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Difference of Injection Point for Local Anesthesia in Alveolar Bone Affects Infiltration and Action of Anesthesia

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In infiltration anesthesia of the jaw bone for oral surgery and dental procedures, injection in the attached gingiva or alveolar mucosa is mainly applied in clinical practice. The anesthetic action on the jaw bone was assessed on injection into the attached gingiva or alveolar mucosa.

The subjects were 30 Japan white rabbits. General anesthesia was induced by 5% sevoflurane, and maintained by 3% sevoflurane after tracheotomy and cannulation to the femoral artery for arterial pressure monitoring. Local anesthesia (2% lidocaine with 1/80,000 adrenaline) was injected at 0.5 mL into the right attached gingiva and left alveolar mucosa in the upper jaw third molar buccal area respectively. The injection pressure was monitored during local anesthesia. After 5, 10, 15, 20, 25, and 30 minutes, bilateral alveolar bone which had undergone infiltration anesthesia was removed by bone forceps as the sample. At that time, the change in arterial pressure was measured. The intra-bone lidocaine concentration in the sample was measured by high-performance liquid chromatography.

Changes in the mean arterial pressure were 14.0 (attached gingiva) and 40.0 (alveolar mucosa) mmHg at 30 minutes ($p<0.01$). Intra-bone lidocaine concentrations were 131.8 (attached gingiva) and 11.4 (alveolar mucosa) μg/g at 30 minutes ($p<0.01$). Injection pressures during infiltration anesthesia were 450.4 (attached gingiva) and 80.1 (alveolar mucosa) mmHg ($p<0.01$).

Major changes in arterial pressure correlated with low intra-bone lidocaine concentrations. If the major change in arterial pressure and low intra-bone lidocaine concentrations reflect strong pain, this means less effective infiltration anesthesia. Therefore, this result suggests that infiltration anesthesia to attached gingiva is more effective. The infiltrating of local anesthetics into the alveolar bone may depend on the anatomical characteristics and injection pressure.

Key words : infiltration anesthesia, local anesthesia, jaw bone, attached gingiva, alveolar mucosa

INTRODUCTION

For dental treatment and oral surgery, subperiosteal infiltration anesthesia of the jaw bone is effective and most frequently used,[1,2] but only a few studies on the most effective injection site have been performed,[3] and the injection point varies among dentists.[3] Regarding

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infiltration anesthesia of the jaw bone, only a single study has been reported, in which local anesthetic dispersion in tissue after the injection of infiltration anesthetics into the upper jaw in rats was investigated,[3] but the correlation between the actual effect of infiltration anesthesia depending on the injection site and local anesthetic infiltration level in the jaw bone has not been sufficiently investigated. In this study, using a rabbit model, we investigated differences in the analgesic effect and local anesthetic infiltration in the jaw bone between subperiosteal infiltration anesthesia induced by injection into the attached gingiva and alveolar mucosa (gingivobuccal fold). The analgesic effect was evaluated by fluctuation of arterial pressure during bone removal,[4] and the local anesthetic infiltration was evaluated by the lidocaine level in the removed jaw bone.[5]

METHODS

1. Animals

Thirty Japanese white rabbits (body weight : 3.1 ± 0.2 kg, 16 weeks of age, male) (Nippon Bio-Supp. Center, Tokyo, Japan) were used (Table 1). Animals were maintained in an animal room controlled at a 23℃ room temperature and 60% humidity, and given free access to pellets (MF, Oriental Yeast, Tokyo, Japan) and drinking water (tap water) until the experiment day. This study was performed in accordance with the Animal Experiment Regulations of Ohu University.

Table 1 Subjects in this study

Species	JapanWhite Rabbit
Number	n=30
Gender	male
Age in weeks (W)	16
Weight(kg)	3.1±0.2

2. General anesthesia and experimental model

General anesthesia was induced by 100% oxygen and 5% sevoflurane using an anesthesia apparatus for small animals, Soft Lander® (Shin-Ei Industries, Tokyo, Japan), followed by tracheotomy. General anesthesia was maintained with 100% oxygen and 3% sevoflurane thereafter. A cannula was inserted into the femoral artery, and the arterial pressure was continuously recorded throughout the experiment using a polygraph (Sanei Sokki, Tokyo, Japan) and a pressure transducer (Nihon Kohden, Tokyo, Japan) (Figure 1).

