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Duration of Action of Bupivacaine Hydrochloride Used for Palatal Sensory Nerve Block in Infant Pigs

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Summary

Bupivacaine hydrochloride is frequently used in veterinary dental procedures to reduce the amount of general anesthesia needed and to reduce post-procedural pain. The aim of this study was to develop a novel method to test local anesthetic duration in mammals. Six infant pigs were placed under deep/surgical anesthesia with 3 % isoflurane and oxygen while 0.5 ml of 0.5 % bupivacaine hydrochloride was injected to block the two greater palatine and the nasopalatine nerves. They were then maintained under light anesthesia with 0.5–1.0 % isoflurane. Beginning 15-minutes after the injection, 7 sites in the oral cavity were stimulated using a pointed dental waxing instrument, including 3 sites on the hard palate. The response, or lack of response, to the stimulus was recorded on video and in written record. The bupivacaine hydrochloride injections lasted 1 to 3-hours before the animals responded to the sensory stimulation with a reflexive movement. This study provides evidence that bupivacaine used to anesthetize the hard palate has a relatively short and variable duration of action far below what is expected based on its pharmacokinetic properties.

Introduction

Bupivacaine hydrochloride^a is a long-lasting, amide-type local anesthetic commonly used for peripheral sensory nerve blocks to provide anesthesia and analgesia during dental procedures in animals.¹ Although the procedures are performed under general anesthesia, the use of a local anesthetic can help reduce the amount of general anesthesia needed and reduce post-procedural pain.^{2,3} Clinically, local anesthetics are regularly used during head and neck surgeries to reduce postoperative pain.^{2–7} Although bupivacaine hydrochloride is typically used, it is not known how long it is effective in practice since it is difficult to evaluate when sensation returns in an animal.

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The pharmacological properties documentation for bupivacaine hydrochloride describe its half-life as 2.7-hours in adults and 8.1-hours in human neonates^a. In infants, protein binding and clearance of bupivacaine hydrochloride is known to be reduced due to immature liver development.⁸ In dentistry, bupivacaine hydrochloride administered as a nerve block in adult humans has a duration of action of 6 to 8-hours, and 5 to 7-hours as a local infiltration.⁹ We hypothesize that bupivacaine hydrochloride would last longer in mammalian infants than adults based on its pharmacokinetic properties.

We aimed to develop a novel method of testing duration of local anesthesia by testing oral reflexes following palatal local anesthesia injection. Using an infant pig (*Sus scrofa*) model, we determined the duration of action of a peripheral sensory nerve block using 0.5 ml of 0.5 % bupivacaine hydrochloride to block the greater palatine and nasopalatine nerves to achieve anesthesia of the hard palate while simultaneously administering isoflurane, a general anesthetic.

Materials and Methods

This study included a total of 6 infant pigs, *Sus scrofa*, 2 to 3-weeks-old weighing 3.0 to 5.5 kg. At this age the animals are comparable to human infants 6-months to 1-year of age as judged by tooth eruption, weaning status, and skeletal development.^{10,11} This experimental design was adapted from a previous study that tested the area of skin desensitization following a mental nerve block in dogs.¹² All procedures were approved by John Hopkins University Institutional Animal Care and Use Committee (IACUC) [SW10M212].

General anesthesia was induced and maintained at stage III, plane III, or a deep/surgical anesthesia with 3% isoflurane and oxygen administered through a mask. The pigs were intubated and continued to receive a lower dose of isoflurane (0.5–1.2 %) until in stage III, plane I, or a lighter stage of anesthesia characterized by occasional movements of the legs, jaw, and tongue. Occasionally a blink reflex or swallow reflex was also observed.

The protocol started with sensory stimulation testing in the control state, prior to placement of the nerve blocks. We performed sensory stimulation testing by using a pointed dental waxing instrument^b to stimulate 7 different regions in the oral cavity, including three on the hard palate: (1) gingiva labial to the maxillary central incisor teeth (CI) at the mucogingival junction (MGJ), (2) gingiva buccal to the eruption site of the permanent maxillary first molar (M1) teeth at the MGJ, (3) gingiva labial to the mandibular CIs, (4) gingiva buccal to the site of the permanent mandibular first molar (M1) teeth at the MGJ (5) gingiva palatal to the maxillary CIs in the region of the incisive papilla on the hard palate, (6) gingiva palatal to the site of the permanent right maxillary M1 tooth on the hard palate and (7) gingiva palatal to the site of the permanent left maxillary M1 tooth on the palate (Fig. 1). Each location was tapped up to 5 times until a clear response was observed or “no response” was recorded. The stimulation testing was also recorded on video so that the responses could be reviewed and verified.

^b3PK Thomas Waxing Instrument, Hu-Friedy, Chicago, IL

During control sensory stimulation testing, a reflexive movement in response to stimulation was observed at the majority of these locations. A positive response was defined as a twitch in a nearby muscle, an extreme and abrupt movement of the head and legs, or a reflexive movement of the jaw.^{13,14} If no stimulation was observed, the concentration of inspired isoflurane was lowered stepwise and testing was repeated at each step until reflexive movements were seen in the majority of the locations. If the pigs moved significantly, the concentration of inspired isoflurane was increased. Vital signs including respiratory rate, temperature, heart rate, oxygen saturation, and electrocardiogram (ECG) were recorded regularly throughout the procedure. We adjusted the concentration of isoflurane to maintain constant heart rate and respiratory rate throughout the study.

