A Histological Study on the Vasoconstrictive Effect of Epinephrine-containing Local Anesthetics in the Jaw Bone

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顎骨内における アドレナリン添加局所麻酔薬による 血管収縮効果の組織学的研究

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Abstract

Introduction

Epinephrine, a vasoconstrictor, is often added to local anesthetics to enhance their efficacy, prolong their action, prevent local anesthetic intoxication, and impart a hemostatic effect. Although this vasoconstrictive effect is well known in the soft tissue, it is unknown in the jaw bone. In this study, we immunohistochemically measured the intravascular lumen area in the mandibular bone and analyzed its effect on vasoconstriction.

Methods

Twelve male Wistar rats (10 weeks old, 300 ± 10 g body weight) were used. General anesthesia was induced and maintained with sevoflurane. After sleep onset, infiltration anesthesia was performed with 0.2 mL of epinephrine-free (condition E−) 2% lidocaine in the left mandibular first molar and with 0.2 mL of epinephrine-containing (condition E+) 2% lidocaine in the right mandibular first molar. Twenty minutes after infiltration anesthesia, perfusion fixation from the left ventricle was achieved with 4% paraformaldehyde. After fixation, the lower jaw bone was removed and decalcified with 10% EDTA. The decalcified specimens were subjected to paraffin fixation, and thin sections were prepared and immunohistologically stained with an anti-smooth muscle actin antibody. The number of blood vessels with smooth muscle compared with the number of

Haversian canals/Volkmann's canals was measured under an optical microscope. The intravascular lumen area was measured with Axio Vision in the mucosa, periodontal membrane, Haversian canal/Volkmann's canal, and bone marrow. A Mann–Whitney U test was used for statistical processing, and $P < 0.05$ was considered to indicate a statistically significant difference.

Results

The ratio of the number of blood vessels with smooth muscle to the number of Haversian canals/Volkmann's canals was 21.3%. The intravascular lumen area in the mucosa was 397 ± 871 μm^2 for condition E− and significantly narrow at 242 ± 667 μm^2 for the epinephrine-containing anesthetic condition E+, and that in the periodontal membrane was 206 ± 297 μm^2 for condition E− and significantly narrow at 131 ± 156 μm^2 for condition E+. On the other tissue, the intravascular lumen area in the Haversian canal/Volkmann's canal was 192 ± 453 μm^2 for condition E− and 156 ± 351 μm^2 for condition E+, and that in the bone marrow was 412 ± 795 μm^2 for condition E− and 364 ± 802 μm^2 for condition E+. Thus, these values were not significantly different between treatments for any region.

Discussion

Although significant vasoconstriction was observed in the soft tissue, no significant difference was found

in the vascular lumen area in the jaw bone. This is likely attributable to a low smooth muscle content of blood vessels in the jaw bone and suggests that the vasoconstrictive effect in the jaw bone is insufficient even with epinephrine-containing local anesthetics.

Key words
Local Anesthesia, Jaw Bone, Epinephrine, Vasoconstriction, Histlogical Study

要　約

緒言

局所麻酔には血管収縮薬であるアドレナリンが添加されていることが多い。その目的は，効果増強および効果延長，局所麻酔中毒の防止，止血効果である。軟組織において，血管収縮効果はよく知られているが，顎骨内において，血管の収縮率は不明である。今回，われわれは顎骨内の血管内腔面積を免疫組織学的に計測し，血管収縮効果を解析した。

方法

ラット（Wistar，オス，10週齢，300±10 g）12匹を使用した。全身麻酔は，セボフルランで導入・維持を行った。入眠後，下顎左側第一臼歯部にアドレナリン無添加（condition E-）2%リドカイン0.2 mlを，下顎右側第一臼歯部にアドレナリン添加（condition E+）2%リドカイン0.2 mLを浸潤麻酔した。浸潤麻酔から20分後に，左心室より生理食塩液灌流後，4%パラホルムアルデヒドで灌流固定を行った。固定後，下顎骨を摘出し，10% EDTAで脱灰を3～6か月間行った。脱灰後，パラフィン包埋を行い，ミクロトームを使って薄切切片を作製，抗SMA抗体を使った免疫組織学的染色を通法により行った。光学顕微鏡下にハバース管・フォルクマン管に対する平滑筋を有する血管数を測定した。粘膜内，歯根膜内，ハバース管・フォルクマン管内，骨髄内の血管内腔面積をAxio Visionで測定した。統計処理はMann-Whitney U testを用い，$P < 0.05$を統計学的有意差ありとした。

結果

ハバース管・フォルクマン管に対する平滑筋を有

する血管の割合は，21.3%であった。　粘膜内の血管内腔面積は，condition E−で 397 ± 871μm^2，condition E+で 242 ± 667 μm^{2}で有意に内腔は狭かった。歯根膜内の血管内腔面積も，condition E−で 206 ± 297 μm^2，condition E+で 131 ± 156 μm^{2}と有意に内腔は狭かった。ハバース管・フォルクマン管内の血管内腔面積は，condition E−で 192 ± 453 μm^2，condition E+で 156 ± 351 μm^2，骨髄内の血管内腔面積は，condition E−で 412 ± 795 μm^2，condition E+で 364 ± 802 μm^{2}とどちらも有意差はなかった。