3. Infiltration anesthetic injection and excision of the jaw bone

Under general anesthesia, using Citoject® (Heraeus, Ecuador) as a quantitative syringe with CARPULE® injection needle (33G, 0.26 x 14) (Heraeus, Ecuador), 0.5 mL of 2% lidocaine (dental Xylocaine cartridge® containing 1/80,000 adrenaline, Dentsply Sankin, Tokyo, Japan) was infused into the bilateral maxillae, for 20 seconds, respectively. The injection site was the buccal side of the third molar on both sides (Figure 2). Local anesthesia was injected into the attached gingiva on the right side (attached gingiva group) and alveolar mucosa on the left side (alveolar mucosa group). On the both sides, needle was inserted at right angle to the mucosa with upturned tip bevel, and subperiosteal infiltration anesthesia was performed by touching the needle tip to the jaw bone surface under the periosteum[1]. The periosteum was dissected at specific time-points (5, 10, 15, 20, 25, and 30 minutes), and the injected maxillary region (from the apical area of third molar to the infrazygomatic crest) was excised approximately 1g using rongeur forceps and stored at -80℃.

4. Experiment 1 : Measurement of infiltration anesthetic injection pressure

The injection pressure for infiltration anesthesia was monitored using a pressure transducer, and the mean injection pressure was calculated from the polygraph record (Figure 3).

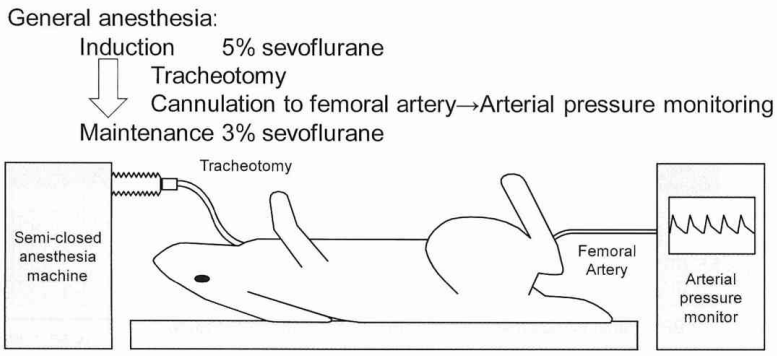


Figure 1 Methods of preparation

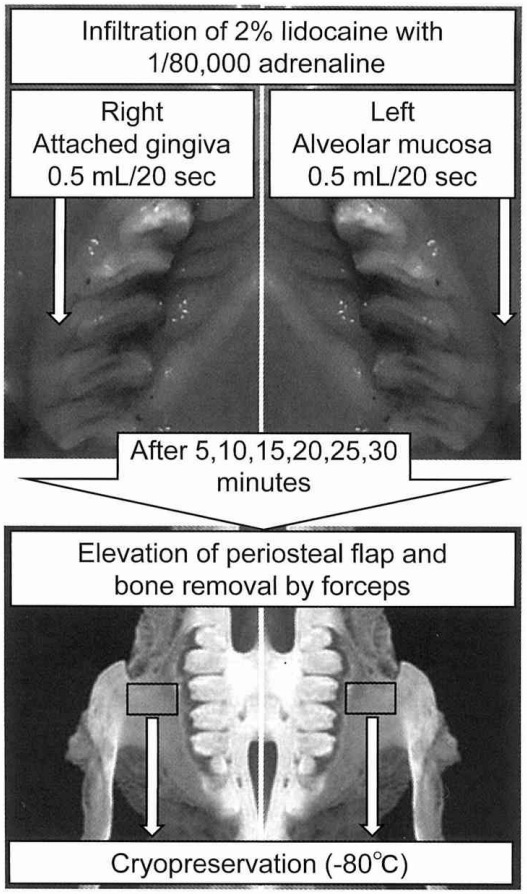


Figure 2 Methods of infiltration anesthesia and sampling

5. Experiment 2 : Measurement of changes in the mean arterial pressure during jaw bone removal

Changes in the arterial pressure during jaw bone removal using rongeur forceps were recorded on the polygraph. Even when under general anesthesia, pain stress changes the arterial pressure.[4] The arterial pressure decreased initially as depressor response and then increased as pressor response (Figure 4). From the polygraphic arterial pressure data, 1/3 pulse pressure + diastolic arterial pressure was calculated as a mean arterial pressure (MAP), and the fluctuations of MAP (depressor response + pressor response) from the baseline MAP (before jaw bone removal) were determined.

