Acta Odontologica Scandinavica. 2014; 72: 265–273

informa
healthcare

ORIGINAL ARTICLE

Effect of the interaction between periodontitis and type 1 diabetes mellitus on alveolar bone, mandibular condyle and tibia

JI-HYE KIM[1,2], DONG-EUN LEE[1,2], K. S. NILUKA DARSHANI GUNAWARDHANA[2], SEONG-HO CHOI[3], GYE-HYEONG WOO[4], JEONG-HEON CHA[1,2,5], EUN-JUNG BAK[5] & YUN-JUNG YOO[1,2]

[1]*Department of Applied Life Science, The Graduate School, Yonsei University, Seoul, Republic of Korea,* [2]*Department of Oral Biology, Yonsei University College of Dentistry, Seoul, Republic of Korea,* [3]*Department of Periodontology, Research Institute for Periodontal Regeneration, Yonsei University College of Dentistry, Seoul, Republic of Korea,* [4]*Department of Clinical Science, Semyung University, Jecheon, Republic of Korea, and* [5]*Oral Cancer Research Institute, Yonsei University College of Dentistry, Seoul, Republic of Korea*

Abstract

Objective. This study examined the effect of the interaction between periodontitis and type 1 diabetes mellitus on alveolar bone, mandibular condyle and tibia in animal models. ***Materials and methods.*** Rats were divided into normal, periodontitis, diabetic and diabetic with periodontitis groups. After injection of streptozotocin to induce diabetes, periodontitis was induced by ligation of both lower-side first molars for 30 days. Alveolar bone loss and trabecular bone volume fraction (BVF) of the mandibular condyle and tibia were estimated via hematoxylin and eosin staining and micro-computed tomography, respectively. Osteoclastogenesis of bone marrow cells isolated from tibia and femur was assayed using tartrate-resistant acid phosphatase staining. ***Results.*** The cemento-enamel junction to the alveolar bone crest distance and ratio of periodontal ligament area in the diabetic with periodontitis group were significantly increased compared to those of the periodontitis group. Mandibular condyle BVF did not differ among groups. The BVF of tibia in the diabetic and diabetic with periodontitis groups was lower than that of the normal and periodontitis groups. Osteoclastogenesis of bone marrow cells in the diabetic groups was higher than that in the non-diabetic groups. However, the BVF of tibia and osteoclastogenesis in the diabetic with periodontitis group were not significantly different than those in the diabetic group. ***Conclusions.*** Type 1 diabetes mellitus aggravates alveolar bone loss induced by periodontitis, but periodontitis does not alter the mandibular condyle and tibia bone loss induced by diabetes. Alveolar bone, mandibular condyle and tibia may have different responses to bone loss stimuli in the diabetic environment.

Key Words: *Bone, periodontitis, type 1 diabetes mellitus*

Introduction

Bone is composed of osteoclasts, osteoblasts and osteocytes. Bone remodeling requires the dual action of bone resorption and bone formation. Osteoclasts resorb bone and osteoblasts form an equivalent amount of new bone [1]. Periodontitis is an inflammatory disease caused by bacteria and alveolar bone loss surrounding the roots of teeth is one of its characteristics. The alveolar bone loss is induced by an imbalance of bone resorption and bone formation caused by bacteria and the immune reaction targeting the bacteria [2,3]. Some diseases such as periodontitis and type 1 diabetes mellitus can disturb the balance of osteoclasts and osteoblasts and cause bone loss. Type 1 diabetes mellitus is characterized by hyperglycemia resulting from defects in insulin secretion and elevated bone fracture risk [4]. Type 1 diabetes mellitus has been associated with bone loss, but a mechanism of action remains undefined. Previous research suggests that bone loss in type 1 diabetes mellitus is caused by reduced bone formation by osteoblasts, but an association with increased bone resorption remains unclear [5–9].

Correspondence: Yun-Jung Yoo, DDS, PhD, Department of Oral Biology, Yonsei University College of Dentistry, 134 Sinchon dong, Seodaemun-gu, Seoul 120-752, Republic of Korea. Tel: +82 2 2228 3060. Fax: +82 2 2227 7903. E-mail: yu618@yuhs.ac
Co-Correspondence: Eun-Jung Bak, D.V.M., Ph.D., Oral Cancer Research Institute, Yonsei University College of Dentistry, 134 Sinchon dong, Seodaemun-gu, Seoul 120-752, Republic of Korea. Tel: +82 2 2228 3062. Fax: +82 2 2227 7903. E-mail: ejbak@yuhs.ac

(Received 17 April 2013; accepted 1 July 2013)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.3109/00016357.2013.822551

There is data suggesting a bidirectional relationship between periodontitis and diabetes. Although some studies fail to find an association between periodontitis and type 1 diabetes mellitus [10,11], several studies suggest that the prevalence of periodontitis is higher in subjects with type 1 diabetes mellitus than those without, suggesting that type 1 diabetes mellitus predisposes to periodontitis [12–15]. Several epidemiological studies report that periodontitis contributes to complications of type 1 diabetes mellitus. Investigators observed a greater risk of ketoacidosis, retinopathy and neuropathy in type 1 diabetes mellitus subjects with periodontitis [16,17]. In addition, there is a significantly higher prevalence of proteinuria and cardiovascular complications in type 1 diabetes mellitus patients with periodontitis [18,19]. Although the association between periodontitis and various complications of type 1 diabetes mellitus has been suggested, the question of whether periodontitis affects systemic bone loss in type 1 diabetes mellitus has not been addressed. To manage bone loss in type 1 diabetes mellitus subjects with periodontitis, it is important to understand the bidirectional relationship between periodontitis and type 1 diabetes mellitus in bone tissue. Therefore, we examined the effect of the interaction between periodontitis and type 1 diabetes mellitus on alveolar bone, mandibular condyle and tibia using streptozotocin (STZ)-induced diabetic rats with ligature-induced periodontitis.

