Bone remodeling during orthodontic tooth movement in rats with type 2 diabetes

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Introduction: Type 2 diabetes is known to affect bone metabolism. In this study, we aimed to determine the effects of type 2 diabetes on bone remodeling during orthodontic tooth movement. **Methods:** The 48 rats were divided into 4 groups: Wistar control group (n = 8), Goto-Kakizaki (GK) control group (n = 8), Wistar appliance group (n = 16), and GK appliance group (n = 16). The distances between the teeth were measured weekly. On day 42, maxillary alveolar bone specimens were obtained for histologic evaluation and determination of the gene expression levels of the receptor activator of nuclear factor κB (RANK), RANK ligand (RANKL), and osteoprotegerin (OPG). **Results:** No significant difference was observed in the levels of tooth movement between the 2 appliance groups. After orthodontic force application, the alveolar bone volume and osteoblast surface in the GK rats were diminished compared with those in the Wistar rats. The increase in the osteoclast surface relative to the control groups was 2.4-fold greater in the GK rats than in the Wistar rats. Significant upregulations of the RANK and OPG gene expression levels in the Wistar appliance group were observed. The RANKL/OPG ratio was increased in the GK appliance group compared with the Wistar appliance group. **Conclusions:** Diminished bone formation and slightly increased bone resorption were observed during orthodontic tooth movement in the rats with type 2 diabetes. (Am J Orthod Dentofacial Orthop 2015;148:1017-25)

Diabetes is a common metabolic disorder that is characterized by chronic hyperglycemia. Changes in bone structure and its metabolism in the diabetic state have been reported. Diabetic osteoporosis has been well documented in type 1 diabetes (T1D).[1,2] In rat models of T1D, decreased rates of bone turnover have been observed.[3,4] In a study conducted by Abbassy et al,[5] fewer osteoclast cells and impaired bone formation were observed in T1D rats compared with the control rats. Additionally, decreased trabecular thickness and trabecular numbers were observed, indicating the deterioration of bone quality in T1D.[5] The association between type 2 diabetes (T2D) and bone mineral density is less clear. Increased, decreased, and unchanged bone volumes have been observed.[6-8] These contradictory findings could be partially attributed to various confounding factors, such as obesity, which tends to be associated with increased bone volume and is prevalent in persons with T2D.[9] In a study in which nonobese, spontaneous T2D Goto-Kakizaki (GK) rats were used, decreased trabecular bone volume and trabecular thickness were found. In the same study, the analysis of bone metabolism biochemical markers in the blood indicated less bone formation and greater bone resorption.[10] In addition, impaired bone formation in the T2D rat model has been associated with the reduced proliferation and diminished function of osteogenic cells.[11] Higher osteoclastic activity might partially contribute to the increase in bone resorption in T2D rats.[10]

Bone remodeling is an essential process for tooth movement during orthodontic treatment. The receptor activator of nuclear factor κB (RANK), its ligand

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All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and none were reported.
Supported by grant number 1000-06-310089 from the Slovenian Research Agency.
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Submitted, September 2014; revised and accepted, May 2015.
0889-5406/$36.00

http://dx.doi.org/10.1016/j.ajodo.2015.05.031

(RANKL), and osteoprotegerin (OPG) interact to regulate the bone remodeling process and, therefore, play roles in orthodontic tooth movement as well.[12] The binding of RANKL, which is expressed on osteoblast cells, to RANK, which is expressed on osteoclast precursor cells, results in the differentiation of these cells into mature osteoclasts. OPG, which competes with RANK for RANKL binding, inhibits osteoclast differentiation. Increases in the RANKL level and the RANKL to OPG ratio in gingival crevicular fluid have been observed during orthodontic tooth movement.[13] In T2D, lower OPG and slightly higher RANKL gene expression levels have been observed in rat tibiae.[14]

Studies regarding orthodontic tooth movement in the diabetic state have been scarce and inconclusive. Decreased numbers of osteoclasts and osteoblasts, but no changes in bone volume, have been observed in a T1D rat model at 48 hours after force application.[15] In contrast, during force application, elevated numbers of osteoclasts were present for a longer time in the T1D rats than in the controls, leading to prolonged alveolar bone resorption.[16] Higher levels of tooth movement, increased numbers of osteoclasts, increased expression of factors involved in osteoclast activity, and decreased expression of osteoblastic markers have been observed in T1D mice.[17] However, to the best of our knowledge, the influence of T2D on orthodontic tooth movement has not yet been studied.