考察

軟組織においては，有意な血管収縮が認められたが，顎骨内においては，血管内腔面積に有意差はなかった。これは，粘膜内と歯根膜内の血管に平滑筋を多く含むのに対し，顎骨内の血管においては平滑筋量が少ない血管である。アドレナリン添加局所麻酔薬投与であっても，顎骨内の血管収縮効果は少ないことが明らかになった。

Introduction

Epinephrine, a vasoconstrictor, is often added to local anesthetics for dental use to enhance and prolong the local anesthetic effect, prevent local anesthetic intoxication, and impart a hemostatic effect [1]. Epinephrine-containing local anesthetics are commonly used for mandibular bone surgeries, such as tooth extraction, radicular cystectomy, implant placement, and the mandibular bone resection[2-5]. The vasoconstrictive effect of epinephrine in the bone 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 bone due to epinephrine by measuring the amount and concentration of lidocaine in the blood and the bone after local anesthesia[8-10]. Yamazaki et al.[9] measured the amount of lidocaine in the bone using radioactive ^{14}C-lidocaine and reported that an increased amount of lidocaine was found in the jawbone when an epinephrine-containing anesthetic preparation was administered. Further, Tanaka et al.[10] directly measured lidocaine levels in the bone using high-performance liquid chromatography and reported that lidocaine levels in the bones treated with an epinephrine-containing lidocaine preparation were somewhat higher than, but did not differ significantly from, those in the bones treated with an epinephrine-free lidocaine preparation.

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

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

Methods

This experiment was conducted with the permission of the Ohu University Animal Experiment Committee (animal experiment permit number 2016-24, 2017-16). The author has no conflicts of interest to disclose (COI).

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 (Fig. 1).

Infiltration anesthesia and tissue removal

After general anesthesia reached stability, 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 four times. The angle between the needle and the mandibular surface was set by 90-degree, and injection pressure about 200 mmHg. Epinephrine-free (condition E−) 2% lidocaine (Xylocaine Injection Polyamp 2%, Astra Zeneca, Tokyo, Japan) was administered on the left side of the jaw, and epinephrine-containing (condition E+) 2% lidocaine (Dental Xylocaine Cartridge containing 1 : 80,000 Epinephrine, Dentsply Sankin, Tokyo, Japan) was administered on the right side of the mandibular.

Twenty minutes after infiltration anesthesia, 0.9% normal saline and 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 perista pump for low flow rate (RP2000, Nihon Rika Kikai, Tokyo, Japan) were used at a flow rate of 2−3 L/h. After completing perfusion fixation, the lower jaw bone was removed and immersed in 4% paraformaldehyde buffer (Wako Junyaku Kougyo, Osaka, Japan) for 24 hours.

Immunohistological staining

Immunohistological staining was used to clearly visualize vascular smooth muscles with anti-alpha smooth muscle actin antibody. Because, the antibody selectively stains vessel smooth muscle [11].

The specimens were decalcified with a 10% EDTA

solution (pH 7.0, 4℃) for 3–6 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, USA). The cutting plane of the paraffin sections was sagittal, frontal, or horizontal for eight specimens (four from the left side and four from the right side) from four animals (Fig. 2).

After deparaffinization and hydration, the sections were treated with 0.3% H_2O_2 in methanol to remove endogenous peroxidase, blocked with goat serum (Vectastain® Elite® ABC Kit, Vecter Lab., CA, USA) for 1 h, and incubated in a solution of rabbit anti-alpha smooth muscle actin antibody (α-SMA Polyclonal Antibody, Bioworld Technology Inc., MN, USA) as the primary antibody for 10 hours. After washing by buffer, the sections were treated with biotin-labeled goat anti-rabbit antibody (Vectastain® Elite® ABC Kit, Vecter Lab., CA, USA) as the secondary antibody for 1 hour and incubated in ABC peroxidase (Vectastain® Elite® ABC Kit, Vecter Lab., CA, USA) for 1 h. DAB color development solution (Peroxidase Substrate Kit, Vecter Lab., USA) 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× 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 jaw bone, and bone marrow using Axio Vision (Carl Zeiss, Tokyo, Japan) (Fig. 3).

The ranges of the measures areas were as follows: for the sagittal sections, a 2 × 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 (Fig. 2a); for frontal sections, a 2 × 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 (Fig. 2b); and for horizontal sections, a 2 × 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 (Fig. 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 <0.05 was considered to be statistically significant.

Results

A total of 4,874 blood vessels were observed in 24 sections (eight sagittal, eight frontal, and eight horizontal sections) from the left and right mandibular bones of 12 rats.

Soft tissue

The intravascular lumen area in the oral mucosa (Fig. 4) was 397 ± 871 μm^2 for E− and 242 ± 667 μm^2 for E+, indicating that the area was significantly smaller for E+ (P=0.00006). The area contraction rate was 36.2% (Fig. 8).

The intravascular lumen area in the periodontal membrane (Fig. 5) was 206 ± 297 μm^2 for E− and 131 ± 156 μm^2 for E+, indicating that the area was significantly smaller for E+ (P = 0.0006). The area contraction rate was 35.2% (Fig. 8).

Jaw bone

The intravascular lumen area in the Haversian canal/Volkmann's canal (Fig. 6) was 192 ± 453 μm^2 for E− and 156 ± 351 μm^2 for E+, indicating no significant differences between the two groups (P = 0.13). The area contraction rate was 18.6% (Fig. 8). The proportion of blood vessels with smooth muscle in the Haversian canal/Volkmann's canal was 21.3% (1,132/5,311).

