Effects of Prostaglandin E1 Application on Rat Incisal Sockets

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Purpose: *Atrophy of the alveolar ridge is a problem in prosthetic and esthetic treatment. In the present study, to examine effects of PGE1 on the alveolar bone after tooth extraction, PGE1 was applied to rat incisal sockets utilizing a slow drug release system using PLGA as the drug carrier.* ***Materials and Methods:*** *Thirty-six male Wistar rats, 10 weeks old, were divided into 3 groups. After all right mandibular incisors were extracted, the sockets were treated in the following manner. The first group was untreated (control group), the second group received a PLGA rod (PLGA group), and the third group was treated with a PLGA rod containing 0.5 mg PGE1 (PGE1 group). Six rats in each group were sacrificed at 4 weeks, and the remaining rats were sacrificed at 8 weeks. For fluorescence labeling, half of the animals were injected with calcein and tetracycline 8 days and 1 day before sacrifice, respectively. After sacrifice, the mandibles were radiologically and histologically examined.* ***Results:*** *In the PGE1 group, the bone volume of the alveolar ridge including the socket was significantly (*$P < .05$*) greater than in the control and PLGA groups at 4 and 8 weeks. At 4 weeks in the PGE1 group, the mineral apposition rate and number of osteoclasts were higher than in the other groups, whereas these parameters were similar in all groups at 8 weeks.* ***Conclusion:*** *Based on this animal study, it appears that local application has the potential to preserve and/or augment the alveolar ridge after tooth extraction.* INT J ORAL MAXILLOFAC IMPLANTS 2008;23:835–840

Key words: *alveolar bone local administration, PLGA, prostaglandin E1, rat, tooth socket*

After tooth extraction, the residual alveolar ridge undergoes marked changes, including the regeneration of the socket and decreased alveolar ridge height. These changes include not only external shape but also internal structure, and most occur within several months after tooth extraction. If the alveolar ridge is highly resorbed and becomes thin and low, the clinical treatment using a prosthesis is particularly difficult and esthetics are inferior. Preserving or augmenting the alveolar ridge after tooth extraction would therefore be clinically beneficial.

Prostaglandins are derivatives of cellular membrane fatty acids and exert complex and multiple physiologic and pathologic effects. They are known to be implicated in inflammation, including periodontitis and peri-implantitis.[1–3] In particular, the E series of prostaglandins are key molecules in inflammation accompanying bone resorption. Indeed, they are highly potent with respect to bone metabolism. However, it has been reported that Prostaglandin E1 (PGE1), one of the E series of prostaglandins, affects differentiation and functions of osteoblasts and osteoclasts.[4–7] Miller and Marks demonstrated that local application of PGE1 to the vicinity of the bone increases bone volume.[8–11] These studies suggest that PGE1 is a compound with potential use for bone augmentation.

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Previous studies of the local application of PGE1 to bone indicate that maintaining a low concentration of PGE1 for a long period in the vicinity of the bone is a prerequisite for increasing bone volume.[8–10] Lactic acid/glycolic acid copolymer (PLGA) is a biodegradable material that has a history of use in medical devices such as bone plates, screws, and sutures for surgery.[12,13] PLGA is a particularly useful polymer for fabricating porous scaffolds, since it biodegrades by hydrolysis of ester bonds into lactic acid and glycolic acid, which are then removed from the body via normal metabolic pathways and are relatively harmless. This investigative team has developed a local drug delivery system based on PLGA that is able to release small quantities of compounds slowly over a long period.[14] The purpose of the present study was to examine the effects of local application of PGE1 in rats on the alveolar bone after incisor extraction utilizing bioabsorbable PLGA scaffold as a drug carrier.

MATERIALS AND METHODS

Animals

Thirty-six male Wistar rats 10 weeks of age (Sankyo Labo Service, Tokyo, Japan), were used. The Animal Experiment Committee at Tokyo Medical and Dental University approved the animal experimental protocol of the present study. The animals were allowed free access to tap water and commercial rat feed throughout the experimental period.