Considering the increasing demand for orthodontic treatment in adults, who are more likely to have T2D, it seems important to investigate the underlying biologic processes involved in orthodontic tooth movement. In this study, we investigated the bone remodeling process during orthodontic tooth movement in both normal and T2D animal models by histologic evaluation of the alveolar bone and determination of the gene expression levels of RANK, RANKL, and OPG in maxillary bone specimens.

MATERIAL AND METHODS

This study was conducted on 24 male Wistar rats (314 ± 10 g, 13-14 weeks old), representing healthy controls, and 24 male GK rats (320 ± 9 g, 13-14 weeks old), which were used as the T2D animal model. In blood samples taken from the tail vein after overnight fasting, we measured the glucose levels using a glucose reagent strip and glucometer (Sensocard; 77 Elektronika Kft, Budapest, Hungary). The inclusion criterion for the GK rats was an increased blood glucose level of 150 mg per deciliter or greater after overnight fasting. Furthermore, to determine serum insulin, the blood samples were collected in sterilized tubes, left to fully coagulate (30-45 minutes), and centrifuged for 10 minutes at 4°C and at 1800 g. Serum insulin was measured by a chemiluminescent immunometric assay (Immulite 2000 XPi; Siemens, Erlangen, Germany).

All animal procedures and the study protocol were approved by the veterinary administration of the Republic of Slovenia (number 34401-62/2008/9) and were in accordance with the Guide for the Care and Use of Laboratory Animals.

The animals were divided into the following 4 groups: Wistar control group (n = 8), GK control group (n = 8), Wistar appliance group (n = 16), and GK appliance group (n = 16). In the Wistar and GK control groups, no orthodontic appliances were placed. The animals in the Wistar and GK appliance groups were fitted with orthodontic appliances.

The orthodontic appliance consisted of a superelastic closed-coil spring (25 cN, 0.15-mm wire diameter; Dentsply GAC International, York, Pa), which was placed with a stainless steel ligature between the maxillary left first and second molars and the incisors as previously described.[18] The orthodontic appliance was placed in an animal under general anesthesia at the beginning of the study and replaced to the correct position every 7 days, ensuring its proper activation and the exertion of constant force on the teeth.[19] To ensure general anesthesia, a mixture of ketamine (50 mg/kg of body weight; Bioketan; Vetoquinol Biowet, Gorzów Wielkopolski, Poland) and medetomidin hydrochloride (67 μg/kg of body weight; Domitorp; Pfizer, Brooklyn, NY) was injected intraperitoneally.

On day 42, all animals in all groups were killed, and the maxillary bones with the 3 molars were obtained. In the control groups, the maxillary left bones were prepared for histomorphometric analysis, and the right bones were used for the gene expression level determination. In the appliance groups, only the maxillary left bones, in which the appliances were fitted, were collected for histomorphometric (n = 8) and gene expression (n = 8) analyses.

The distance between the most mesial point of the maxillary left first molar and the most palatal point of the ipsilateral incisor at the gingival level was measured in all groups. Measurements were obtained weekly while the animals were anesthetized using a digitronic caliper with an accuracy of ±0.01 mm (Wilson & Wolpert, Utrecht, The Netherlands). All measurements were independently obtained twice by 2 investigators (A.P., Š.S.) within a few minutes. Tooth movement was calculated by subtracting the distance between the teeth on each day of measurement (days 0, 7, 14, 21, 28, 35, and 42) from the distance between the teeth measured the previous week.