Materials and methods

Induction of type 1 diabetes mellitus and periodontitis in rats

Forty male rats of the F344 strain were purchased from Oriental Bio (Gyeonggi-do, Korea) and acclimated for 1 week. Food and water were provided *ad libitum*. The rats were maintained in a temperature-controlled room ($22 \pm 2^{\circ}$C) on a 12 h light–dark cycle. Animals were randomly divided into four groups (10 animals per group): normal, periodontitis, diabetic and diabetic with periodontitis. After 18 h of fasting, the diabetic groups were given intravenously 50 mg/kg of STZ (Sigma-Aldrich, St Louis, MO) in 0.1 M citrate buffer and the non-diabetic groups were injected with citrate buffer alone. At day 7 after injection, fasting blood glucose levels were measured from tail with Accu-check pro (Roche Diagnostics, Mannheim, Germany). If glucose levels were greater than 300 mg/dl, the rat was considered diabetic. Seven days after STZ or citrate buffer injection, rats were anesthetized with a 1:2 mixture of Zoletil 50 (Virbac, Carros, France) and Rumpun (Bayer, Ansan, Korea). Thread was placed around the cervix of the right and left first molars in the mandible and knotted mesially. At days 10, 20 and 30 after the ligation, the blood glucose level and body weight were measured and all rats were sacrificed at day 30. All animal procedure protocols were approved by the Institutional Animal Care and Use Committee of Yonsei University (2010-0209).

Histological analysis

For histopathological examination, both mandibles were extracted at day 30 after the ligation. The dissected mandibles were fixed in 10% neutral-buffered formalin for 2 days and decalcified in 5% nitric acid for 1 week. After embedding in paraffin, sections were cut at a thickness of 4 μm. Sections were selected based on the clear appearance of dental pulp of the mesial and distal roots of the first molars and were stained with hematoxylin and eosin. The degree of alveolar bone loss in the distal area and furcation of the first molar was examined with a light microscope ($\times$100). Alveolar bone loss in the distal area of the first molar was examined by measuring the distance from cemento-enamel junction (CEJ) to alveolar bone crest (ABC) using Image Pro Plus (Media Cybernetics, Silver Spring, MD). Alveolar bone loss in the furcation was estimated by measuring a ratio of the periodontal ligament (PDL) area to the region of interest (ROI) of furcation. The height of the ROI is 0.8 mm from the most apical furcation and the PDL area was calculated by subtracting the remaining alveolar bone area from the ROI area. The number of inflammatory cells, included polymorphonuclear leukocytes (PMNs) and mononuclear cells, was counted in a standardized site (0.1 mm $\times$ 0.1 mm) located under the junctional epithelium of the distal area of the ligated first molar.

Micro-computed tomography (micro-CT) analysis

Fixed tibia and mandible were scanned using micro-CT (Skyscan, Antwerp, Belgium) to evaluate the trabecular bone loss. After three-dimensional images of the mandibular condyle and tibia were reconstructed, the ROI was determined. The ROI of tibia was sized at 2.5 mm (1.5 mm below the growth plate toward the diaphysis and excluding the outer cortical shell). The ROI of condyle was extended 0.45 mm from the highest point of the condyle and excluded the outer cortical shell. Trabecular bone loss was defined as the trabecular bone volume fraction (BVF) and it was determined by dividing the total trabecular bone volume by the total ROI volume.

Osteoclast formation assay

Bone marrow cells were isolated from the tibia and femur. Cells were flushed out with a syringe and collected in α-minimum essential media (Welgene, Deagu, Korea). Bone marrow cells were plated at a constant density (2×10^5) in 96-well plastic

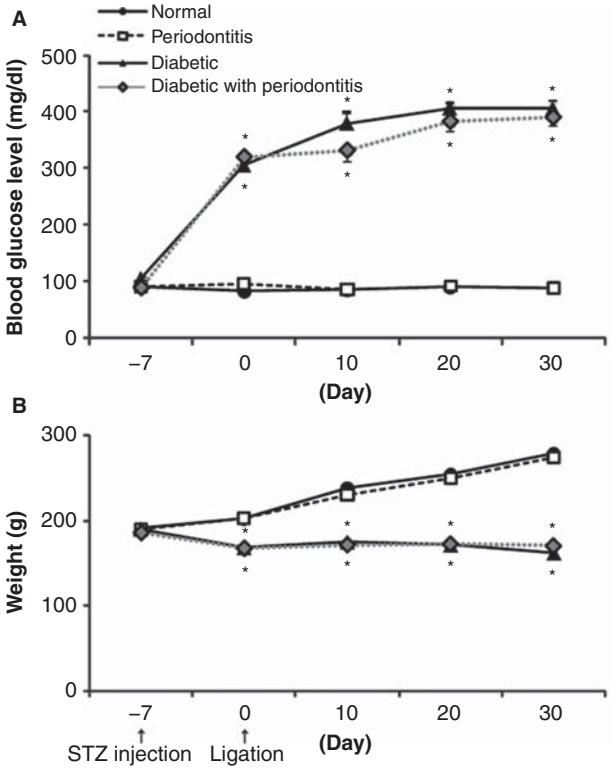

Figure 1. Blood glucose level and body weight. Rats were divided into four groups: normal, periodontitis, diabetic and diabetic with periodontitis. Diabetic groups and non-diabetic groups were injected with STZ or vehicle, respectively, and at day 7 after injection, periodontitis was induced by ligation of tooth in periodontitis groups. Before ligation and at day 10, 20 and 30 after ligation, fasting blood glucose level (A) and weight (B) were estimated. Data are represented with mean ± SEM. * $p < 0.05$ compared with normal group.

plates and cultured in media containing 30 ng/ml macrophage colony-stimulating factor (M-CSF; Pepro Tech., Rocky Hill, NJ) and 60 ng/ml receptor activator of nuclear factor-kappa B ligand (RANKL; Pepro Tech.). Cells were maintained for 5 days and the medium was refreshed every 2 days. Osteoclast formation was evaluated by staining with tartrate-resistant acid phosphatase (TRAP), an enzyme that marks osteoclasts. For this stain, cells were fixed with 10% formaldehyde in phosphate-buffered saline, treated with ethanol-acetone (50:50), and stained with TRAP. Via light microscopy, osteoclasts were counted as TRAP-positive cells with more than three nuclei.