PGE1 Rod Preparation

Rods were prepared according to the technique reported by Ara et al.[14] PLGA carrier with PGE1 was fabricated using PLGA (PLGA 5-50; Mitsui Chemicals, Tokyo, Japan). The ratio of lactic acid to glycolic acid was equivalent (50:50w%, molecular weight of 16000 to 18000 kDa). PLGA was dissolved in an organic solvent, 1, 4-dioxane (Wako Pure Chemical Industries, Osaka, Japan) at a concentration of 10% (w/v) overnight. PLGA solution was mixed with β-tricalcium phosphate (β-TCP; Wako Pure Chemical Industries), calcium carbonate (Wako Pure Chemical Industries), and PGE1 (Wako Pure Chemical Industries). The mass ratio of PLGA, β-TCP, and calcium carbonate was 2:1:1. The mixture was vortex mixed and loaded into a disk mold 15 cm in diameter, freeze-dried, and then compressed to yield a solid 1.5-mm-thick disk. The disks were vacuum treated for 24 hours to remove any residual 1,4-dioxane and then cut into 1.5 mm × 1.5 mm × 15 mm rods. The average weight of the rods was about 32 mg. Rods containing 0.5 mg of PGE1 were prepared. Each rod was bent slightly to fit into the incisor socket. PLGA carrier without PGE1 was also prepared in the same manner.

Mandibular Incisor Extraction and PGE1 Application

The animals were divided into 3 groups. The right incisor was cut at the level of the interdental papilla of the mandibular incisors using a small diamond disk with a dental micromotor handpiece under ether anesthesia every 3 days. Three days after the last cutting, the right incisor was pulled out without any rotation and extracted in the vertical direction along the long axis of the incisor using a conventional needle-holder under ether anesthesia. After incisor extraction, the animals in the 3 groups were treated differently. The first group was untreated (the control group), the second group received only a PLGA rod (the PLGA group), and the third group was treated with a PLGA rod containing 0.5 mg PGE1 (the PGE1 group).

Radiologic and Histologic Examinations

For fluorescence labeling, half of the animals were subcutaneously injected with 3.0 mg/kg calcein (Sigma-Aldrich, Tokyo, Japan) 8 days before sacrifice and 6.0 mg/kg tetracycline-HCl (Sigma-Aldrich) 1 day before sacrifice, respectively. The animals were sacrificed under chloroform anesthesia at 4 and 8 weeks after incisor extraction. The mandibles were dissected and fixed in 10% neutralized formalin solution.

First, a soft x-ray radiograph of each mandible was taken using a soft x-ray radiographic apparatus (SPO-M50; Sofron, Tokyo, Japan). Second, the bone mineral density of the right mandibular alveolar ridge, including the socket, was measured with a dual-energy x-ray absorptiometer (DEXA) for small animals (DCS-600R; Aloka Co, Ltd, Tokyo, Japan). The bone mineral density of the area from the anterior edge of the alveolar ridge to the proximal border of the first molar was measured, since this area does not contain any tooth root. The mandibles were then analyzed by microcomputer tomography (SMX-90CT; Shimadzu, Kyoto, Japan). Three-dimensional images were constructed using 3D-BON software (Ratoc System Engineering, Tokyo, Japan), the bone of the same area in DEXA analysis was extracted (Fig 1), and the bone volume was measured.

Fluorescence-labeled mandibles were dehydrated in ethanol and embedded in glycidyl methacrylate and methyl methacrylate. The remaining mandibles were decalcified with 10% EDTA, dehydrated in ethanol, and embedded in paraffin wax. Transverse sections of the sockets were prepared. Acrylic resin sections were stained with toluidine blue, and paraffin sections were stained with hematoxylin-eosin.

