Investigation of a maxillary nerve block technique in healthy New Zealand White rabbits (Oryctolagus cuniculus)

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Received November 12, 2019.
Accepted March 17, 2020.

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OBJECTIVE
To investigate use of a candidate maxillary nerve block in rabbits.

ANIMALS
13 healthy New Zealand White rabbits (Oryctolagus cuniculus).

PROCEDURES
In phase 1, the maxillary nerve block procedure was performed in 7 sedated rabbits with 2 volumes (0.25 and 0.5 mL) of a saline (0.9% NaCl)–tissue marker dye solution (1 injection/side by random assignment). Rabbits were euthanized and dissected; numeric scales were used to rate injection accuracy and extent of staining. In phase 2, the nerve block was performed with articaine hydrochloride–epinephrine solution (0.5 mL) on a randomly assigned side in 6 sedated rabbits, with the contralateral side used as a control. Sensory function of the relevant dermatome was tested in triplicate with an algesiometer 0, 30, and 90 minutes after recovery from sedation. Statistical methods were used to compare results between injection volumes (phase 1) and between treated and control sides (phase 2).

RESULTS
In phase 1, dye was in contact with the targeted nerve after 13 of 14 injections. Accuracy and extent of staining did not differ significantly between volumes. In phase 2, algesiometer-applied force tolerance differed significantly between treated and control sides 30 minutes after recovery from sedation (56 to 145 minutes after the nerve block procedure). No adverse effects were detected in either study phase.

CONCLUSIONS AND CLINICAL RELEVANCE
The described technique for a maxillary nerve block was accurate and effective for desensitization of the relevant dermatome as assessed by algesiometry in healthy rabbits. Additional studies are needed to assess use of this procedure in rabbits of other breeds and its efficacy for clinical use. (Am J Vet Res 2020;81:843–848)
suggested for rabbits.\textsuperscript{8,13} However, small skull size, unique craniofacial anatomy, and an inability to access and identify intraoral anatomic structures make these techniques impractical in rabbits.\textsuperscript{2} Species variations in skull anatomy make the infraorbital foramen difficult to locate. In addition, results from a pilot study performed by the authors revealed that the infraorbital nerve (a continuation of the maxillary nerve) occupied most of the infraorbital canal so that any attempt to block the nerve via the opening of the infraorbital foramen might be associated with a substantial risk of direct damage to the nerve. The bone plate between the infraorbital canal and nasal cavity is also extremely thin and cavitated. During attempts to access the maxillary nerve with a retrograde infraorbital approach in the pilot study, the needle frequently migrated into the nasal cavity, potentially rendering this technique unsuccessful. Those preliminary results suggested that the approach to this nerve as recommended for dogs is difficult to perform in rabbits and could be associated with complications.

The purpose of the study reported here was to investigate a novel approach for a maxillary nerve block in rabbits in 2 study phases. In the first phase, we aimed to assess the injection technique and extent of injectate distribution at 2 volumes (0.25 and 0.5 mL), and in the second phase, we sought to determine the efficacy of this technique with a local anesthetic (volume selected in phase 1) as assessed by algesiometry. The hypotheses were that the larger volume of injectate would have greater accuracy (with success defined as injectate in contact with the maxillary nerve) than the smaller volume in phase 1 and that the algesiometer forces tolerated following a unilateral maxillary nerve block procedure would be significantly greater than the forces tolerated on the contralateral (untreated) side for the same rabbits in phase 2.

**Materials and Methods**

**Animals**

Thirteen healthy adult female New Zealand White rabbits (*Oryctolagus cuniculus*) were used in the study (7 in phase 1 and 6 in phase 2). Prior to experiments, rabbits were deemed healthy on the basis of physical examination findings. The study procedures were approved by the Cornell University Institutional Animal Care and Use Committee (No. 2013-0077). Food and water were not withheld during the study.

**Phase 1**

Seven healthy female New Zealand White rabbits (mean ± SD weight, 4 ± 0.21 kg) were included in the evaluation of the candidate maxillary nerve block injection technique. Two volumes (0.25 and 0.5 mL) were used. One volume was randomly assigned (by a coin toss) to the left or right side so that each rabbit received a 0.25-mL injection in the targeted region on one side of the maxilla and a 0.5-mL injection on the contralateral side. Saline (0.9% NaCl) solution\textsuperscript{a} mixed with a permanent tissue-marking dye\textsuperscript{b} was used as injectate. Each rabbit was sedated with a combination of dexmedetomidine hydrochloride\textsuperscript{c} (30 µg/kg, IM) and ketamine hydrochloride\textsuperscript{d} (5 mg/kg, IM). Once the rabbits were deemed sedated, the candidate maxillary nerve block approach was used for injection of the saline-marker dye solution. One investigator performed all injections (LC).