After the control sensory stimulation testing, the inspiratory concentration of isoflurane was increased to 2–3.5 % to return the animal to the stage III, plane III, or a deep/surgical anesthesia, and the sensory nerve blocks were administered. The nasopalatine foramina, where the nasopalatine nerves exit the maxilla, are located just dorsal to the incisive papilla on the hard palate, palatal to the central incisor teeth. The greater palatine foramina, where the greater palatine nerves exit the maxilla, are located approximately 1-cm palatal to the maxillary M1 tooth. At the nasopalatine foramina and each greater palatine foramen, 0.5 ml of 0.5 % bupivacaine hydrochloride was injected slowly using a 25 g needle. For consistency, the same investigator (SDH) administered all regional blocks. The maximum dose recommended was 2.0 mg/kg¹⁵. As the animals were approximately the same weight, the total dose of bupivacaine hydrochloride was the same for each animal (7.5 mg). After the injections were completed, the animal was returned to stage III, plane I, or a lighter stage of anesthesia, for the sensory stimulation testing.

Starting 15-minutes following injection, the investigator (SDH) started the sensory stimulation testing and the location of the reflexive responses were noted. This was done every 10 to 30-minutes until a response was seen from any of the three regions of the palate. The amount of time between sensory testing was maximized to prevent over-stimulation of the site. Once a response was observed, testing was then performed every 10 to 20-minutes until a reflexive movement was seen in response to the palatal stimuli in at least 2 of the 3 locations for three consecutive sensory stimulation tests. At this point, the sensory nerve block was determined to be ineffective and the animal was allowed to recover. Three pigs underwent the same procedure twice, at least 3 days apart.

Two animals in this study, Pigs A and B, were also used for a feeding study. The local anesthetic testing described was performed twice each for pigs A and B, once before and once after the feeding study, 6-days apart. As part of the feeding study, we surgically implanted electromyographic (EMG) electrodes in several hyolaryngeal muscles, and radio-opaque markers on the hyoid bone, hard palate, and thyroid cartilage as well as in the tongue, and maxillary gingiva. A hemoclip^c was placed onto the epiglottis by an intraoral approach. After this surgery, the animals were administered ampicillin (8.0 mg/kg) and buprenorphine (0.01 mg/kg) BID, and metacam (0.1 mL, 0.1 mg/kg) SID.

^cPilling Weck, Research Triangle Park, NC

For Pig B's first sensory stimulation testing, the pig could not be intubated after repeated attempts by a veterinary technician and a veterinarian, so intravenous injections of propofol were given for sedation as recommended by the veterinarian. The pig was administered 10.0 mg, half the dose usually recommended to maintain light anesthesia, every 5 to 10-minutes. When the pig started moving more frequently and to a larger extent, the next dosage was administered. Sensory stimulation testing was always performed 5-minutes after the previous injection of propofol.

Pigs C, D, E and F were also used for a feeding study, and the duration of action of the nerve blocks was tested the day before that study commenced. Prior to testing the duration of local anesthetic action, a short (10-minute) surgery was performed to suture radio-opaque markers to the hyoid bone and thyroid cartilage. A hemoclip was placed onto the arytenoid cartilages by an intraoral approach. In Pigs C and F, additional radio-opaque markers were inserted intraorally into the maxillary gingiva, hard palate, soft palate, posterior pharyngeal wall, and tongue. After these markers were placed, the animals were administered the same postoperative medication protocol as described previously. Pig C underwent sensory stimulation testing twice, 4-days apart. The other three pigs could not be tested again due to the feeding study protocol.

Results

Bupivacaine hydrochloride's duration of action in the infant pigs ranged from 65 to 190-minutes, or approximately 1 to 3-hours (Fig. 2, Table 1). This is based on the time until the first response from any region of the hard palate. The average time of the last sensory stimulation test where there was no response in the hard palate was 94-minutes (SD: 39-minutes, range: 45 to 150-minutes) after bupivacaine hydrochloride was administered. The first response to the sensory stimulation test was seen an average of 119-minutes (SD: 39-minutes, range: 65 to 190-minutes) after bupivacaine hydrochloride injection (standard deviation: 39-minutes, range: 65 to 190-minutes). A consistent response in at least 2 of the 3 regions of the hard palate tested was seen an average 167-minutes (SD: 64-minutes, range: 115 to 310-minutes) after administering bupivacaine hydrochloride. The bupivacaine hydrochloride consistently lasted at least 1-hour in all pigs. Individual variation was clear, as well as variation between trials in the 2 animals with replicates. In a few instances, response to sensory stimulation testing could be elicited from only one location, and then during the next test there would be no response at the same location. There was always a response from the site buccal to the eruption site of the permanent maxillary M1 at the MGJ.