Quantitative real time polymerase chain reaction

Whole femurs were homogenized in Trizol reagent (Invitrogen, Carlsbad, CA) and RNA was extracted according to the manufacturer's protocol. Synthesis of complementary DNA (cDNA) was performed by reverse transcription with 3 μg of total RNA using the RT premix kit (Bioneer, Daejon, Korea) with oligo DT primers. Real-time PCR was performed using 2 μl of cDNA with SYBR-Green Real-time PCR Master Mix plus (Applied Biosystems, Warrington, UK). The primers for real-time PCR were as follows: TRAP sense primer, 5′-AATGCCTCGACCTGGG A-3′ and antisense primer, 5′-CGTAGTCCTCCTT GGCTGCT-3′; GAPDH sense primer, 5′-AGTC TACTGGCGTCTTCAC-3′ and antisense primer 5′-TCATATTTCTCGTGGTTCAC-3′. The amplification program was comprised of the initial denaturation step which is 1 cycle for 30 s at 95°C and followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. Relative quantification on gene expression was performed in relation to GAPDH mRNA expression by the application of the $2^{\Delta\Delta C}{}_t$ analysis method [20]. Melting curve and gel analyses were used to verify specific products of appropriate size.

Statistical analysis

All statistical analyses were performed using SPSS 12.0 (SPSS Inc, Chicago, IL). One-way analysis of variance (ANOVA) was used to determine significant differences. A p-value of less than 0.05 was considered statistically significant. Data are expressed as the mean ± standard error (SEM).

Results

Changes in blood glucose level and body weight by STZ

To investigate the induction and maintenance of type 1 diabetes mellitus, we measured fasting glucose level and body weight during the experimental period after STZ or vehicle (citrate buffer) injection. The normal and periodontitis groups injected with vehicle had average blood glucose levels of 100 mg/dl during the experimental period, while the diabetic and diabetic with periodontitis groups injected with STZ had fasting glucose levels of more than 300 mg/dl, typical of diabetic hyperglycemia (Figure 1A). There was no significant difference in fasting glucose levels between the diabetic group (406 ± 14 mg/dl) and the diabetic with periodontitis group (390 ± 11 mg/dl). Seven days after STZ injection, body weight in both the diabetic and diabetic with periodontitis groups were lower than that in the normal and periodontitis groups followed during the 30 days after ligation (Figure 1B).

Effect of type 1 diabetes mellitus on alveolar bone loss induced by periodontitis

The alveolar bone level of the distal and furcation area of the first molar was estimated by measuring the CEJ-ABC distance and a ratio of the PDL area, respectively. The CEJ-ABC distance increased in order from diabetic with periodontitis, to periodontitis, to diabetic to normal (Figure 2). The CEJ-ABC distance in the diabetic with periodontitis and periodontitis groups increased 3.9-fold and 2.9-fold