The intravascular lumen area in the bone marrow (Fig. 7) was 412 ± 795 μm^2 for E− and 364 ± 802 μm^2 for E+, indicating no significant difference between the

two groups (P = 0.31). The area contraction rate was 11.6% (Fig. 8).

Discussion

The mandibular of rat is much smaller than that of human. Therefore, the finest needle (33G) in the marketplace was chosen for this study. The insertional degree (90-degree) and the injection pressure (150mmHg) were adopted from the method of Yoshida et al[12].

The results of this study indicated increased SD values. This is likely because of variability due to the fact that all blood vessels of various sizes were measured, including thick central vessels and thin peripheral vessels that underwent branching.

Significant vasoconstriction of blood vessels in the mucosa and periodontal membrane, which are soft tissues, was observed, whereas no significant vasoconstriction of those in the Haversian canal/Volkmann's canal in the jaw bone or of those in the bone marrow was observed. 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 in the jaw bone have a low smooth muscle mass. This makes it unlikely that epinephrine would exert vasoconstrictive effect on blood vessels in the jaw bone.

As new finding, this study revealed that blood vessels in the Haversian canal/Volkmann's canal in the jaw bone had a low vascular smooth muscle mass. This suggests that a sufficient vasoconstrictive effect cannot be expected with an epinephrine-containing

local anesthetic, which exerts a hemostatic effect through vasoconstrictive action. However, in tooth extraction or implant surgery using an epinephrine-containing local anesthetic, reduced contraction of blood vessels in bones surrounding the extraction socket or implant placement socket could actually be beneficial for healing and Osseo integration as it prevents dry socket after tooth extraction[13-15].

No report has directly confirmed the vasoconstrictive effect of an epinephrine-containing local anesthetic in the jaw bone using immunohistology. Soejima[16] stated that in his study, the vasoconstrictive effect 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 and used no statistical method.

Although some studies used a local anesthetic along with a radioisotope[9] and measured approximate amounts of the local anesthetic in tissues, its local amounts in the jaw bone are difficult to measure, and the results will not accurately represent vasoconstriction in the jaw bone.

In this study, vasoconstriction in the pulp was not in the scope of observation. Kim et al.[17] reported that the blood flow in the pulp decreased when an epinephrine-containing local anesthetic was used in the jaw bone of dogs. The results in the present study

suggested that vasoconstriction in the periodontal membrane was involved in blood flow in the pulp because blood vessels in the periodontal membrane were constricted, whereas vasoconstriction in the jaw bone was insufficient. However, the anesthetic effect on the pulp has been reported to be shorter than that on the soft tissue[18,19]. This is presumably because even if the blood flow to the pulp was reduced due to vasoconstriction in the periodontal membrane, the anesthetic effect in the bone would not be as prolonged as in the gingiva, with quick loss of efficacy. It is inferred that lidocaine is absorbed faster in the jaw bone than in the soft tissue because of less vasoconstriction.

Ogawa et al.[20] have reported that the amount of lidocaine in the jaw bone is markedly reduced after periosteal elevation and washing with physiological saline. This appears to be related to the fact that lidocaine can be retained in the jaw bone for a reduced length of time because of less vasoconstriction in the jaw bone. Furthermore, if there is less vasoconstriction in the jaw bone and the periosteal elevation promotes a decrease in the amount of lidocaine in the jaw bone, it is conceivable that the amount of lidocaine in the jaw bone may depend on infiltration from the gingival side. In the future, detailed studies on the mode of lidocaine infiltration into the jaw bone need to be conducted.

However, the vasoconstriction also has a correlation with NOS as the compound of nitrogen[21].

Therefore, the study about the correlation between NOS and vasodilatation by local anesthetics or vasoconstriction by epinephrine was expected for the future.

Conclusions

In this study, we anesthetized rats through infiltration of epinephrine-containing lidocaine into the jaw bone and immunohistochemically analyzed the vasoconstrictive effect of epinephrine in the soft tissues and jaw bone. As a result, the vasoconstrictive effect with epinephrine in the jaw bone is lower clearly than in the soft tissue could be proved newly.

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Figure Legend

Fig. 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.

Fig. 2 Planes of the sections made in this study
a. Sagittal section
b. Frontal section
c. Horizontal section

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

Fig. 4 Immunostaining of rat oral mucosa with anti-alpha-SMA antibody
The condition E+ had a significantly smaller vascular lumen area than the condition E−.

Fig. 5 Immunostaining of rat periodontal membrane with anti-alpha-SMA antibody
The condition E+ had a significantly smaller vascular lumen area than the condition E−.

Fig. 6 Immunostaining of rat the Haversian canal and Volkmann's canal with anti-alpha-SMA antibody
No significant intergroup difference was observed in the intravascular lumen area.

Fig. 7 Immunostaining of rat bone marrow with anti-alpha-SMA antibody
No significant intergroup difference was observed in the intravascular lumen area.