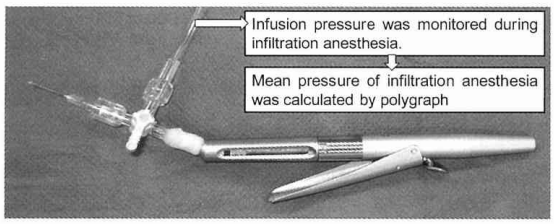


Figure 3 Pressure monitoring during infiltration anesthesia

6. Experiment 3 : Measurement of lidocaine level in the jaw bone

Jaw bone samples were thawed immediately before measurement, ground using a bone mill, TK-CM20S® (Tokken, Tokyo, Japan), suspended with 0.01 M boric acid at pH 9.18, and homogenized for 2 minutes using POLYTRON PT2100® (Kinematica, Switzerland). The supernatant (0.5 mL) was combined with 100 μL of 10 μg/mL mexiletine and then 5 mL of

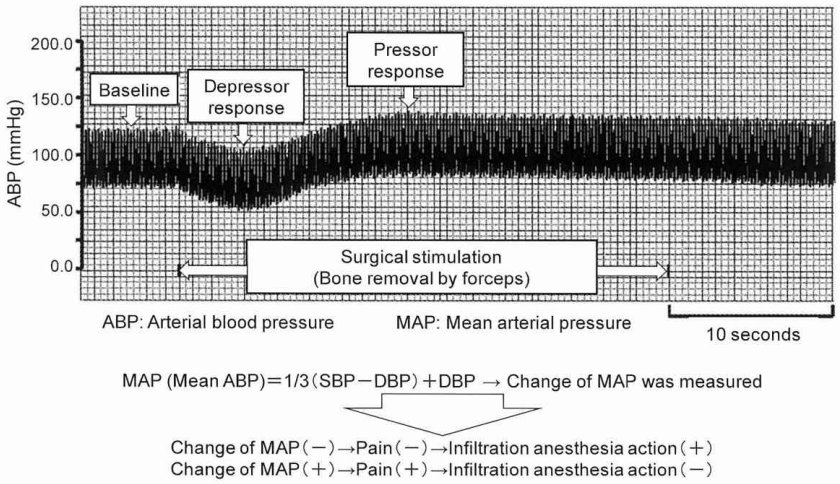
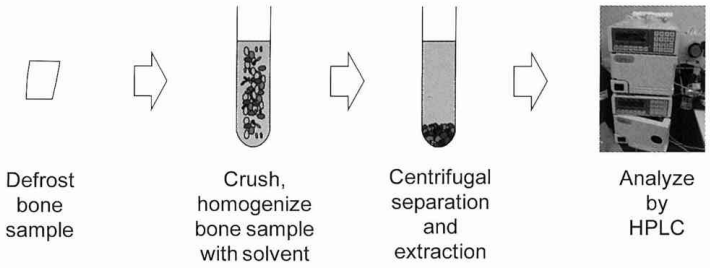


Figure 4 ABP monitoring during bone removal



The intra-bone lidocaine concentration was shown in units (μg/g) as the amount of lidocaine (μg) per 1 g of bone.

Figure 5 Measurement of intra-bone lidocaine concentration by high-performance liquid chromatography (HPLC)

chloroform: methanol (8 : 2). After mixing, the solution was centrifuged at 3,000 rpm (1,000 G) for 10 minutes, and 3 mL of the organic layer was collected and dried under a reduced pressure at 35℃ for 35 minutes using a rotary evaporator, EYELA® (Tokyo Rikakikai, Tokyo, Japan). The sample was then dissolved in 250 μL of the mobile phase (50 mM KH_2PO_4 : CH_3CN=4 : 1), stirred using a mixer, extracted, and applied to high-performance liquid chromatography (HPLC) (Jasco PU-2080 Plus®, JASCO, Tokyo, Japan) to measure the lidocaine level in the jaw bone, according to the method reported by Piwowarska *et al.*[5] The measurement procedure and detailed HPLC conditions are shown in Figure 5 and Table 2, respectively. The typical chromatogram of lidocaine from rabbit bone sample is shown in Figure 6. The jaw bone lidocaine data were converted to the lidocaine level per g jaw bone.