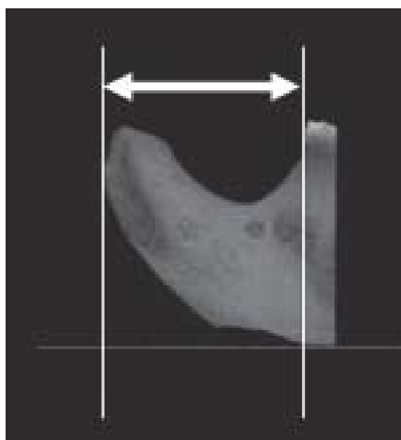

Fig 1 Bone volume measurement. After taking images with micro-CT, 3-dimensional images were constructed. Bone volume from the anterior edge of the mandible to the proximal border of the first molar root was measured.

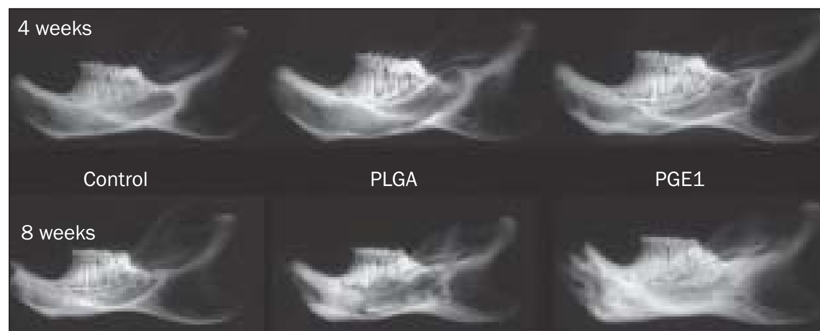

Fig 2 Soft radiographs of the mandibles at 4 and 8 weeks.

Table 1a Bone Mineral Density (mg/cm^2)

	4 weeks		8 weeks	
	Mean	SE	Mean	SE
Control	85.1	1.4	93.4	3.0
PLGA	96.5	2.7[a]	105.0	2.7[a]
PGE1	117.5	2.1[a,b]	123.3	1.6[a,b]

Number of samples is 6 per group. The *a* means significantly different from the control, and *b* means significantly different from the PLGA group ($P < .05$).

Table 1b Bone Volume (mm^3)

	4 weeks		8 weeks	
	Mean	SE	Mean	SE
Control	42.7	1.2	46.7	1.7
PLGA	49.4	4.7[a]	58.4	1.0[a]
PGE1	63.4	2.8[a,b]	73.5	2.7[a,b]

Number of samples is 6 per group. The *a* means significantly different from the control, and *b* means significantly different from the PLGA group ($P < .05$).

The mineral apposition rate (MAR) was obtained by dividing the distance between the 2 fluorescent labels (calcein and tetracycline) by the time between the application of the 2 bone markers (7 days). To identify osteoclasts, the paraffin sections were also stained with tartrate-resistant acid phosphatase (TRAP). TRAP-positive multinucleated cells with more than 3 nuclei were counted.

Statistical Analysis

Statistical differences between treatments were determined by analysis of variance using Statcel software (OMS, Saitama, Japan). All statistical analyses were done using the Fisher exact test. $P < .05$ was regarded as the level of significance.

RESULTS

Soft radiographs demonstrated the increase of the width of alveolar bone in the PGE1 group at 8 weeks, although this was not clearly observed at 4 weeks (Fig 2). Tables 1a and 1b show bone mineral density and bone volume at 4 and 8 weeks. Corresponding to the images of soft radiographs, both bone mineral density and bone volume of the PGE1 group were significantly higher than those of the control and PLGA groups at 4 and 8 weeks.