Fig. 8 Intravascular lumen area
*P < 0.05 **P < 0.01
Mean ± SD
VR: Vasoconstriction rate

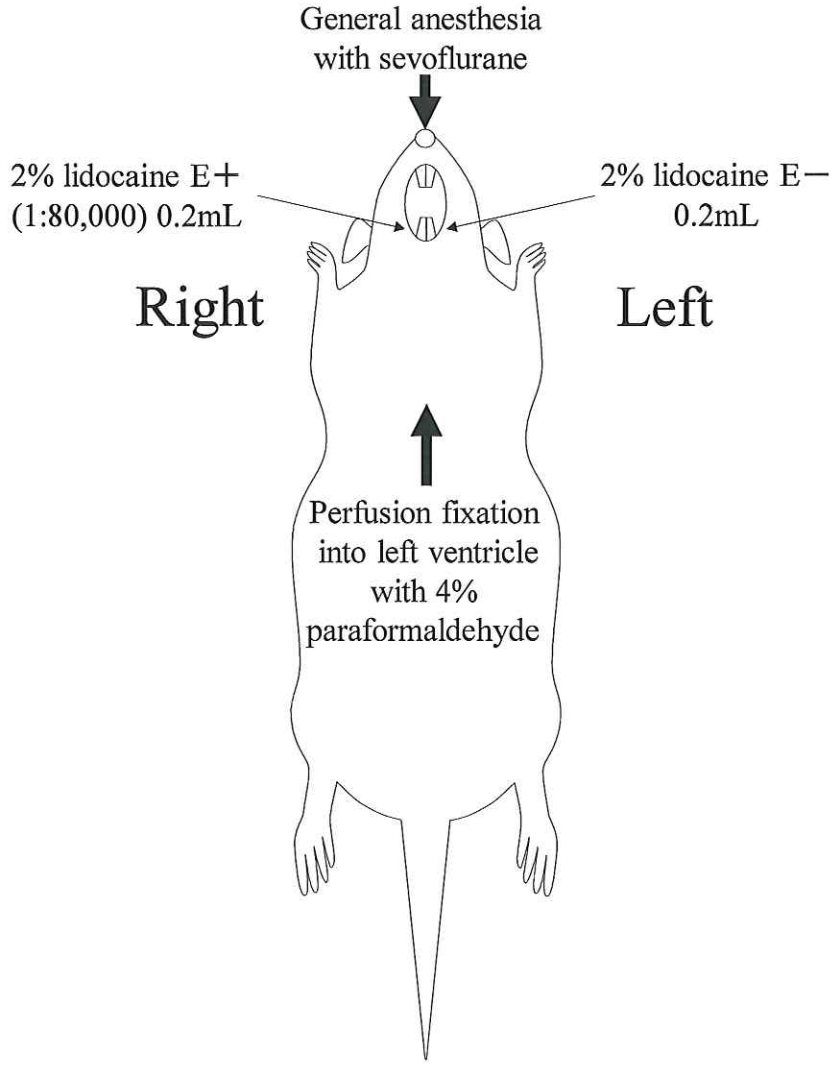

Fig. 1 Preparation for the study of rats

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.