A 25-gauge, 5/8-inch needle\textsuperscript{e} attached to a T-port\textsuperscript{f} and a 1-mL syringe\textsuperscript{g} were used for the injections. The puncture site was located subzygomatically, 2 to 3 mm caudal to the facial tuber by palpation. The needle was directed dorsally, medially, and caudally until contact with the alveolar bulla was felt. The needle was subsequently walked off caudally until the caudal aspect of the alveolar bulla was felt. At this point, the needle was advanced medially approximately 1 mm to deposit the injectate at the lateral aspect of the maxillary nerve, caudal and medial to the alveolar bulla. An image is provided to show the anatomic landmarks and needle orientation used in a rabbit cadaver (Figure 1).

After all injections were completed, the rabbits were euthanized by IV administration of an overdose of pentobarbital.\textsuperscript{h} Immediately after euthanasia, the heads were dissected to evaluate the accuracy and distribution of the injections. Measurements were made by an observer who was blinded to the injectate volumes (TP). Numeric rating scales were used to subjectively assess the accuracy (location relative to the maxillary nerve) of each injection and the extent to which the injectate stained the nerve and surrounding tissues (Appendix). Additionally, visual evaluation of possible complications such as vessel laceration or unwanted injectate migrations was conducted.

**Phase 2**

Six healthy female New Zealand White rabbits (mean ± SD weight, 3.9 ± 0.23 kg) were used to evaluate effectiveness of the candidate maxillary nerve block with a local anesthetic. Each rabbit was sedated with the same combination of medications and the same doses as described for phase 1. A maxillary nerve block was subsequently performed as previously described on a side chosen at random (by coin toss). The contralateral side served as the control for the same rabbit. A solution of 4% articaine hydrochloride with epinephrine\textsuperscript{i} (0.5 mL; approx 5 mg of articaine/kg determined on the basis of mean body weight) was used as injectate. The volume was chosen on the basis of findings in phase 1. All injections were performed by the same individual who performed the injections for phase 1.

Once rabbits spontaneously recovered from sedation (defined as having normal posture, mentation, and activity level), each rabbit was blindfolded and gently positioned by an assistant who held the blindfold in place, and a previously calibrated electronic von Frey-
Type algesiometer \( j,k \) was applied at the maxillary gingival mucosa next to the incisor teeth. The applied force was gradually increased until a purposeful response was obtained or a maximum force of approximately 250 g was reached. The force at which the animal first purposefully responded (ie, head movement away from the person performing the test) was recorded. This process was repeated in triplicate on both sides of the mouth, and the mean of the readings was calculated. Gingival sensitivity was tested at the time of recovery from sedation (0 minutes; T0) and 30 (T1) and 90 (T2) minutes later. Additionally, rabbits were monitored for complications such as abnormal facial rubbing, hematomas, and signs of oral or ocular trauma for 1 hour after the last algesiometry test.

**Statistical analysis**

Data were analyzed for normality with the Shapiro-Wilk test. Results for parametric data are reported as mean ± SD, and results for nonparametric data are reported as median and range. A nonparametric test for paired data (the Wilcoxon signed rank test) was used to analyze whether the numeric rating scores for accuracy and extent of staining in phase 1 were significantly different between the 2 candidate injectate volumes. In phase 2, the effects of treatment, time, and treatment-by-time interaction on algesiometry results were assessed with mixed-effect models, with rabbit as the random effect and time and treatment (and their interaction) as fixed effects. Values of \( P \leq 0.05 \) were considered significant. Statistical analyses were carried out with commercially available software.\(^{1,m}\)

**Results**

**Phase I**

Accuracy evaluation indicated maxillary perineural infiltration was successful (grade 2) in 13 of 14 injection sites. The median (range) grade was 2 (0 to 2) for the 0.5-mL injection, and all 0.25-mL injections had an accuracy grade of 2. An example of a successful injection is shown (Figure 2). One injection (0.5 mL) in 1 rabbit was scored as a failure (grade 0). The median (range) grades for extent of staining were 1 (0 to 1) and 1 (0 to 2) for the 0.5- and 0.25-mL volumes, respectively. The injection considered a failure for accuracy was also considered a failure for extent of staining. No differences were found between the 0.25- and 0.5-mL volumes for accuracy (\( P = 0.5 \)) or extent of staining (\( P = 0.5 \)) grades. No evidence of complications was found at any of the 14 injection sites on gross examination.

**Phase 2**

The median time elapsed between administration of sedatives and administration of the candidate maxillary nerve block with articaine-epinephrine solution was 22 minutes (range, 19 to 25 minutes). The median times from completion of the nerve block to T0, T1, and T2 were 41 minutes (range, 31 to 51 minutes), 77 minutes (range, 56 to 145 minutes), and 145 minutes.
minutes (range, 116 to 220 minutes), respectively. Significant effects of time (P = 0.008) and treatment (P = 0.004) were observed for the algesiometry results.