7. Anatomical characteristics of the rabbit jaw bone around the injection site

The jaw bone was excised from a rabbit, and the cortical bone width around the injection site (average of range 5mm[3]) was measured using a compact X-ray CT device, 3DX-multi

Table 2 Condition for HPLC analysis of lidocaine

Pump	JascoPU-2080 Plus
Detector	JascoUV-2075 Plus
Sensitivity	0.001AUFS
Column	TOSOH TSK-GEL ODS-100V 15 cm×4.6 mm
Column oven	SugaiV-630
Column temperature	40℃
Mobile phase	50mMKH_2PO_4 : CH_3CN=4 : 1
Flow rate	1.0 mL/min
Wavelength	205 nm
Degasser	AZZOTAAG-12

image micro CT type-F (Morita, Tokyo, Japan). The bone density around the injection site (average of range 5mm^3) was measured by the DXA method using a densitometer, DCS600 (Aloka, Tokyo, Japan). In addition, close-up photographs of the rabbit alveolar bone were taken to observe the anatomical characteristics around the alveolar bone.

8. Statistical analysis

The attached gingiva and alveolar mucosa groups were compared in Experiment 1 (anesthetic injection pressure), Experiment 2 (changes in the mean arterial pressure during jaw bone removal), and Experiment 3 (jaw bone lidocaine level) using the Mann-Whitney U-test, and setting the significance level at $P<0.05$.

RESULTS

1. Infiltration anesthetic injection pressure (Figure 7)

The mean infiltration anesthetic injection pressure was high (450.4 ± 145.7 mmHg) in the attached gingiva group, but low (80.1 ± 37.2 mmHg) in the alveolar mucosa group, showing a marked significant difference between the groups.

2. Changes in the mean arterial pressure during jaw bone removal (Figure 8)

The changes in the mean arterial pressure during jaw bone removal at 5 minutes after infiltration anesthetic injection were 3.3 ± 0.3 and 8.0 ± 3.5 mmHg in the attached gingiva and alveolar mucosa groups, respectively, showing no significant difference between the groups. However, marked significant differences were observed at all time-points thereafter, and the changes were 14.0 ± 1.0 and 40.0 ± 4.9 mmHg at 30 minutes, respectively, i.e., changes in the mean arterial pressure were significantly smaller in the attached gingiva group after 10 minutes of infiltration anesthesia. The difference between the groups was 4.7 mmHg at 5 minutes, but it increased to 26.0 mmHg at 30 minutes, showing that the difference increased with time.

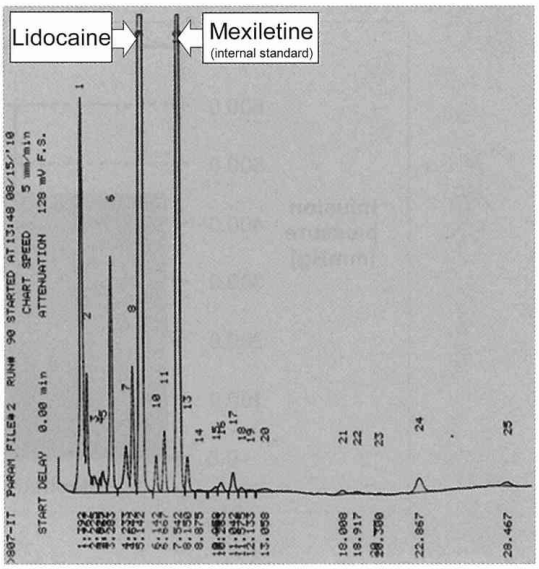


Figure 6 Typical chromatogram of lidocaine from rabbit bone sample

3. Lidocaine level in the jaw bone (Figure 9)

The lidocaine levels in the jaw bone at 5 minutes of infiltration anesthesia were 342.5 ± 17.6 and 168.5 ± 6.6 μg/g in the attached gingiva and alveolar mucosa groups, respectively, showing a marked significant difference. Marked significant differences were