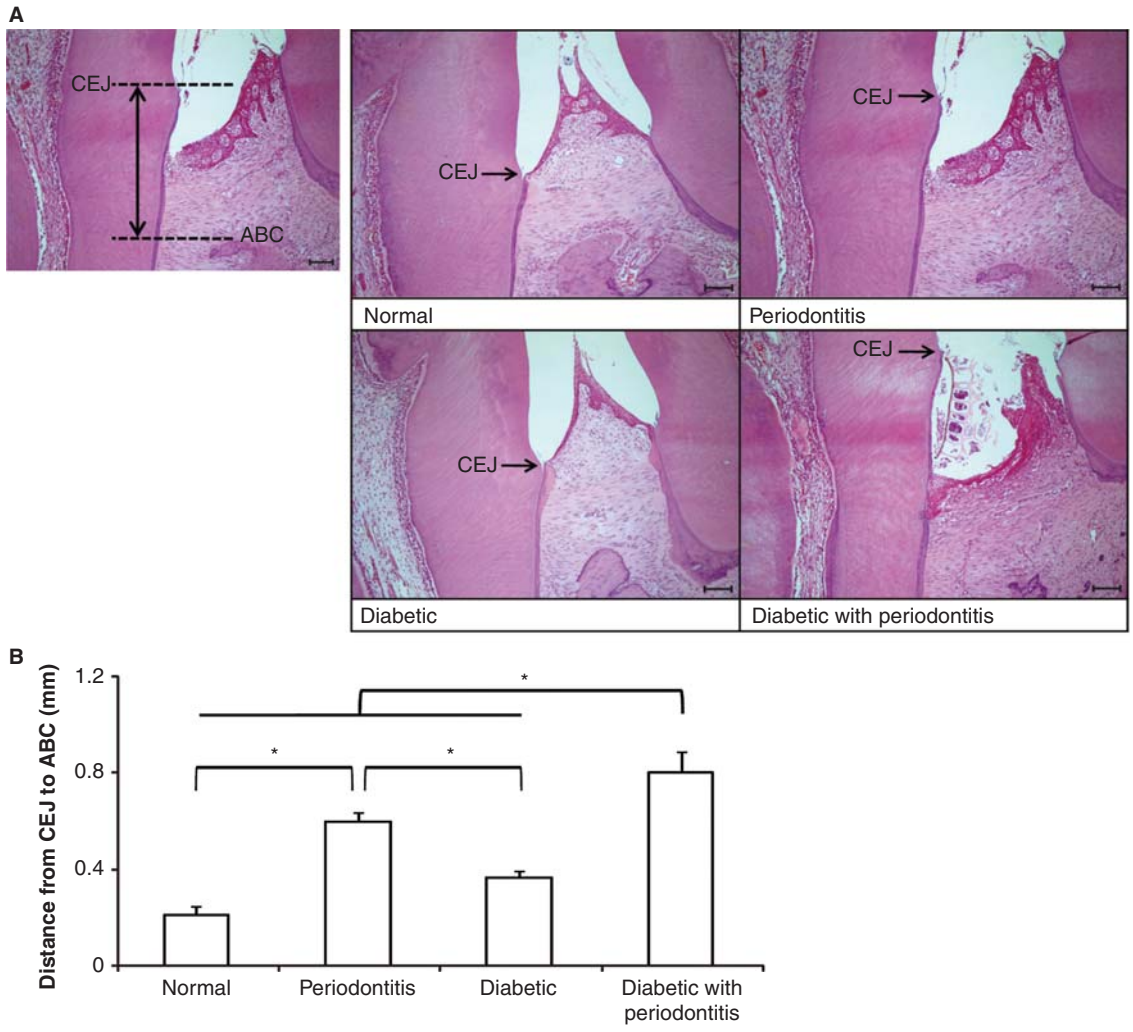

Figure 2. Alveolar bone level in distal area of ligated tooth. At day 30 after tooth ligation, alveolar bone level in the distal area of the first molar was examined with light microscopy (A, ×100 magnification). Alveolar bone loss was estimated by measuring the distance from CEJ to ABC (B). Data are represented with mean ± SEM. *$p < 0.05$ (Bar = 100 μm).

from that in the normal group, respectively. Further, the distance in the diabetic with periodontitis group was significantly increased by 2.2-fold over the diabetic group. Although it was not significant, the CEJ-ABC distance in the diabetic group was slightly increased compared with that of the normal group. The PDL area of the four groups showed a similar trend to the CEJ-ABC distance (Figure 3). There was a 2.3-fold and 1.7-fold increase over normal in the ratio of the PDL area in the diabetic with periodontitis group and the periodontitis group, respectively. There was no difference observed between the normal and diabetic groups.

Effect of type 1 diabetes mellitus on inflammation in gingival tissue

We estimated the number of inflammatory cells, such as PMNs and mononuclear cells, which had infiltrated the connective tissue beneath gingival epithelium. The number of inflammatory cells was increased in the periodontitis, diabetic and diabetic with periodontitis groups when compared to the normal group (Figure 4). However, there was no significant difference observed in the number of inflammatory cells between these three groups.

Effect of periodontitis on loss of mandibular condyle and tibia induced by type 1 diabetes mellitus

The trabecular BVF of the mandibular condyle and tibia was analyzed using micro-CT. The trabecular BVF in the condyle of the diabetic, periodontitis and diabetic with periodontitis groups did not differ from that observed in the normal group (Figure 5). In contrast, the trabecular BVF of the tibia showed a significant decrease in the diabetic and diabetic with periodontitis groups compared to the normal and periodontitis groups (Figure 6). However, there was no difference in the trabecular BVF of the tibia between the normal and periodontitis groups (0.25 ± 0.01, and 0.24 ± 0.01, respectively) or between the