On slice analysis, the maximum applied force was significantly different between the treated and untreated sides at T1 (P = 0.026; Figure 3). No complications were found at the maxillary dermatome in any rabbits. No other complications were observed.

Discussion

The present study was performed to investigate an anatomic landmark-based injection technique for a candidate maxillary nerve block with 2 different injectate volumes in healthy adult New Zealand White rabbits and to evaluate the efficacy of the block with a local anesthetic by use of a von Frey-type algesiometer. The described technique was determined to be accurate and, when performed with a local anesthetic (4% articaine solution with epinephrine), was associated with significant desensitization of the associated dermatome in the study rabbits at a time point 30 minutes after rabbits were deemed recovered from sedation (T1; measured 56 to 145 minutes after the nerve block procedure). No adverse effects associated with the injection procedure or the injectate were noted in either phase of the study.

The results of phase 1 of the study indicated that the injection method was accurate for both volumes used in 6 of 7 rabbits (13/14 procedures). No significant differences in the subjectively assessed grades for accuracy and extent of staining were detected between the 0.25- and 0.5-mL volumes in phase 1, and the larger volume was chosen for phase 2 of the study to prolong the limited duration of action for the chosen local anesthetic. Articaine is commonly the local anesthetic of choice in human dentistry because of its capacity to penetrate bone tissue and its short duration of action. This particular local anesthetic was used in the present study owing to its availability from a previous study. The articaine manufacturer’s published toxic dose for rabbits is 80 mg/kg. Our phase 1 results indicated that an injection of 0.25 mL had an accuracy and extent of perineural infiltration similar to those for the 0.5-mL injection, suggesting that if bilateral maxillary nerve blocks are needed, use of the smaller volume bilaterally might yield similar results while keeping the overall dose the same as for the single 0.5-mL injection; however, the effects and duration of a maxillary nerve block with the smaller volume were not evaluated in the present study, and further research would be needed to confirm this.

In phase 2 of the study, results indicated that the maxillary nerve block effectively desensitized the relevant maxillary nerve dermatome as assessed by algesiometry at 1 measurement time (T1) 30 minutes after rabbits recovered from sedation. This suggested the maxillary nerve block approach used in this study may be beneficial in providing short-term analgesia to rabbits affected by orofacial pain in this region. The lack of a significant difference in algesiometer force tolerance between the treated and untreated sides at the time of recovery may have been attributable to the relatively small cohort of rabbits enrolled in phase 2 of the study or to some remaining analgesic effects from the drugs used for sedation masking a difference. However, results of a post hoc power analysis suggested that the sample size of 6 rabbits was adequate to detect a significant difference. The lack of a significant difference between treated and untreated sides at the last measurement time (T2; 90 minutes after recovery from sedation) was most likely attributable to the relatively small cohort of rabbits enrolled in phase 2 of the study or to some remaining analgesic effects from the drugs used for sedation masking a difference. Considering that T2 measurements took place a median of 145 minutes (range, 116 to 220 minutes) after the nerve block was performed, it was possible that the effect of the local injection had decreased to an undetectable level.

Variability in the algesiometer readings was a limiting factor in the study reported here. It has been shown that repeated testing with this specific type of algesiometer (von Frey filament) can lead to changes
in sensitivity and reactions at lower pressures.\textsuperscript{24} It is possible that this results from learned behavior causing reactions to non-noxious stimuli because of annoyance or avoidance. Although the rabbits in the present study were blindfolded with the intent of decreasing the possible learned behaviors, repeated probing of 1 region of the mucosa may also cause injury to the tissue and local inflammation. This can lead to hyperalgesia and bias the results. This bias can be reduced when a similar testing pattern is used for the controls and each animal serves as its own control. However, an apparent decrease in the amount of force needed to elicit a reaction was observed on both the treated (blocked) and untreated sides at the 2 later time points. Additionally, we decided not to include a period of acclimatization to the algometer or to make baseline measurements but introduced a cutoff in the force applied to minimize tissue inflammation and damage. This cutoff was arbitrarily chosen on the basis of previously reported mechanical withdrawal thresholds in rabbits (60 to 80 g),\textsuperscript{24} approximately tripled for the study. Interestingly, Renno et al\textsuperscript{26} reported a maximum pressure withdrawal threshold of up to 250 g in rabbits following a (sciatic) nerve block with a liposomal formulation of local anesthetic.

No gross evidence of injection site hemorrhage, obvious nerve or muscle damage, orbital penetration, or other complications were detected during either study phase. This result could not completely eliminate concerns related to use of the nerve block as performed in our study, as even commonly performed nerve blocks in human and animal medicine can lead to complications.\textsuperscript{27–29} The candidate maxillary nerve block was extensively studied and practiced on rabbit cadavers prior to its use in the study rabbits. Although no complications were found during the study, complications such as hemorrhage or orbital penetration may occur if the procedure is improperly performed.