Histologic images are presented in Fig 3. At 4 weeks in the control group, the socket was filled with fibrous tissue, and erythroid tissue had accumulated in spots. At 8 weeks in the control group, newly

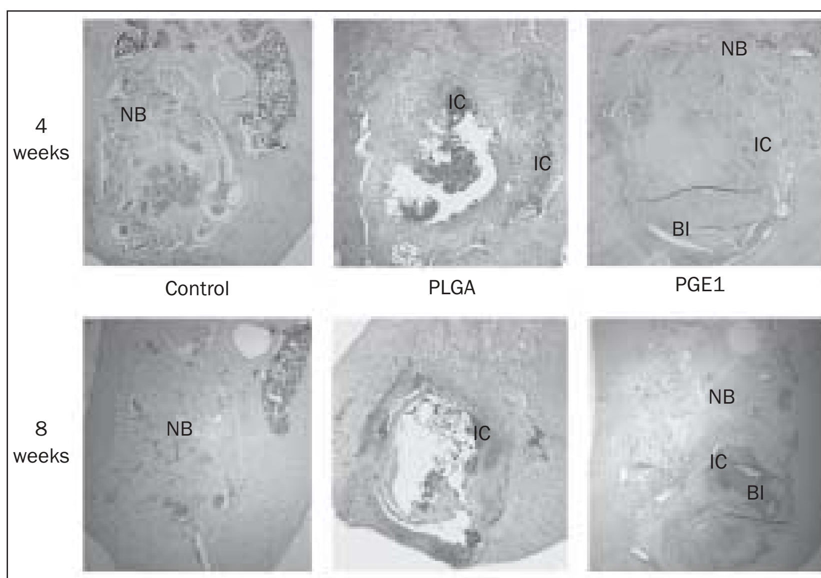

Fig 3 Histologic images of transverse sections of the sockets. Hematoxylin-eosin staining. Newly formed bone (NB), bone islands (BI), and inflammatory cell filtration (IC) are noted.

Table 2a Mineral Apposition Rate (μm/day)

	4 weeks		8 weeks	
	Mean	SE	Mean	SE
Control	3.28	0.30	2.72	0.32
PLGA	2.76	0.49	3.41	0.41
PGE1	4.74	0.33[a,b]	3.39	0.49

Number of samples is 3 per group. The letter *a* means significantly different from the control group and *b* means significantly different from the PLGA group ($P < .05$).

Table 2b Number of Osteoclasts (mm)

	4 weeks		8 weeks	
	Mean	SE	Mean	SE
Control	9.0	0.6	9.0	0.6
PLGA	12.0	0.6	13.0	1.2
PGE1	20.0	2.0[a,b]	12.0	1.2

Number of samples is 3 per group. The letter *a* means significantly different from the control group and *b* means significantly different from the PLGA group ($P < .05$).

formed bone occupied the socket. On the other hand, in the PLGA group, although newly formed bone had appeared, the presence of inflammatory cells was evident in the central part of the socket at 4 and 8 weeks. In the PGE1 group at 4 weeks, although the socket was filled with fibrous tissue, new bone appeared in the socket as islands and inflammatory cells had accumulated around these bone islands. Furthermore, the alveolar bone had thickened. In the PGE1 group at 8 weeks, the socket was still filled with fibrous tissue and inflammatory cells had accumulated around the bone islands. Newly formed bone extended from the original alveolar bone, and the alveolar ridge had thickened.

Histomorphometric data are presented in Tables 2a and 2b. At 4 weeks in the PGE1 group, mineral apposition rate and number of osteoclasts were higher than in the other groups, whereas these parameters were similar in all groups at 8 weeks.

During the experiments, the body weight of the animals was measured and there was no difference in body weight change between the 3 groups (data not shown). Visually, no differences were observed in the socket or the surrounding gingiva between the 3 groups during the experimental period. At 4 weeks, gingiva completely covered the socket in all 3 groups.