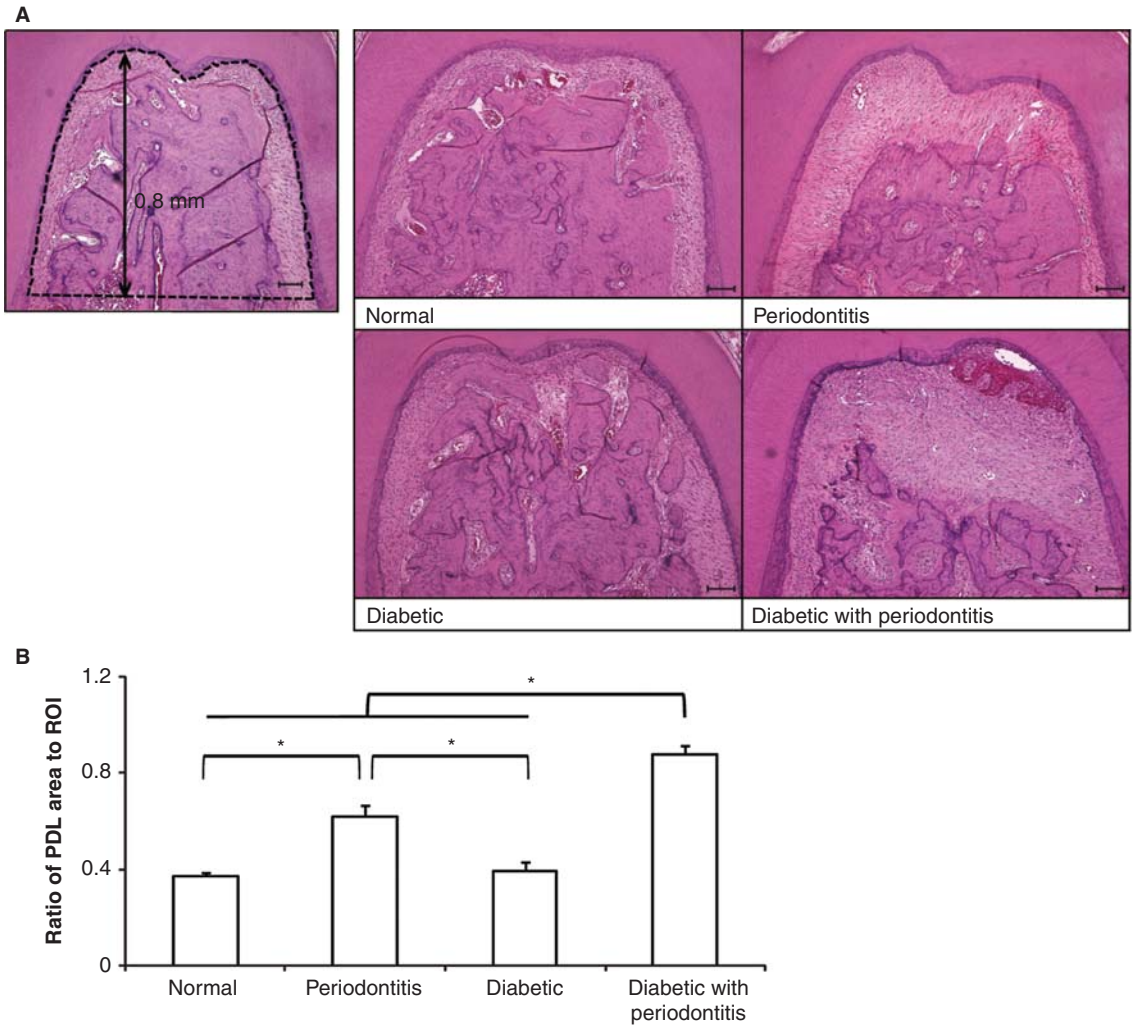

Figure 3. Alveolar bone level in the furcation of ligated tooth. At day 30 after tooth ligation, alveolar bone level in the furcation of the first molar was examined using light microscopy (A, ×100 magnification). Alveolar bone loss in the furcation was represented as a ratio of the PDL area to the ROI. The height of the ROI was 0.8 mm from the apical side of the furcation and the PDL area was calculated by subtracting the alveolar bone area from the ROI (B). Data are represented with mean ± SEM. * $p < 0.05$ (Bar = 100 μm).

diabetic and diabetic with periodontitis groups (0.13 ± 0.02, and 0.12 ± 0.02, respectively), regardless of the induction of periodontitis.

Effect of periodontitis on osteoclast formation of bone marrow cells in tibia and femur induced by type 1 diabetes mellitus

The osteoclast formation ability of bone marrow cells in the tibia and femur was estimated by culturing bone marrow cells in the presence of RANKL and M-CSF and then staining for TRAP. Although the number of TRAP-positive multinucleated cells in the diabetic and diabetic with periodontitis groups was markedly increased compared to the normal and periodontitis groups (Figure 7A), there was no significant difference between the diabetic group and diabetic with periodontitis group. We next compared the TRAP mRNA expression level in the femur between the normal and diabetic groups using real time PCR. We found that the mRNA expression level of TRAP was increased by 2-fold in the diabetic group compared to the normal group (Figure 7B).

Discussion

In this study, we studied the interaction between periodontitis and type 1 diabetes mellitus in bone tissues such as alveolar bone, mandibular condyle and tibia using animal models of periodontitis, diabetes and both periodontitis and diabetes. To induce diabetes experimentally, we injected rats with STZ intravenously. STZ is cytotoxic to pancreatic β cells and thus induces type 1 diabetes mellitus [21]. While the normal and periodontitis groups had normal blood glucose levels and body weight gain during the experiment, the diabetic and diabetic with periodontitis groups exhibited hyperglycemia (300 mg/dl) and severe weight loss. These data confirmed that the symptoms of type 1 diabetes mellitus were maintained

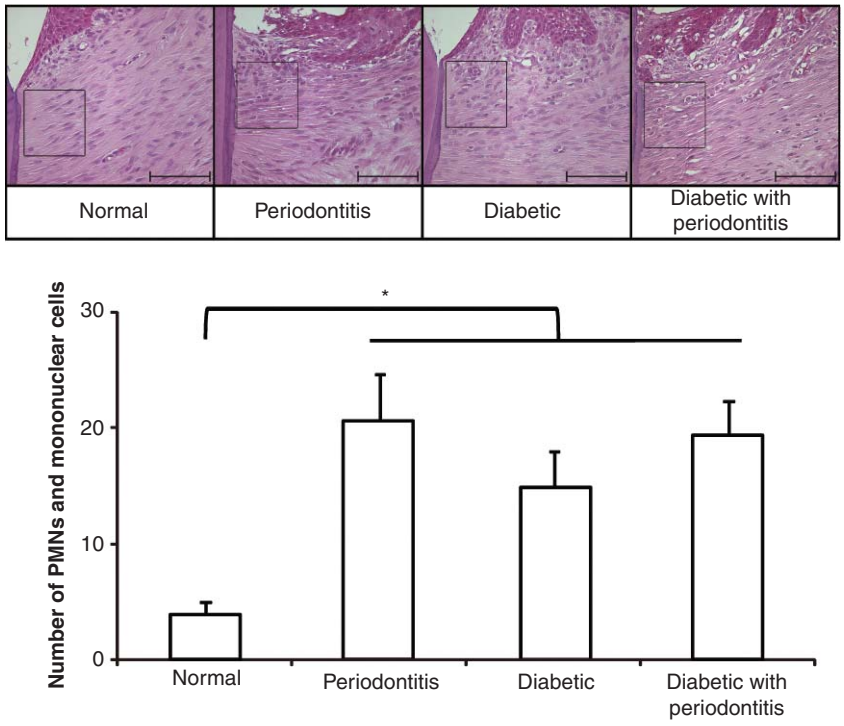

Figure 4. Inflammatory cells in the distal area of the ligated tooth. At day 30 after tooth ligation, the number of inflammatory cells in the area (0.1 mm × 0.1 mm) located under the junctional epithelium were counted. Data are represented with mean ± SEM. * $p < 0.05$ (Bar = 100 μm).

in the STZ-injected rats for the duration of the experiment. In animal models, periodontitis can be induced by various methods including ligation, periodontopathogen swabbing or lipopolysaccharide injection [22,23]. Duarte et al. [22] analyzed the composition of the biofilm that had accumulated around ligatures using probes of 40 human periodontal species in rats. None of the 40 species was detected in the sulcus of the un-ligated teeth, while 25 bacterial species were found in the biofilm around the ligature, suggesting that ligation plays an important role in the deposition of periodontopathogens. Therefore, we selected the ligature method to induce periodontitis in rats. In ligated teeth, we observed a significant increase in both the distance from CEJ to ABC in distal area and in the ratio of the PDL area in the furcation. These data confirmed that alveolar bone loss is significantly induced by tooth ligation, as previously reported [22].