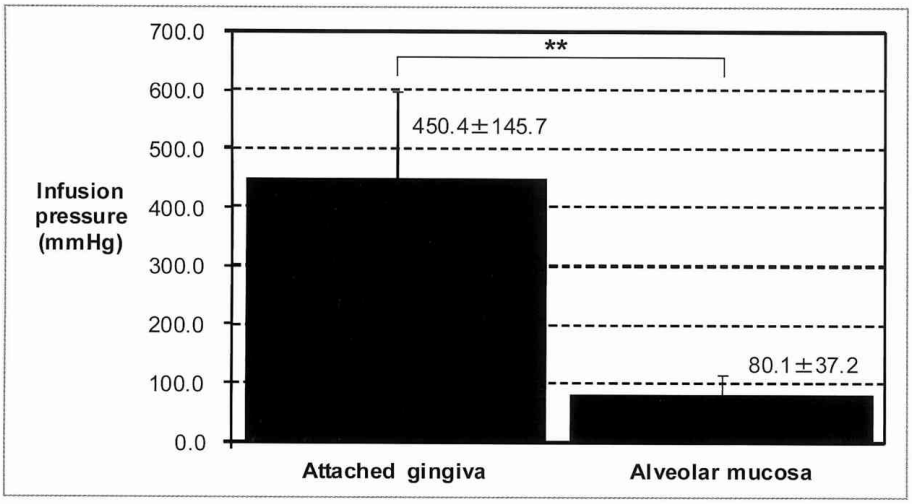


Figure 7 Mean infusion pressure during infiltration anesthesia

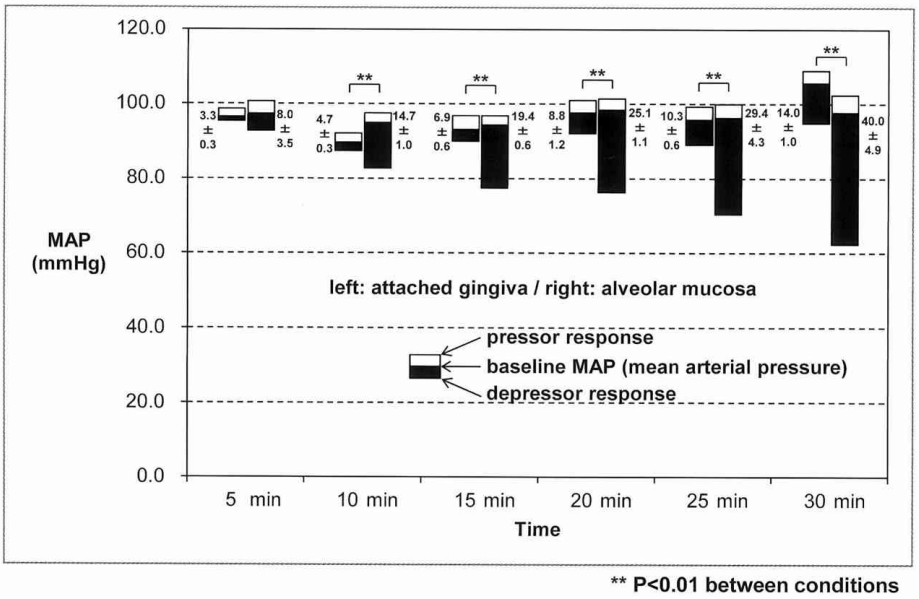
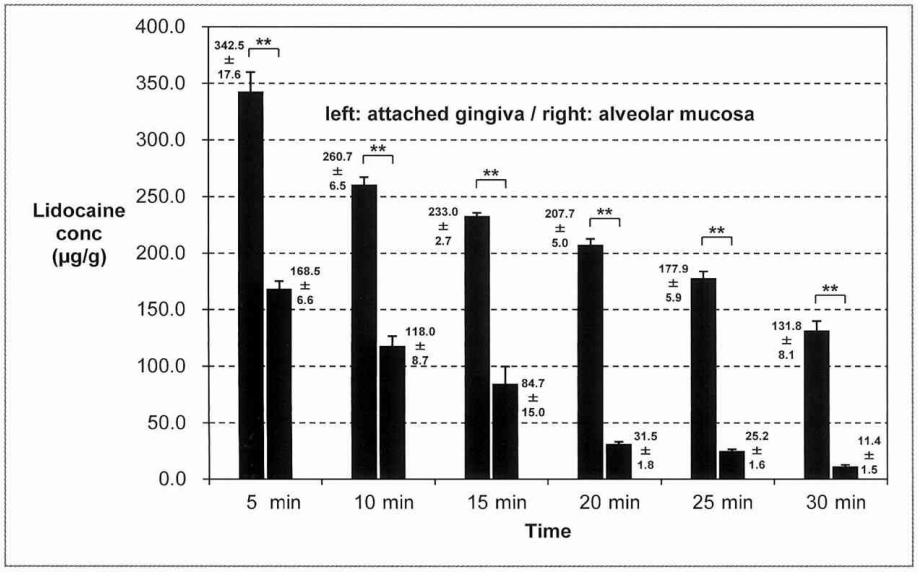


Figure 8 MAP fluctuation during bone removal

also noted thereafter, and the final levels at 30 minutes were 131.8 ± 8.1 and 11.4 ± 1.5 μg/g, respectively. The jaw bone lidocaine level was significantly higher in the attached gingiva group at all time-points after infiltration anesthetic injection, and the difference between the groups was 174.0 μg/g at 5 minutes and 120.4 μg/g at 30 minutes.