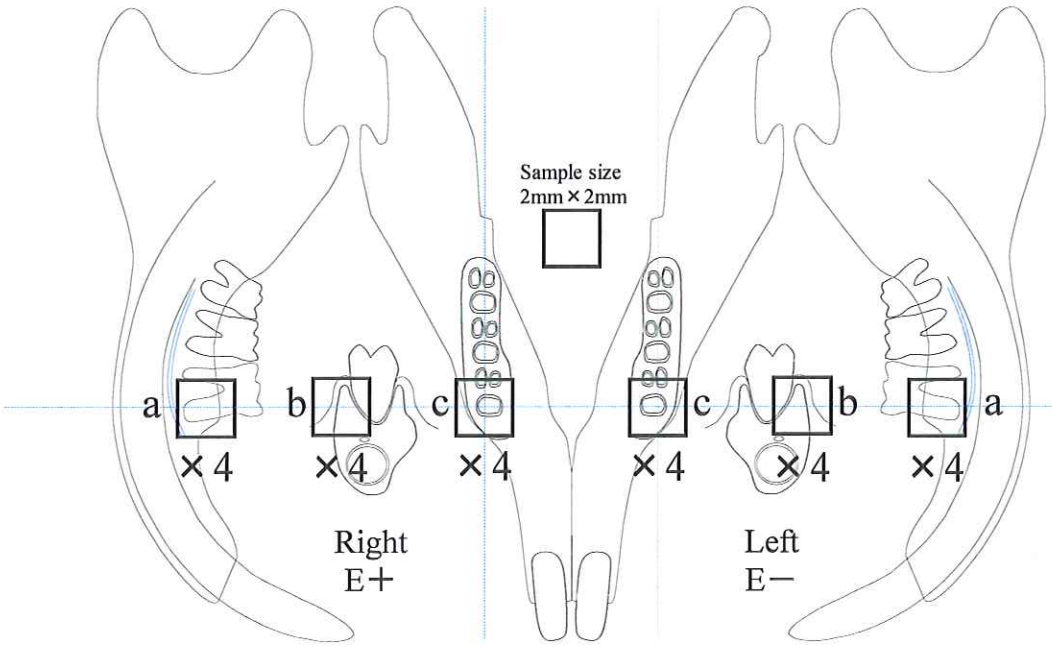

Fig. 2 Planes of the sections made in this study

a. Sagittal section

b. Frontal section

c. Horizontal section

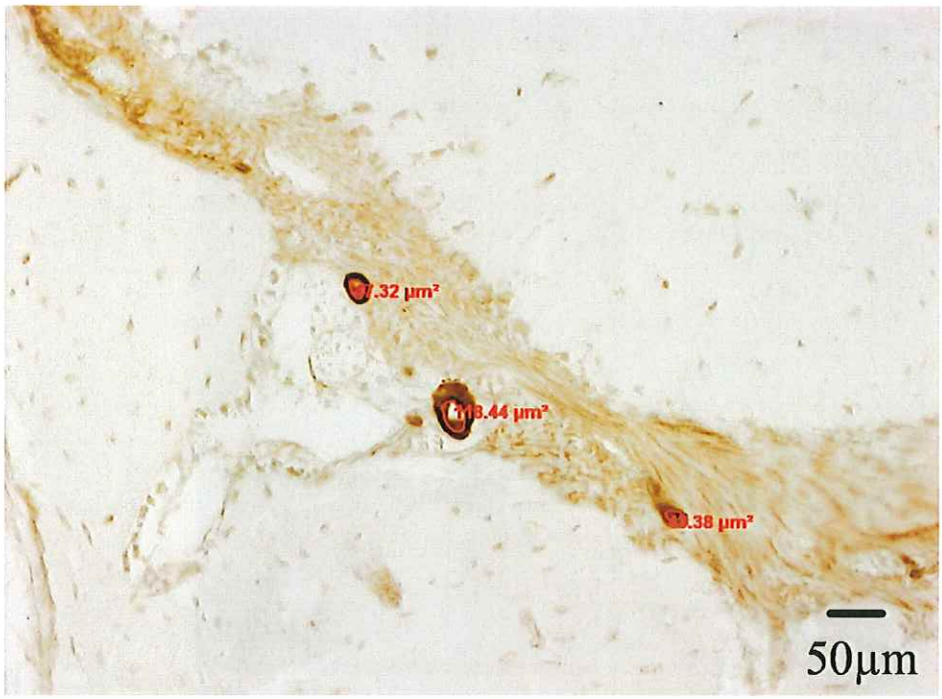

Fig. 3 Measurement of the intravascular lumen area by axio vision

Dark brown regions denote vascular smooth muscles. Values in red indicate intravascular lumen areas.

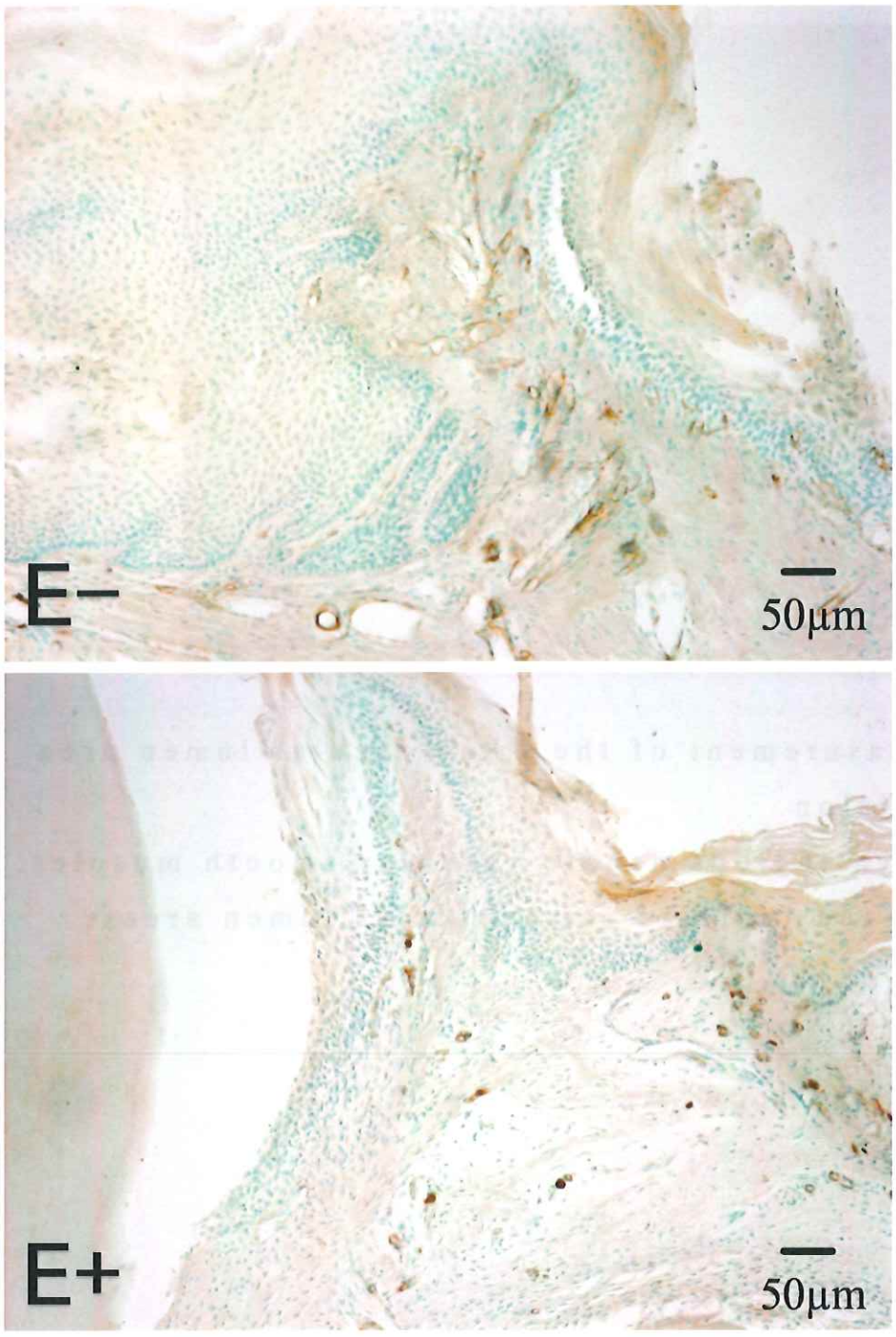

Fig. 4 Immunostaining of rat oral mucosa with anti-alpha-SMA antibody
The condition E+ had a significantly smaller vascular lumen area than the condition E−.

