr>
<td>4R</td>
<td>Min CI</td>
<td>Ni</td>
</tr>
<tr>
<td>5L</td>
<td>Min CI</td>
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</tr>
<tr>
<td>8R</td>
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<td>Mild CI</td>
</tr>
<tr>
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<td>Ni</td>
<td>Ni</td>
</tr>
<tr>
<td>12R</td>
<td>Mild ACI</td>
<td>Ni</td>
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<tr>
<td>13R</td>
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ACI = acute and chronic inflammation; AI = acute inflammation; CI = chronic inflammation; L = left; R = right; Min = minimal; Mod = moderate; Nl = normal.

Fig. 4. Sample histology slides. (Left) Low power. (Right) High power. (Top) Dog 14R (24 hours, 0.6 mg) showing moderate acute inflammation. (Bottom) Dog 13R (14 days, 0.6 mg) showing normal histology with complete resolution of inflammation.
CONCLUSION  
Intramuscular injections of vincristine, injected into the PCA muscle, did not show any significant local toxicity at the doses tested in this canine study. There was no mucosal ulceration and no effect on movement of the cricoarytenoid joint or adductor muscle strength. Local acute inflammatory changes, seen primarily at the higher dose tested, were largely resolved at the secondary time interval of two weeks. These findings suggest that IM vincristine may be safe to use clinically as an inhibitor of reinnervation as proposed. A phase I clinical trial would be the appropriate next step.

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BIBLIOGRAPHY  

This study suggests that fears of injecting vincristine into the laryngeal muscles due to local toxicities may be ill founded. The proposed use of vincristine as an inhibitor of reinnervation involves only a single injection, and our prior studies found this effect was achieved with the lower dose tested in this toxicity trial. The single dose of 0.4 mg, or even 0.6 mg, is much lower than the vincristine dose that would typically be given as a cancer chemotherapeutic drug. It is possible that prior patient experiences with local toxicities from this drug were caused by much higher doses or concentrations than were used in this study. If an intended IM injection at this dose was inadvertently given intravenously, it would be a subclinical dose and thus would not be expected to cause any systemic toxicity.

This animal study cannot determine whether there was any local effect that would make a PCA muscle injection uncomfortable for the patient (i.e., by causing pain or other irritation). There was some local acute inflammatory change seen on histology suggesting that there may have been some short-term irritation from the injection, although not enough to cause ulceration or tissue slough. The dogs in this study did not show any outward signs of discomfort, but if they were experiencing sore throats, for example, we might not be able to detect it. The injections did not cause the dogs to stop eating or drinking and they did not lose any weight or have other signs of throat problems. But the only way to be certain that patients will tolerate IM injections into the PCA muscle would be to carry out a phase I clinical trial, with gradually increasing doses. The potential value of the proposed new application of vincristine in reducing postreinnervation synkinesis makes such a clinical trial attractive. Based on the sizes of the PCA muscles in adult humans compared with the canines in this study, we would anticipate the effective human dose would be about 0.5 mg; but a phase I safety trial would start at a much lower dose and gradually escalate to this level.

DISCUSSION

All 16 dogs had samples drawn at the end of the day of injection (a few hours later), and 14/16 had vincristine levels below the limit of quantitation (i.e., <0.2 ng/mL). For the nine dogs that had samples drawn for 2 days, only one had any detectable vincristine in the last plasma sample.

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