Discussion

Bupivacaine hydrochloride is a long-lasting local anesthetic drug commonly used in veterinary dental procedures to reduce intraoperative and post-procedure pain.^{2,3} The duration of action of bupivacaine hydrochloride was determined to be 1 to 3-hours in the infant pig before oral reflexes returned, which is substantially less than predicted based on its pharmacokinetic properties.

It is most likely that our method of sensory stimulation testing elicited oral reflexes that had low thresholds for excitation. When bupivacaine hydrochloride is administered, it first blocks the C fibers (pain), then A δ fibers (pain/temperature) and last A β fibers (mechanoreceptors). As it begins to be metabolized, sensation returns in the reverse order. The C fibers are pain and temperature receptors while the A β are mechanoreceptors. Bupivacaine hydrochloride's duration of action will be different depending on the outcome measured. It is unclear exactly which types of sensory fibers were stimulated during sensory testing when a reflexive response was observed. One explanation for the relatively short duration of action is that the oral reflexes observed were elicited by stimulating mechanoreceptors and not nociceptors. The duration of action may have been longer if we were measuring an outcome based on nociceptor stimulation. Alternatively, the short duration of action observed in our study relative to past studies could reflect variation in the metabolism rate of local anesthetics between infant pigs and infant humans, although this is unlikely based on their shared anatomy and development.

Variability in our findings may be explained due to a fluctuating level of inhalational anesthesia or physiological variation across individuals in how they metabolize bupivacaine hydrochloride. For Pig B, the second sensory testing was done with propofol instead of isoflurane which could have influenced the duration of action of bupivacaine hydrochloride. It is not known how general anesthetics can influence local anesthetic metabolism. There were instances during the sensory stimulation testing where a response was positive and then could not be elicited on subsequent testing. In one case, Pig E had a strong response (2 out of 3 sites) to the sensory stimulation test which could not be reproduced on two following tests. In this case, the site may have been over-stimulated and the animal stopped responding. The animal did eventually respond.

Local anesthetics are a valuable tool for animal researchers. In particular, local anesthetics have been used to understand the functional significance of sensory input from the oral cavity and pharynx during feeding.¹⁶ In order to use local anesthetics as a research tool, the agent's pharmacological properties and duration of action must be understood. Using the methods established in this study, any local anesthetic could be tested in any mammalian animal model. In the future, this will allow local anesthetic properties to be validated so that they can be used effectively in a wide variety of animal studies.

It is not known how the duration of action of bupivacaine hydrochloride in infant pigs will compare to adult pigs or other mammals. This study demonstrates a novel methodology for testing local anesthetic duration in mammals and could be performed in different species of different ages and weights to determine the range of variation. It is also noteworthy that the sensory testing procedure does not result in any visible damage to tissues, such as bleeding or swelling. Using local anesthetics as a research tool is especially useful since they have a limited duration, and testing them using the methods described also result in no visible injury to oral structures.

Our study had an objective and reflexive endpoint to determine the duration of the sensory nerve blocks. The duration of action of bupivacaine hydrochloride in infants pigs reported in this study, as measured by their oral reflexes, should be taken into consideration for

veterinary procedures that aim to maximally reduce postoperative pain. Further research is needed to understand how combining general anesthesia or monitored anesthetic care with propofol can increase the duration of a bupivacaine hydrochloride sensory nerve block in the oral cavity. The infant pig model can be used for future studies to answer these clinical questions.

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Figure 1.

Photographs showing the 7 locations stimulated for the sensory stimulation testing: (1) gingiva labial to the maxillary central incisor teeth (CIs) at the mucogingival junction (MGJ), (2) gingiva buccal to the eruption site of the permanent maxillary molar (M1) tooth at the MGJ (A). (3) gingiva labial to the mandibular CIs, (4) gingiva buccal to the eruption site of the permanent mandibular M1 at the MGJ (B). (5) gingiva palatal to the maxillary CIs in the region of the incisive papilla on the hard palate, (6) gingiva palatal to the eruption site of the permanent maxillary right M1 on the hard palate, and (7) gingiva palatal to the eruption site of the permanent maxillary left M1 on the palate (C).

Duration of action for bupivacaine blocks of the nasopalatine and greater palatine nerves.

Figure 2.
Duration of action for bupivacaine blocks of the nasopalatine and greater palatine nerves.

Table 1

Duration of action for bupivacaine blocks of the nasopalatine and greater palatine nerves in infant pigs.

	Last test with no response (minutes)*	First test with response (minutes) [†]	Consistent response (minutes) [^]
Pig A- 1	100	130	310
Pig A- 2	75	125	145
Pig B- 1	135	150	150
Pig B- 2	170	190	190
Pig C- 1	95	115	115
Pig C- 2	60	80	120
Pig D	45	65	120
Pig E	65	85	220
Pig F	105	135	135
Average	94	119	167
SD	39	39	64
Range	45 – 170	65 – 190	115 – 310

* last time where no response was seen in any of three palatal regions tested following sensory stimulation test.

[†] time of the first sensory stimulation test where at least one maxillary region responded to the test.

[^] time when the response to the sensory stimulation test showed a response in two or more of the three palatal regions and was repeatable for three tests in a row.