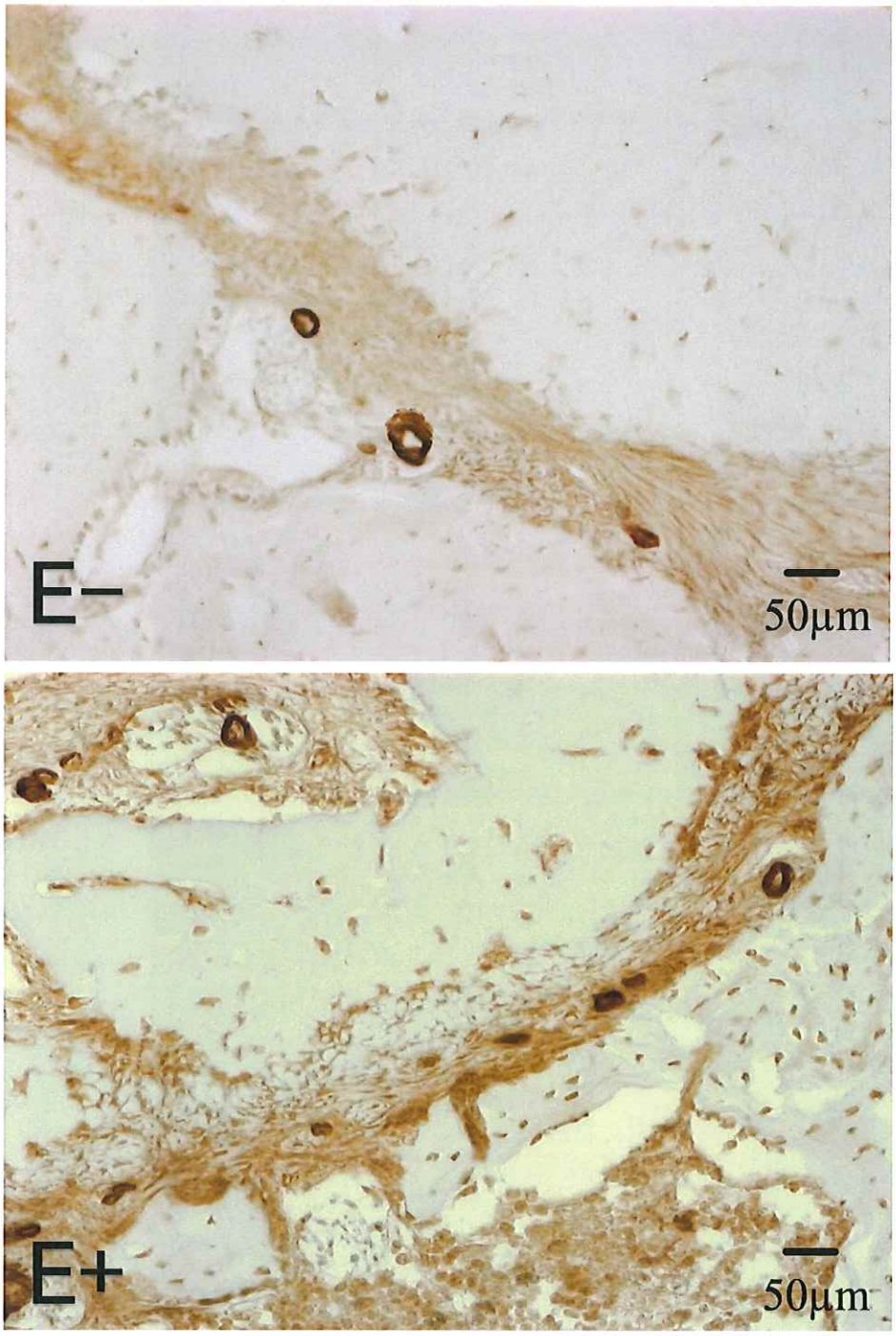

Fig. 5 Immunostaining of rat periodontal membrane with anti-alpha-SMA antibody
The condition E+ had a significantly smaller vascular lumen area than the condition E−.

