A Histological Study of Vasoconstriction by Local Anesthetics in Mandible

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To assess the effect of epinephrine-containing local anesthetics on vasoconstriction, we immunohistochemically measured the intravascular lumen area in different regions of the mandible. Twelve male Wistar rats were used. General anesthesia was induced and maintained with sevoflurane. Infiltration anesthesia was performed with 0.2 mL of epinephrine-free 2% lidocaine (E−) near the left mandibular first molar and with 0.2 mL of epinephrine-containing 2% lidocaine (E+) near the right mandibular first molar. After decalcification, the specimens were paraffinized, and thin sections were prepared and immunohistologically stained with an antismooth muscle actin antibody. The intravascular lumen area was measured in the mucosa, periodontal membrane, Haversian/Volkman's canal, and bone marrow. A Mann-Whitney *U* test was used for statistical processing, and $p < .05$ was considered to indicate a statistically significant difference. In the oral mucosa and the periodontal membrane, E+ had a significantly smaller vascular lumen area than E−. In the Haversian/Volkman's canal and the bone marrow, no significant intergroup difference was observed in the intravascular lumen area. We postulate that this is due to a low smooth muscle content of blood vessels in the mandible and suggest that the vasoconstrictive effect of epinephrine-containing local anesthetics within the mandible is ineffective.

Key Words: Local anesthesia; Infiltration anesthesia; Epinephrine; Vasoconstriction; Mandible.

Epinephrine, a vasoconstrictor, is often added to local anesthetics for dental use to enhance and prolong the local anesthetic effect, prevent local anesthetic systemic toxicity, and impart a hemostatic effect.¹ Epinephrine-containing local anesthetics are commonly used for surgical procedures involving the oral cavity and facial bones, such as tooth extraction, radicular cystectomy, implant placement, and jawbone resection.^{2–5} The vasoconstrictive effect of epinephrine in the maxilla and mandible has not been sufficiently described, although some reports on its effect in the soft tissue have been published.^{6,7} For example, there are reports describing the indirect observation of vasoconstriction in the mandible and maxilla due to epinephrine by measuring the amount and concentra-

tion of lidocaine in the blood and mandible after local anesthesia.^{8–10} Yamazaki et al⁹ previously measured the amount of lidocaine in the mandible using radioactive ¹⁴C-lidocaine and reported that an increased amount of lidocaine was found in the mandible when an epinephrine-containing anesthetic preparation was administered. Further, Tanaka et al¹⁰ directly measured lidocaine levels in the mandible using high-performance liquid chromatography and reported that lidocaine levels in mandibles treated with an epinephrine-containing lidocaine preparation were somewhat higher than, but did not differ significantly from, those in mandibles treated with an epinephrine-free lidocaine preparation.

All the above studies assumed that epinephrine induced vasoconstriction in the mandible. No study has evaluated where vasoconstriction in the mandibular tissue is histologically observed directly when an epinephrine-containing local anesthetic is used.

In order to quantitatively analyze the vasoconstrictive effect, we immunohistologically measured differences in the intravascular lumen area in the mandibles of rats that underwent mandibular infiltration anesthesia with an epinephrine-containing local anesthetic.

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METHODS

This experiment was conducted with the permission of the Ohu University Animal Experiment Committee (animal experiment permit number 2016-24, 2017-16).

Animals

Twelve male Wistar rats (10 weeks old, 300 ± 10 g body weight) were used in this study (Clea Japan, Tokyo, Japan). Until the day of experimentation, the rats were housed at a room temperature of 23°C and humidity of 60% with free access to food (MF, Oriental Yeast, Tokyo, Japan) and water.

General Anesthesia

General anesthesia in the rats was induced by inhalation of 5% sevoflurane via an anesthesia machine for small animals (Soft Lander, Shin-Ei Industries, Tokyo, Japan) and of 5 L/min oxygen via a nasal mask. Sevoflurane (3%) and oxygen (3 L/min) were used for maintaining general anesthesia (Figure 1).

Infiltration Anesthesia and Tissue Removal

After stable general anesthesia was reached, a Sopira Citoject Syringe (Kulzer, Hanau, Germany) and a needle for infiltration anesthesia (33 G, 0.26×12 mm) (Niproject Dental Needle, Nipro, Tokyo, Japan) were used to control the injection amount of local anesthetic by performing one push (delivering 0.05 mL) per second 4 times. Epinephrine-free 2% lidocaine (Xylocaine Injection Polyamp 2%, Astra Zeneca, Tokyo, Japan) (E–) was administered on the left side of the jaw, and 2% lidocaine (Dental Xylocaine Cartridge containing 1:80,000 Epinephrine, Dentsply Sankin, Tokyo, Japan) (E+) was administered on the right side of the jaw.