New Zealand White is a medium-sized rabbit breed, and these animals are commonly used in laboratory settings. The anatomy and physiology of this breed have been well documented.\textsuperscript{50} In contrast, pet rabbits can vary widely in size. The American Rabbit Breeders Association recognizes 49 different breeds of rabbits ranging from Netherland dwarfs, which typically weigh <1 kg, to Flemish giants, which can weigh up to 10 kg.\textsuperscript{31} The anatomic variations of the maxillofacial anatomy among rabbit breeds has not been well researched. In addition, the small skull size of many pet rabbit breeds may make landmark identification difficult so that use of the technique and supplies described here could lead to potentially dangerous consequences. Further research is required to assess the effects of anatomic variations and the need to adjust the described maxillary nerve block technique for maximum efficacy while minimizing the potential for adverse effects.

Animals used in the present study were healthy and had no clinically relevant medical history. Rabbits with orofacial pain caused by processes such as endodontal or periodontal disease would be expected to benefit from a maxillary nerve block in a clinical setting. These animals may have changes in the supporting alveolar bone resulting from elongation of the reserve crown and tooth apices, destruction of the supporting bone secondary to infection, or both.\textsuperscript{10–12} These disease processes may alter craniofacial anatomy, disrupt blood flow, and alter the normal pathway of maxillary nerves, making it challenging to identify landmarks. This could increase the risk of damage to this nerve or other anatomic structures. Additional research is needed to assess the effects of local disease processes in soft tissues and bone structures at the proposed nerve block site and the need to adjust the described technique for maximum nerve block efficacy and minimization of the risk for potential adverse effects. Until our understanding of the behavior of this block is improved and the possibility of unanticipated local anesthetic migration and effects can be assessed, we cannot recommend the use of longer-lasting local anesthetics such as bupivacaine.

\textbf{Acknowledgments}

No third-party funding or support was received in connection with this study or the writing or publication of the manuscript. The authors declare that there were no conflicts of interest.


\textbf{Footnotes}

\begin{itemize}
  \item a. Hospira Inc, Lake Forest, Ill.
  \item b. Davison Marking System, Bradley Products Inc, Bloomington, Minn.
  \item c. Zoetis Inc, Kalamazoo, Mich.
  \item d. Henry Schein Animal Health, Dublin, Ohio.
  \item e. BD PrecisionGlide, Becton, Dickinson and Co, Franklin Lakes, NJ.
  \item f. Smiths Medical ASD, Dublin, Ohio.
  \item g. BD Tuberculin Slip Tip, Becton, Dickinson and Co, Franklin Lakes, NJ.
  \item h. Fatal-Plus Solution, Vortech Pharmaceuticals Ltd, Dearborn, Mich.
  \item i. Septicaine 4\% and epinephrine 1:100,000, Novococ Pharmaeutical of Canada Inc, Cambridge, ON, Canada.
  \item j. Almena 2450, Ahlborn, Woodland Hills, Calif.
  \item k. Von Frey Analgesia Meter 2390 series, IITC Life Science, Woodland Hills, Calif.
  \item l. Prism 6, GraphPad Software, La Jolla, Calif.
  \item m. JMP, version 13.1.0, SAS Institute Inc, Cary, NC.
\end{itemize}

\textbf{References}

\begin{enumerate}
  \item Jekl V, Redrobe S. Rabbit dental disease and calcium metabo-
\end{enumerate}
20. Septicaine 4% and epinephrine 1:100,000 [package insert].

### Appendix

Subjective grading system used to grossly assess the accuracy of a candidate maxillary nerve block technique and extent to which the injectate stained the maxillary nerve and surrounding tissues when the procedure was performed with 0.25 or 0.5 mL of a saline (0.9% NaCl)–tissue marker dye solution in healthy adult New Zealand White rabbits (*Oryctolagus cuniculus*).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>0</td>
<td>Failure: no dye present or dye present far from the maxillary nerve</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Partial success: dye in close proximity to, but not in direct contact with, the maxillary nerve</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Success: dye in direct contact with the maxillary nerve</td>
</tr>
<tr>
<td>Extent of staining</td>
<td>0</td>
<td>Low: nerve site partially stained in diameter or circumference</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Adequate: nerve site completely stained with minimal dye in surrounding tissues</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Excessive: nerve site completely stained; staining of substantial amounts of surrounding tissue</td>
</tr>
</tbody>
</table>

Rabbits (*n* = 7) were sedated for the procedure; each rabbit underwent injection of both volumes (1/side by random assignment) and was euthanized immediately after the injections for gross examination by an investigator who was blinded to the injectate volumes.