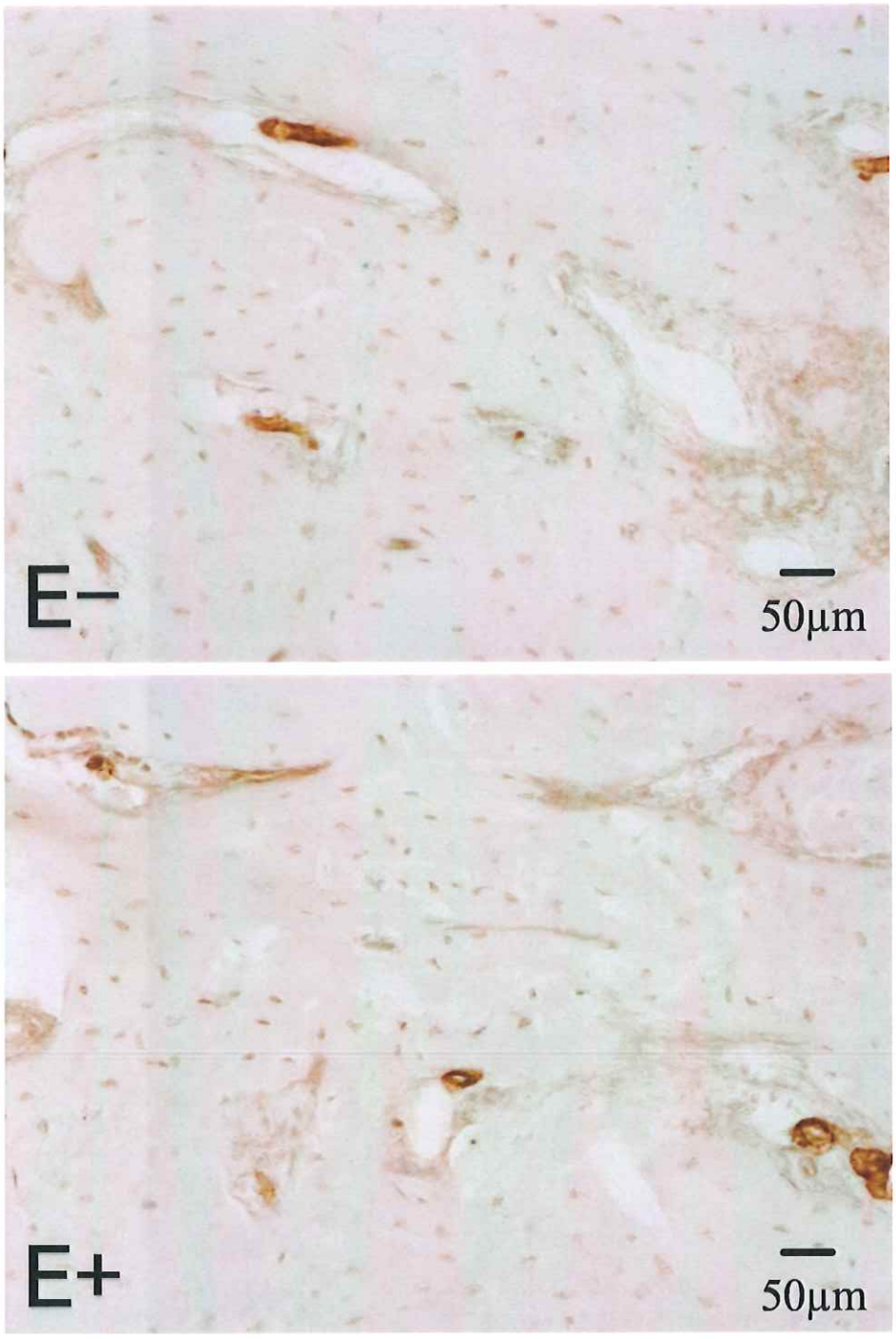

Fig. 6 Immunostaining of rat the Haversian canal and Volkmann's canal with anti-alpha-SMA antibody No significant intergroup difference was observed in the intravascular lumen area.