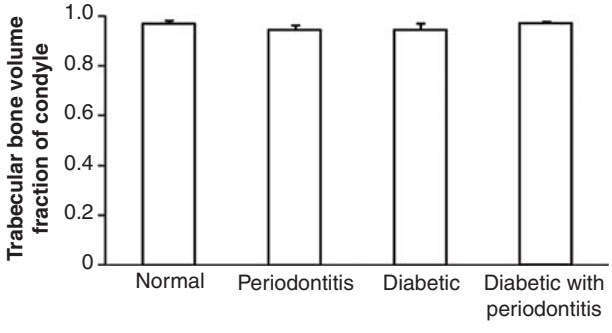

Figure 5. Trabecular bone level in the mandibular condyle. At day 30 after tooth ligation, mandibles were fixed with 10% buffered neutral formalin and scanned using micro-CT. The trabecular bone level was evaluated by the trabecular bone volume fraction, which was calculated by dividing the trabecular bone volume by the total ROI volume. Data are represented with mean ± SEM. * $p < 0.05$.

The effects of type 1 diabetes mellitus on periodontitis have been investigated in epidemiological studies and using animal models. In some epidemiological studies, no significant differences in gingivitis, pocket depth and radiographically estimated alveolar bone loss are observed between the type 1 diabetes mellitus group and the group without diabetes [10,11]. However, other studies report the number of teeth with evidence of attachment loss is significantly greater in subjects with type 1 diabetes mellitus, suggesting that diabetes significantly increases the risk for periodontal destruction [12,24]. In studies using a STZ-induced type 1 diabetes mellitus model, alveolar bone loss induced by *Porphyromonas gingivalis* is aggravated by type 1 diabetes mellitus, but the effects on alveolar bone loss by ligation remain controversial [23,25,26]. These animal studies used different methods to measure alveolar bone loss and this may account for the discrepancy in the data reported. To estimate the exact bone loss in this study, we measured not only the distance from CEJ to ABC, but also a ratio of the PDL area. Although the distance from CEJ to ABC of the distal area and a ratio of the PDL area of furcation in the diabetic group were not significantly different from the normal group, they were greater in the diabetic with periodontitis group than in the periodontitis group. These results indicate that

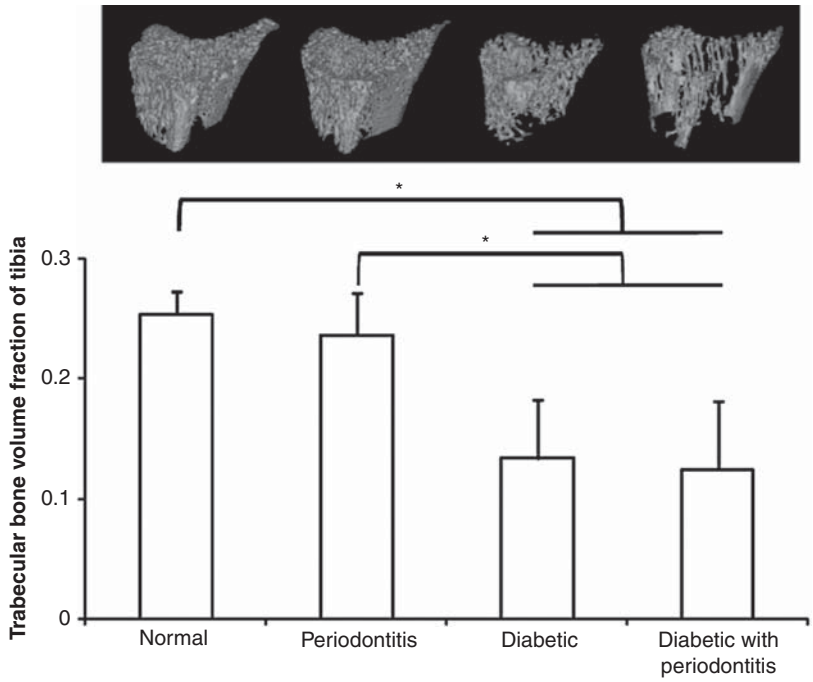

Figure 6. Trabecular bone level in tibia. At day 30 after tooth ligation, the tibia was fixed with 10% buffered neutral formalin and scanned using micro-CT. The trabecular bone level was evaluated by trabecular bone volume fraction which was calculated by dividing the trabecular bone volume by the total ROI volume. Data are represented with mean ± SEM. * $p < 0.05$.