Twenty minutes after infiltration anesthesia, 4% paraformaldehyde buffer (Wako Junyaku Kougyo, Osaka, Japan) was injected via the left ventricle, and perfusion fixation was performed. For perfusion fixation, a 24G intravenous catheter (BD Insyte IV Catheter, Japan BD, Tokyo, Japan) and a constant-flow pump for low flow rate (RP2000, Nihon Rika Kikai, Tokyo, Japan) were used at a flow rate of 2 to 3 L/h. After completing perfusion fixation, the lower mandible was removed and immersed in 4% paraformaldehyde buffer (Wako Junyaku Kougyo) for 24 hours.

Figure 1. Preparation for the study of rats. General anesthesia was induced and maintained by sevoflurane. Infiltration anesthesia of 0.2 mL of 2% lidocaine without epinephrine was injected in the left side, and 0.2 mL of 2% lidocaine with 1:80,000 epinephrine was injected in the right side. Twenty minutes after injection, fixation was performed by 4% paraformaldehyde via a left ventricle.

Immunohistological Staining

Immunohistological staining was used to clearly visualize vascular smooth muscle.¹¹

The specimens were decalcified with a 10% EDTA solution (pH 7.0) for 3 to 6 months. During decalcification, trial cutting was performed on the incisor teeth to check the decalcification depth.

The decalcified specimens were embedded in paraffin, and 10-μm sections were obtained using a Microm HM 360 machine (Thermo Scientific, Waltham, MA). The cutting plane of the paraffin sections was sagittal, frontal, or horizontal for 8 specimens (4 from the left side and 4 from the right side) from 4 animals (Figure 2).

After deparaffinization and hydration, the sections were treated with 0.3% H₂O₂ in methanol to remove endogenous peroxidase, blocked with goat serum (Vectastain Elite ABC Kit, Vector Lab, Burlingame, CA) for 1 hour, and incubated in a solution of rabbit anti-α smooth muscle actin antibody (α-SMA Polyclon-

Figure 2. Planes of the sections made in this study. (a) Sagittal section. (b) Coronal section. (c) Horizontal section.

al Antibody, Bioworld Technology, Inc, St Louis Park, MN) as the primary antibody for 10 hours. After washing, the sections were treated with biotin-labeled goat anti-rabbit antibody (Vectastain Elite ABC Kit, Vector Lab) as the secondary antibody for 1 hour and incubated in ABC peroxidase (Vectastain Elite ABC Kit, Vector Lab) for 1 hour. DAB color development solution (Peroxidase Substrate Kit, Vector Lab) was used for staining, and nuclei were stained with methyl green (Muto Pure Chemicals Co, Tokyo, Japan).

Measurement of the Intravascular Lumen Area

Under an optical microscope (at 20 $\times$ magnification), the intravascular lumen areas at E– and E+ effect sites were measured in the oral mucosa, periodontal membrane, Haversian canal/Volkmann’s canal in the mandible, and bone marrow using Axio Vision (Carl Zeiss, Tokyo, Japan). The inner circumference of the vascular smooth muscle, which was stained dark brown, was drawn by a red line, and the lumen area was calculated automatically by Axio Vision (Figure 3).

The ranges of the measured areas were as follows: For the sagittal sections, a 2 $\times$ 2 mm area located superiorly to the mandibular canal and anteriorly to the interalveolar septum of the first molar tooth of sections of the buccolingual center of the first molar mesial root (Figure 2a); For frontal sections, a 2 $\times$ 2 mm area located superiorly to the mandibular canal and buccally to the midline of the first molar mesial root of sections of the mesiodistal center of the first molar mesial root (Figure 2b); And for horizontal sections, a 2 $\times$ 2 mm area located anteriorly to the interalveolar septum and buccally to the

center of the first molar mesial root at the height of the center of the first molar mesial root (Figure 2c).

The proportion of blood vessels with smooth muscle in the Haversian canal/Volkmann’s canal was also measured.

Mann-Whitney *U* test was used for statistical analysis, and a *p* value of < .05 was considered to be statistically significant.

RESULTS

A total of 4874 blood vessels were observed in 24 sections (8 sagittal, 8 frontal, and 8 horizontal sections) from the left and right mandibles of 12 rats.

Soft Tissue

The intravascular lumen area in the oral mucosa was $397 \pm 871 \mu\text{m}^2$ for E– and $242 \pm 667 \mu\text{m}^2$ for E+, indicating that the area was significantly smaller for E+ ($p < .006$). The calculated area contraction rate was 36.2% (Figure 4).

The intravascular lumen area in the periodontal membrane was $206 \pm 297 \mu\text{m}^2$ for E– and $131 \pm 156 \mu\text{m}^2$ for E+, indicating that the area was significantly smaller for E+ ($p < 0.001$). The calculated area contraction rate was 35.2% (Figure 4).