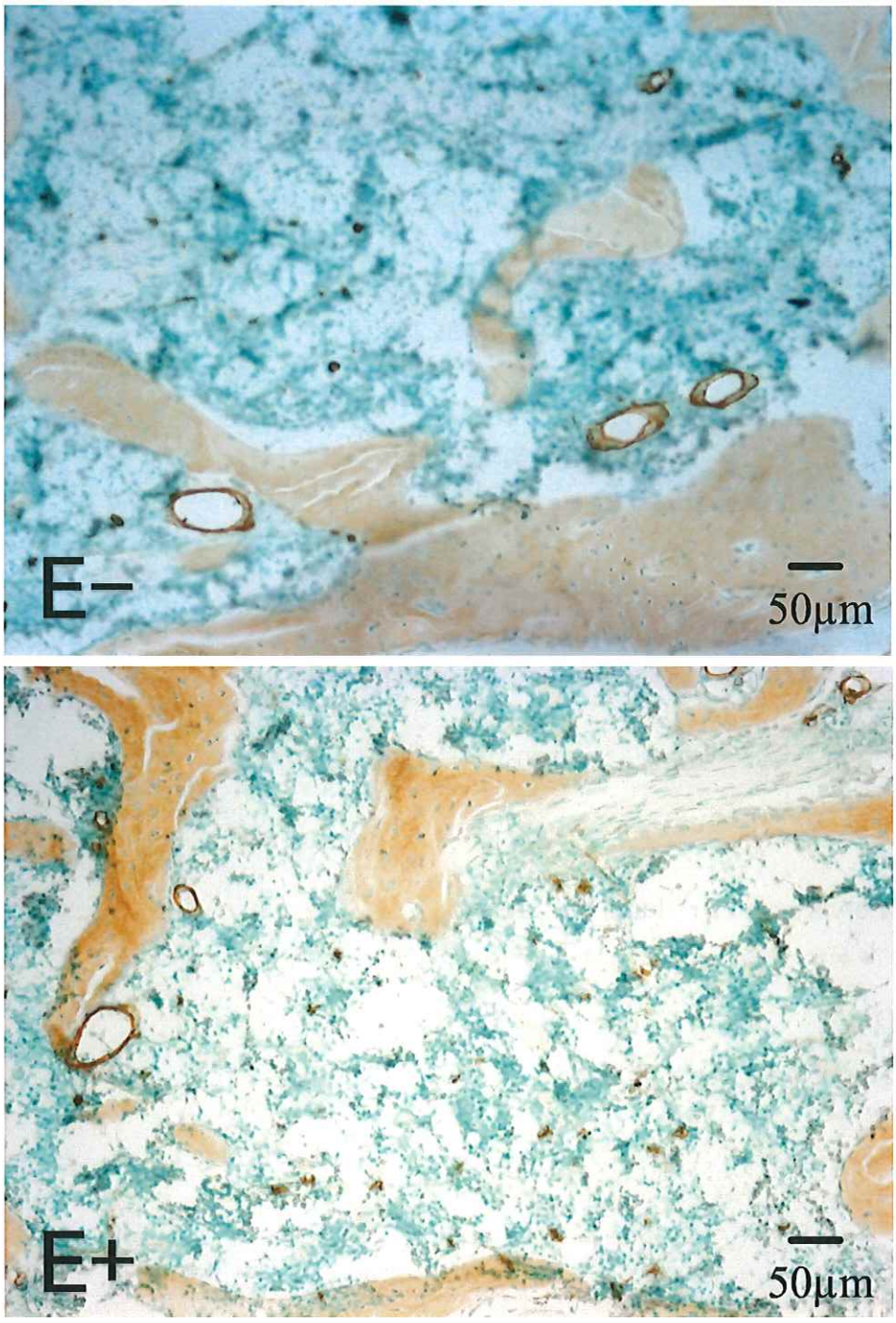

Fig. 7 Immunostaining of rat bone marrow with anti-alpha-SMA antibody

No significant intergroup difference was observed in the intravascular lumen area.

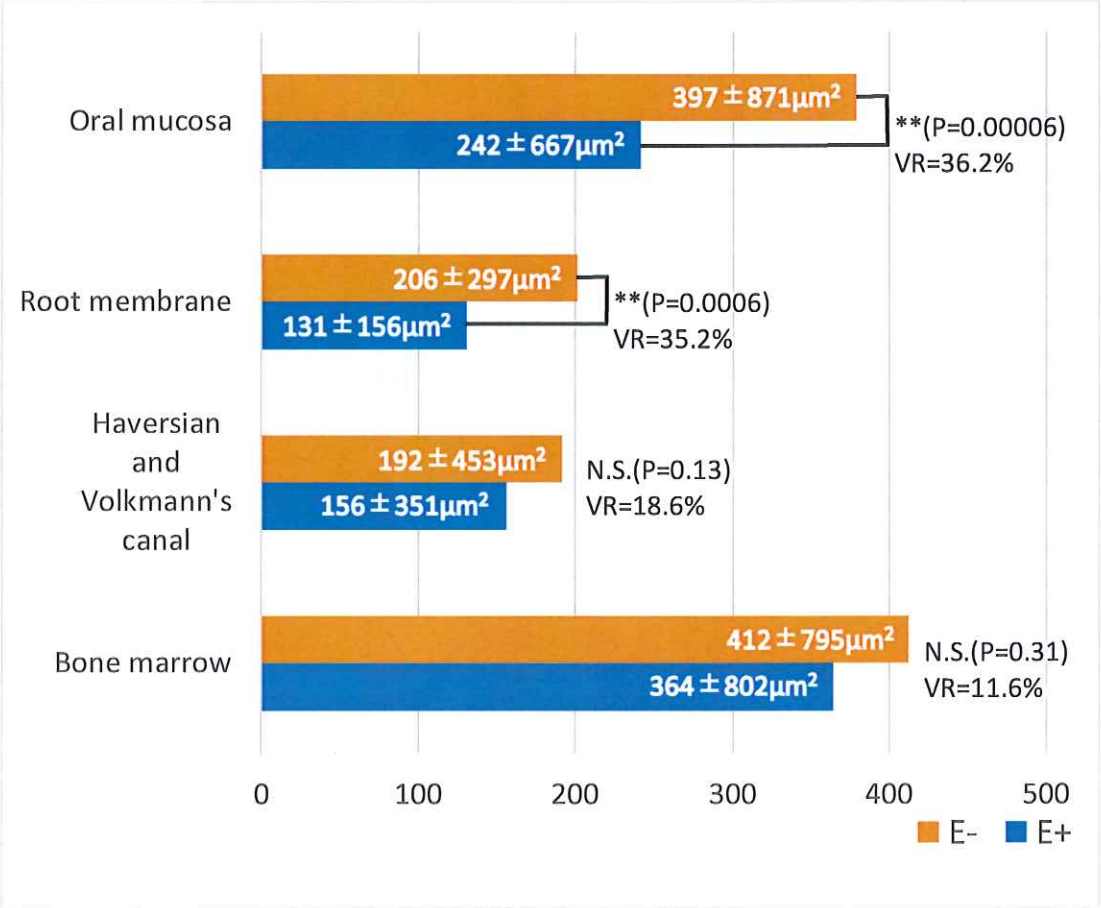

Fig. 8 Intravascular lumen area

*P < 0.05 **P < 0.01

Mean ± SD

VR: Vasoconstriction rate