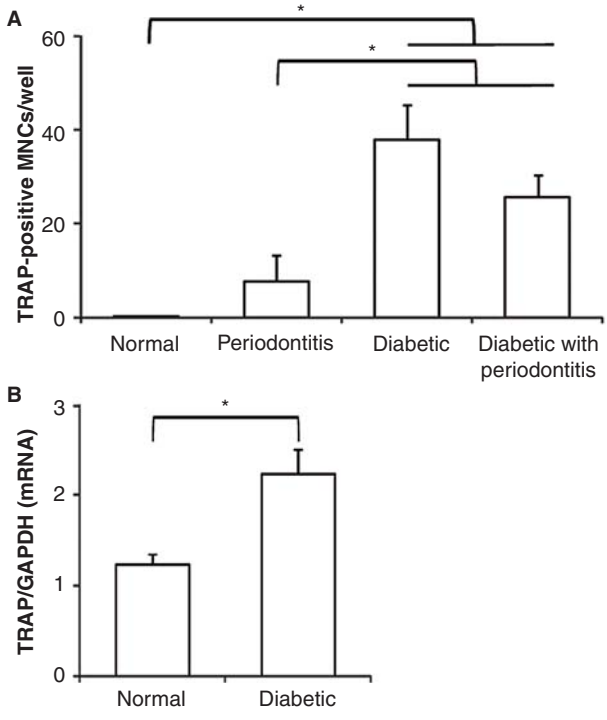

Figure 7. Osteoclastogenesis of tibia and femur. At day 30 after tooth ligation the bone marrow cells were isolated from the tibia and femur. Bone marrow cells were cultured in the presence of M-CSF (30 ng/ml) and RANKL (60 ng/ml) for 5 days and the number of osteoclasts was estimated by TRAP staining (A). TRAP-positive cells with more than three nuclei were counted as osteoclasts and the data are represented with mean ± SEM. * $p < 0.05$. RNA was extracted from the femur of the normal and diabetic groups and real-time PCR for TRAP and GAPDH was performed. The level of TRAP expression was normalized to GAPDH (B). Data are represented with mean ± SEM. * $p < 0.05$.

type 1 diabetes mellitus aggravates the alveolar bone loss observed with periodontitis.

As inflammation in periodontal tissue is associated with alveolar bone resorption in periodontitis, we measured the degree of inflammation. The diabetic, periodontitis and diabetic with periodontitis groups all had an increased number of inflammatory cells when compared to the normal group. These data suggest that, although type 1 diabetes mellitus alone does not induce alveolar bone loss, it significantly stimulates inflammation in gingival tissue. In other study using type 2 diabetic animal model, inflammation in the periodontitis group peaks at day 7 after removal of the ligation and then decreased gradually. In contrast, the diabetic with periodontitis group maintained high inflammation levels until 15 days after removal of the ligation and then the levels began to decrease [27]. In this study, periodontitis was sustained for 30 days by ligation and long maintenance of periodontitis may be related with no difference of inflammation degree between the periodontitis and the diabetic with periodontitis groups.

Systemic bone loss is a complication of type 1 diabetes mellitus. Decreased bone mineral density is found in more than 50% of type 1 diabetes mellitus patients [28,29]. To evaluate the effects of periodontitis on skeletal bone in type 1 diabetes mellitus, we analyzed trabecular BVF using micro-CT in mandibular condyle and tibia which are located near or far away from the periodontal lesion, respectively. A significant decrease in tibia trabecular BVF was observed in the diabetic group compared to normal,

but no differences were observed between the diabetic with periodontitis group and the diabetic group. These data suggested that type 1 diabetes mellitus induces bone loss in the tibia, but the addition of periodontitis does not aggravate trabecular bone loss induced by type 1 diabetes mellitus. On the other hand, the trabecular BVF in mandibular condyle did not differ between any groups. These results showed that, although diabetes had an obvious effect on tibia bone loss, it did not induce mandibular condyle bone loss. These findings are consistent with a previous study in which mandibular bone loss was found to be less than tibia bone loss induced by ovariectomy [30]. Mandibular bone arises from a different embryonic germ layer, neuroectoderm, compared to tibia which arises from the mesoderm. Mavropoulos et al. [30] explained that the difference in embryological origin between the two skeletal sites might lead to different responses to bone stimuli. To ascertain the effects of periodontitis on bone loss induced by type 1 diabetes mellitus, we evaluated the osteoclast formation ability of bone marrow cells isolated from tibia and femur in the presence of the osteoclast differentiation factors M-CSF and RANKL. Although bone marrow cells from the diabetic group had an increased ability to form osteoclasts, there was no significant difference between the diabetic with periodontitis group and the diabetic group. This indicates that type 1 diabetes mellitus increases osteoclast formation, which may be related with tibia bone loss, but periodontitis has no effects on osteoclast formation induced by type 1 diabetes mellitus. The increase of osteoclast formation in the type 1 diabetes mellitus rats may mean an increase in the number or potential of osteoclast progenitor cells that respond to M-CSF and RANKL in the type 1 diabetes mellitus rats. Although it is known that impaired bone formation contributes to bone loss of type 1 diabetes mellitus, the involvement of increased bone resorption is still unclear. Results of TRAP mRNA expression, a marker of osteoclast, in type 1 diabetes mellitus, are controversial [5,7–9]. To confirm the involvement of bone resorption in type 1 diabetes mellitus-induced bone loss in the tibia, we examined TRAP mRNA expression of the femur. In the diabetic group, the level of TRAP mRNA expression was elevated compared with the normal group, as in previous studies [7,8]. This suggests that bone resorption in the tibia is enhanced by type 1 diabetes mellitus.

In conclusion, using STZ-induced type 1 diabetes mellitus rats with ligature-induced periodontitis, we observed a bidirectional effect between periodontitis and type 1 diabetes mellitus in alveolar bone, mandibular condyle and tibia. These results suggest that type 1 diabetes mellitus increases the severity of alveolar bone loss induced by periodontitis, but periodontitis does not affect the severity of tibia loss induced by type 1 diabetes mellitus. The mandibular condyle is less sensitive to type 1 diabetes mellitus than the tibia.

Acknowledgments

The authors thank Jang Gi Cho, Department of Oral Biology, Yonsei University College of Dentistry, for assistance with the micro-CT analysis and graphics. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science and Technology (2010-0008221).