4. Anatomical characteristics of the rabbit jaw bone around the injection site (Figures 10 and 11)

Based on the X-ray CT findings and bone



** P<0.01 between conditions

Figure 9 Intra-bone lidocaine concentration

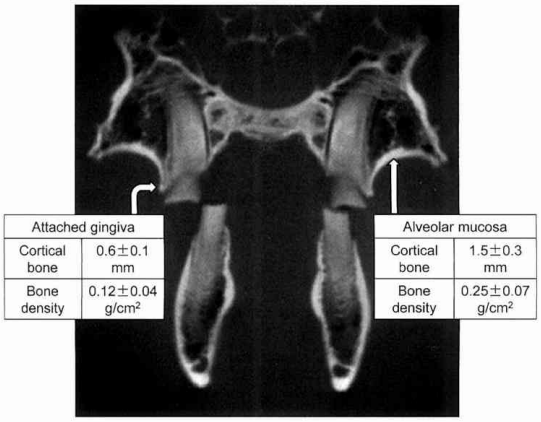


Figure 10 Bone characteristics in both injected positions

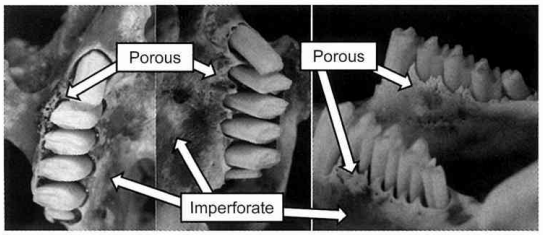


Figure 11 Characteristics of rabbit jaw bone Around attached gingiva (alveolar crest) shows porous bone. Around alveolar mucosa shows imperforate bone.

density measurement, the cortical bone width was 0.6 ± 0.1 mm and bone density was 0.12 ± 0.04 g/cm² in the attached gingiva, and 1.5 ± 0.3 mm and 0.25 ± 0.07 g/cm² in the alveolar mucosa, respectively. In the close-up photograph, the alveolar bone in the attached gingival region (alveolar crest) was porous, whereas the bone in the alveolar mucosal region distant from the crest was imperforate.

DISCUSSION

1. Local anesthetic infiltration

In the employed subperiosteal infiltration anesthesia method, the periosteum is punctured by a needle tip, and local anesthetics are injected between the periosteum and bone.[1] Local anesthetics injected and retained in the subperiosteal region infiltrate, passing through the cortical bone, reach the bone marrow and dental pulp, and act on the target nerve.[2] Finally, the residual local anesthetic (lidocaine)

in the tissue is entirely absorbed into the general circulation through capillary blood vessels, and metabolized by cytochrome P-450 IIIA4 in the liver.[6] Reportedly, when a local anesthetic (lidocaine) infiltrates into the jaw bone, it does not readily infiltrate into regions with thick cortical bone and a high bone density, whereas it readily infiltrates into the jaw bone in regions with thin cortical bone and a low bone density.[7] It has been reported that in surgery with irrigation of periosteum-dissected bone with water or saline, lidocaine retained in the subperiosteum of the jaw bone was washed out in an early phase, which prevented elevation and rather markedly reduced the jaw bone lidocaine level,[7] markedly shortening the duration of the local anesthetic action.[8] Recently, oral surgeries, such as oral implant placement and impacted tooth extraction, are performed with irrigation of periosteum-dissected bone with water or saline, in which the effect of local anesthesia action may be reduced because of the above reason. Therefore, sufficient elevation of the jaw bone lidocaine level before surgery is important, for which an effective infiltration anesthesia method is necessary.