Mandible

The intravascular lumen area in the Haversian canal/Volkmann’s canal was $192 \pm 453 \mu\text{m}^2$ for E– and $156 \pm$

Figure 3. Measurement of the intravascular lumen area. Dark brown regions denote vascular smooth muscles. Values in red indicate intravascular lumen areas.

351 μm^2 for E+, indicating no significant differences between the 2 groups ($p = .13$). The calculated area contraction rate was 18.6% (Figure 4). The proportion of blood vessels with smooth muscle in the Haversian canal/Volkmann's canal was 21.3% (1132/5311).

The intravascular lumen area in the bone marrow was $412 \pm 795 \mu\text{m}^2$ for E– and $364 \pm 802 \mu\text{m}^2$ for E+, indicating no significant difference between the 2 groups ($p = .31$). The calculated area contraction rate was 11.6% (Figure 4).

DISCUSSION

This study found that epinephrine-containing local anesthetic solution caused a significant vasoconstriction of soft tissue blood vessels in the mucosa and periodontal membrane, whereas no significant vasoconstriction of blood vessels in the Haversian canal/Volkmann's canal or of those in the bone marrow of the mandible was observed.

An interesting new finding was that in the Haversian canal/Volkmann's canal, the proportion of blood vessels with vascular smooth muscle was low at approximately 21%, suggesting that blood vessels within the mandible have a low smooth muscle mass. This makes it much less likely that epinephrine exerts a significant vasoconstrictive effect on blood vessels in the mandibular bone.

However, in tooth extraction or implant surgery using an epinephrine-containing local anesthetic, less contraction of blood vessels in bone surrounding the extraction socket or implant placement socket could actually be beneficial for healing and osseointegration as it prevents dry socket after tooth extraction.^{12–14}

No report has directly confirmed the vasoconstrictive effect of an epinephrine-containing local anesthetic in the

Figure 4. Intravascular lumen area. * $p < .05$; ** $p < .01$; mean $\pm$ SD. VR, vasoconstriction rate.

mandible using immunohistology. In a study by Soejima,¹⁵ the vasoconstrictive effect of epinephrine was observed by counting the number of migrating leukocytes in the bone marrow before and after the injection of epinephrine alone into the nutritional artery in the femur of rabbits. However, the study used arterial injection and leukocyte counting as an indirect measurement method without statistical analysis. Although some studies used a local anesthetic along with a radioisotope⁹ and measured approximate amounts of the local anesthetic in tissues, its local amounts in the mandible are difficult to measure, and the results may not accurately represent vasoconstriction in the mandible.

In this study, we did not measure vasoconstriction in the dental pulp. However, Kim et al¹⁶ reported that the blood flow in the dental pulp decreased when an epinephrine-containing local anesthetic was used in the mandible of dogs. The results in the present study suggest that vasoconstriction in the periodontal membrane may be in part responsible for decreased pulp blood flow because blood vessels in the periodontal membrane were constricted, whereas vasoconstriction in the mandibular bone was poor. It has been reported that the anesthetic effect on the pulp has been reported to be shorter than that on the soft tissue.^{17,18} This may be because even if the blood flow to the pulp was reduced due to vasoconstriction in the periodontal membrane, the anesthetic effect in mandibular bone is poor leading to rapid loss of efficacy in the pulp. The lack of vasoconstriction in the mandibular bone may lead to faster systemic absorption of lidocaine.

Ogawa et al¹⁹ have reported that the amount of lidocaine in the mandible is markedly reduced after periosteum elevation and washing with physiological saline. This appears to be related to the fact that lidocaine

can only be retained in the mandible for a reduced length of time because of decreased bony vasoconstriction. Furthermore, if there is less vasoconstriction in the mandible and the periosteum elevation promotes a decrease in the amount of lidocaine, it is conceivable that the amount of lidocaine in the mandible may depend on infiltration from the gingival side. In the future, more detailed studies on the mode of lidocaine infiltration into the mandible should be conducted.

One concern in our study was the high standard deviation values of the vascular luminal areas. We consider this variability to have resulted from the fact that all blood vessels of various sizes were measured, including thick central vessels and thin peripheral vessels that underwent branching. Nevertheless, the reliability appears to be high because the measurements involved >4000 blood vessels.

CONCLUSIONS

The mandibles in rats were infiltrated with epinephrine-containing and non-epinephrine-containing lidocaine and immunohistochemically analyzed for the vasoconstrictive effect of epinephrine in the soft tissues and mandibular bone. Based on intravascular lumen areas, a significant vasoconstrictive effect of approximately $\geq 30\%$ was observed in the soft tissue, whereas the observed vasoconstrictive effect in the mandible was not significant. This suggests that epinephrine vasoconstrictive effects of soft tissue infiltration local anesthesia are inadequate within mandibular bone likely due to the lack of vascular smooth muscle.

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