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Matsuo K. Cross-talk among bone cells. Curr Opin Nephrol Hypertens 2009;18:292–7.
[2] Graves DT, Oates T, Garlet GP. Review of osteoimmunology and the host response in endodontic and periodontal lesions. J Oral Microbiol 2011;3.
[3] Behl Y, Siqueira M, Ortiz J, Li J, Desta T, Faibish D. Activation of the acquired immune response reduces coupled bone formation in response to a periodontal pathogen. J Immunol 2008;181:8711–18.
[4] Mastrandrea LD, Wactawski-Wende J, Donahue RP, Hovey KM, Clark A, Quattrin T. Young women with type 1 diabetes have lower bone mineral density that persists over time. Diabetes Care 2008;31:1729–35.
[5] Motyl KJ, Botolin S, Irwin R, Appledorn DM, Kadakia T, Amalfitano A. Bone inflammation and altered gene expression with type I diabetes early onset. J Cell Physiol 2009;218: 575–83.
[6] Motyl KJ, McCabe LR. Leptin treatment prevents type I diabetic marrow adiposity but not bone loss in mice. J Cell Physiol 2009;218:376–84.
[7] Hie M, Yamazaki M, Tsukamoto I. Curcumin suppresses increased bone resorption by inhibiting osteoclastogenesis in rats with streptozotocin-induced diabetes. Eur J Pharmacol 2009;621:1–9.
[8] Hie M, Tsukamoto I. Increased expression of the receptor for activation of NF-κB and decreased runt-related transcription factor 2 expression in bone of rats with streptozotocin-induced diabetes. Int J Mol Med 2010;26:611–18.
[9] Botolin S, Faugere MC, Malluche H, Orth M, Meyer R, McCabe LR. Increased bone adiposity and peroxisomal proliferator-activated receptor-γ2 expression in type I diabetic mice. Endocrinology 2005;146:3622–31.
[10] Barnett ML, Baker RL, Yancey JM, MacMillan DR, Kotoyan M. Absence of periodontitis in a population of insulin-dependent diabetes mellitus (IDDM) patients. J Periodontol 1984;55:402–5.
[11] Rylander H, Ramberg P, Blohme G, Lindhe J. Prevalence of periodontal disease in young diabetics. J Clin Periodontol 1987;14:38–43.
[12] Lalla E, Cheng B, Lal S, Tucker S, Greenberg E, Goland R. Periodontal changes in children and adolescents with diabetes: a case-control study. Diabetes Care 2006;29:295–9.

[13] Lalla E, Cheng B, Lal S, Kaplan S, Softness B, Greenberg E. Diabetes-related parameters and periodontal conditions in children. J Periodontal Res 2007;42:345–9.
[14] Ruiz DR, Romito GA, Dib SA. Periodontal disease in gestational and type 1 diabetes mellitus pregnant women. Oral Dis 2011;17:515–21.
[15] Hodge PJ, Robertson D, Paterson K, Smith GL, Creanor S, Sherriff A. Periodontitis in non-smoking type 1 diabetic adults: a cross-sectional study. J Clin Periodontol 2012;39:20–9.
[16] Rosenthal IM, Abrams H, Kopczyk A. The relationship of inflammatory periodontal disease to diabetic status in insulin-dependent diabetes mellitus patients. J Clin Periodontol 1988; 15:425–9.
[17] Noma H, Sakamoto I, Mochizuki H, Tsukamoto H, Minamoto A, Funatsu H. Relationship between periodontal disease and diabetic retinopathy. Diabetes Care 2004;27:615.
[18] Thorstensson H, Kuylenstierna J, Hugoson A. Medical status and complications in relation to periodontal disease experience in insulin–dependent diabetics. J Clin Periodontol 1996; 23:194–202.
[19] Sadzeviciene R, Paipaliene P, Zekonis G, Zilinskas J. The influence of microvascular complications caused by diabetes mellitus on the inflammatory pathology of periodontal tissues. Stomatologija 2005;7:121–4.
[20] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta\ C_T}$ Method. Methods 2001;25:402–8.
[21] Ganda OP, Rossini AA, Like AA. Studies on streptozotocin diabetes. Diabetes 1976;25:595–603.
[22] Duarte PM, Tezolin KR, Figueiredo LC, Feres M, Bastos MF. Microbial profile of ligature-induced periodontitis in rats. Arch Oral Biol 2010;55:142–7.
[23] Lalla E, Lamster IB, Feit M, Huang L, Schmidt AM. A murine model of accelerated periodontal disease in diabetes. J Periodontal Res 1998;33:387–99.
[24] Lalla E, Cheng B, Lal S, Kaplan S, Softness B, Greenberg E. Diabetes mellitus promotes periodontal destruction in children. J Clin Periodontol 2007;34:294–8.
[25] Holzhausen M, Garcia DF, Pepato MT, Marcantonio E Jr. The influence of short-term diabetes mellitus and insulin therapy on alveolar bone loss in rats. J Periodontal Res 2004;39:188–93.
[26] Fu YW, He HB. Apoptosis of periodontium cells in streptozototocin- and ligature-induced experimental diabetic periodontitis in rats. Acta Odontol Scand 2013. [Epub ahead of print].
[27] Pacios S, Kang J, Galicia J, Gluck K, Patel H, Ovaydi-Mandel A. Diabetes aggravates periodontitis by limiting repair through enhanced inflammation. FASEB J 2012;26:1423–30.
[28] McCabe LR. Understanding the pathology and mechanisms of type I diabetic bone loss. J Cell Biochem 2007;102: 1343–57.
[29] Martin LM, McCabe LR. Type I diabetic bone phenotype is location but not gender dependent. Histochem Cell Biol 2007;128:125–33.
[30] Mavropoulos A, Rizzoli R, Ammann P. Different responsiveness of alveolar and tibial bone to bone loss stimuli. J Bone Miner Res 2007;22:403–10.