DISCUSSION

In the present study, the effects of PGE1 on the alveolar ridge were examined after mandibular incisor extraction in the rat. The reasons for using this animal experimental model were as follows. First, the use of small animals like rats permits larger sample numbers than is feasible for large animals. Second, large tooth sockets can be constantly prepared

in rats by extracting the mandibular incisors. Rat incisors continuously erupt, and the removal of occlusal contact by cutting the incisal edges every 3 days increases eruption speed and reduces the mechanical strength of the periodontal ligament.[15] Following the procedure of a previous study, extraction of the incisors without fracturing the tooth roots was carried out to permit easy extraction.[15] Finally, precise histologic changes in the alveolar ridge after rat mandibular incisor extraction have been reported by Sato and Kasugai.[16]

PGE1 has been used in infants with ductus dependent cyanotic congenital heart disease.[17] Ueda et al reported 2 patients with cortical hyperostosis, that occurred following long-term administration of PGE1 at low doses.[18] Marks and Miller performed an experiment involving local application of PGE1 to the mandibles of beagles. They showed that local infusions of PGE initiated a marked osteogenic response that resulted in the production of new primary lamellar bone. This osteogenic response is rapid, producing a substantial amount of new primary bone, which is localized at the site of infusion, and occurs without any appreciable side effects. Although in vitro effects of PGE1 on proliferation and differentiation are controversial, and although PGE1 stimulates osteoclast formation and activation in culture, it is obvious that PGE1 exerts anabolic actions in bone when it is systemically or locally administered.

In the present study, radiologic analyses demonstrated that the width of the alveolar bone increased in the PGE1 group. Histologically, thicker alveolar bone was observed in the PGE1 group than in the other 2 groups. Histomorphometric data revealed an increase in both mineral apposition rate and number of osteoclasts at 4 weeks in the PGE1 group, clearly indicating enhancement of bone remodeling. Miller and Marks applied control-release pellets containing PGE1 to the surface of the mandibles of beagles.[9] In his experiment, bone formation followed a resorption phase, indicating that PGE1 stimulates bone remodeling. Consequently, cortical bone porosity and single- and double-labeled surfaces increase. The present results confirm the finding of Miller and Marks[9] using a different experimental model.

At 8 weeks there was no difference in mineral apposition rate or number of osteoclasts among the 3 groups. It has been reported that the period of complete absorption of the PLGA carrier, which was used in the present study, is approximately 35 days. As was expected, PLGA together with PGE1 had been completely absorbed by the socket at 8 weeks. It would thus be expected for the mineral apposition rate and number of osteoclasts in the PGE1 group to be similar to those of the other 2 groups at 8 weeks, although bone volume and bone mineral density was still higher in the PGE1 group at 8 weeks. These findings indicate that even if the effects of PGE1 disappear and the normal bone remodeling cycle returns, increased bone volume and bone mineral density are maintained.

In the present study, the body weight of the animals and the gingiva of the alveolar ridge did not differ among the 3 groups. PGE1 is a highly effective molecule; however, it is unlikely in the present study that local application of PGE1 would affect systemic bone metabolism and elicit severe systemic side effects, since the dose of PGE1 was 0.5 mg and it was locally applied and slow released over a long period.

PLGA is a biodegradable biomaterial with a long history of use as bone plates, screws, and sutures for surgery. Negative effects of PLGA on tissues have not been extensively reported; however, biodegradation of PLGA produces lactic acid and glycolic acid, which may irritate the surrounding tissues. To compensate for the decrease of pH following the degradation of PLGA, calcium carbonate was added to the PLGA carrier. In the present study, inflammatory cells were histologically observed in the PLGA and PGE1 groups but not in the control group. It is thus possible that PLGA itself influenced socket healing. Although a negative influence of PLGA on socket healing was speculated, PGE1 markedly increased alveolar bone. Bone mineral density and bone volume in the PLGA group were higher than in the control group because β-TCP in the carrier increased these values.

CONCLUSION

In summary, the present study demonstrates that local application of PGE1 to the incisal tooth socket activates both osteoclast differentiation and osteoblast activity, consequently increasing the bone volume and bone mineral density of the alveolar ridge. Although further studies are required, PGE1 has the potential to preserve and/or augment alveolar bone after tooth extraction.

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