2. Durations of action of local anesthetics and treatment

Two percent lidocaine containing 1/80,000 adrenaline is a local anesthetic widely used in dental practice, and the optimum local anesthetic effect is exhibited at this compounding ratio.[9] In addition to the local anesthetic effect, this drug also exhibits a superior hemostatic effect,[10] and its clinical duration has been reported to be about 100 minutes.[11] When it was injected into the alveolar mucosa, the effect had already disappeared at 30 minutes after administration in this study, suggesting that it is dangerous to indiscriminately consider that the duration of the infiltration anesthetic action in the jaw bone is 100 minutes.

3. Infiltration anesthetic injection pressure

The mean pressure of infiltration anesthetic injection was high (450.4 ± 145.7 mmHg) in the attached gingiva group and low (80.1 ± 37.2 mmHg) in the alveolar mucosa group, showing a marked significant difference between the groups. Hochman *et al.*[12] reported the pressure of local anesthetic injection into the gingiva, in which they considered that the injection pressure and tissue permeability were high in the attached gingiva because the gingiva was thicker than the alveolar mucosa, and the periosteum and bone surface strongly and closely joined, whereas local anesthetics can be infused at a low pressure and readily disperse into soft tissue in the alveolar mucosa because the gingiva is thin and soft tissue is flexible,[12] suggesting that the difference in the injection pressure observed in our study was due to histological differences between the injection sites. Tateno *et al.*[3] also reported that local anesthetic injected into the rat buccal alveolar mucosa widely dispersed in soft tissue. Therefore, although infiltration anesthetic injection into the attached gingiva required a high pressure, the anesthetic may readily infiltrate into the jaw bone with little leakage into soft tissue.

4. Changes in the mean arterial pressure during jaw bone removal

Changes in the mean arterial pressure are correlated with the severity of pain.[4] Pain may be mild when the change in the mean arterial pressure is small, exhibiting a high infiltration anesthetic effect, whereas pain is severe when the change is large, reducing the infiltration anesthetic effect. Changes in the mean arterial pressure were significantly smaller in the attached gingiva group, suggesting a significantly stronger analgesic effect. In contrast, changes in the mean arterial pressure were significantly larger and increased with

time in the alveolar mucosa group, suggesting that the analgesic effect is significantly weak and rapidly disappears. Iba *et al.*[13] reported that the nerve fibers extensively innervate bone such as periosteum, compact and trabecular bone, and bone marrow space. Regarding as nerve fibers, there are myelinated fibers as Aβ and Aδ fibers, and unmyelinated fibers as C fiber, further, sympathetic nerve fibers also innervated bone tissue. In addition to nerve fibers, several receptors such as nociceptors at the terminal of the fibers, and released many kinds of neuropeptides were identified in the bone.[13] This report suggests that the bone resection without anesthetic effect produces painful nociceptive stimulation.

5. Anatomical characteristics of jaw bone and local anesthetic infiltration

At all time-points, the jaw bone lidocaine level was significantly higher in the attached gingiva than in the alveolar mucosa group, and this may have been related to the anatomical characteristics of rabbits : The attached gingiva located at the alveolar crest is porous, and the cortical bone is thin with a low bone density in this region, whereas the bone is thick with a high bone density in the alveolar mucosal region (Figures 10 and 11). Considering these characteristics, more local anesthetic may have infiltrated into the jaw bone in the attached gingiva than in the alveolar mucosa group, elevating the jaw bone lidocaine level. Actually, Ogawa *et al.*[7] reported that local anesthetics did not readily infiltrate into regions with a high bone density and thick cortical bone. These findings of the jaw bone lidocaine level were also strongly demonstrated by changes in the mean arterial pressure representing the severity of pain. These anatomical characteristics of the jaw bone are similar to those in humans.[14] In the attached gingival region, many branches communicating with the inner region of the bone marrow are present because the bone surface is very porous and rich in nutrient foramens. The cortical bone becomes thick, dense, and imperforate as the region become distant from the alveolar crest, and these anatomical characteristics have been reported for a long time.[14] Therefore, it is likely that the jaw bone infiltration pattern of local anesthetics in humans is similar to that observed in rabbits.

CONCLUSION

1) The infiltration anesthetic injection pressure was high in the attached gingiva group, suggesting that, although injection is not easy, the jaw bone lidocaine level readily rises and exhibits a strong analgesic effect.

2) In the alveolar mucosa group, the infiltration anesthetic injection pressure was low, suggesting that, although injection is easy, local anesthetics are likely to be retained in the submucosal or subperiosteal region outside the jaw bone, reducing elevation of the jaw bone lidocaine level and the subsequent analgesic effect.

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