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Intermittent administration of parathyroid hormone ameliorated alveolar bone loss in experimental periodontitis in streptozotocin-induced diabetic rats

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ABSTRACT

Objective: Intermittent administration of parathyroid hormone (PTH) has been demonstrated to have anabolic effects on bone metabolism and is approved for use in the treatment of osteoporosis. This study evaluates the role of intermittent PTH administration on alveolar bone loss in streptozotocin (STZ)-induced diabetic rats.

Design: Fifty male Sprague Dawley rats were randomly divided into the following five groups: (1) a control group (saline placebo without ligature and STZ injection), (2) a PTH group (PTH administration without ligature and STZ injection), (3) an L group (saline placebo with ligature), (4) an L + STZ group (saline placebo with ligature and STZ injection), and (5) an L + STZ + PTH group (PTH administration with ligature and STZ injection). PTH was administered at 75 μg/kg per dose four times a week for 28 days. Subsequently, all rats were sacrificed, and their mandibles were extracted for micro-computed tomography (micro-CT) scanning, as well as histological and immunochemical evaluation.

Results: Micro-CT scanning demonstrated the anabolic effect of PTH on alveolar bone metabolism in STZ-induced diabetic rats ($P < 0.05$), and histomorphometry indicated that PTH inhibited inflammation of the periodontium and increased the level of osteoblastic activity ($P < 0.05$). Immunochemical evaluation showed that rats subjected to both ligature placement and STZ injection had the highest receptor activator of nuclear factor kappa B ligand (RANKL)/osteoprotegerin (OPG) ratio and that PTH administration decreased this ratio.

Conclusion: Intermittent systemic PTH administration effectively reduced alveolar bone loss and ameliorated the manifestation of experimental periodontitis in STZ-induced diabetic rats.

1. Introduction

Periodontal disease, one of the most common chronic inflammatory diseases, involves the gradual destruction of connective tissue surrounding the teeth and eventually leads to tooth loss (Kim, Sang, Choi, & Won, 2013). The clinical features of periodontitis include clinical attachment loss, alveolar bone loss, periodontal pockets and gingival inflammation (Armitage, 2004). Several mechanisms of periodontal tissue destruction have been proposed, including a complex array of factors, such as those derived from direct bacterial influence and the immune and non-immune responses of the host system to damage inflicted by bacteria (Pradeep, Agarwal, Bajaj, & Rao, 2013). During the process of periodontal ligament and bone destruction, the host immune system responds to direct bacterial infection, which results in increased production of osteoclastogenic factors in immune cells and eventually contributes to alveolar bone loss.

Diabetes is a metabolic disease characterized by hyperglycaemia, which is caused mainly by insulin resistance and relative or absolute lack of insulin. It is widely accepted in the dental literature that diabetes mellitus (DM) is a risk factor for periodontitis (Kinane & Bouchard, 2008), and periodontitis is recognized as the sixth complication of DM (Löe, 1993). Epidemiological studies demonstrate that the risk of periodontitis in diabetics is almost 3–4 times higher than that in non-diabetic subjects (Preshaw & Bissett, 2013) and that the

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observed severity of periodontitis is greater in patients with DM than in those without (Leite, Marlow, & Fernandes, 2013). More specifically, DM can enhance gingival inflammation (Graves, Naguib, & Lu, 2005), contribute to clinical attachment loss (Al-Khabbaz, 2014) and accelerate alveolar bone resorption (Kim, Lee, & Gunawardhana, 2014). As part of the skeletal system, alveolar bone can also be lost due to an imbalance between bone formation and resorption. More osteoclasts were observed in rats with diabetes and periodontitis than in control rats (Pacios, Kang, & Galicia, 2012). This increase in osteoclastogenesis is thought to result from the enhancement of local inflammation levels (Duarte, de Oliveira, & Tambeli, 2007; Pacios et al., 2012). Patients with diabetes and periodontitis have been observed to have elevated levels of local inflammatory cytokines, such as IL-1β, IL-6 and TNF-α, which eventually result in osteoclastogenesis (Duarte et al., 2007; Southerland, Taylor, & Offenbacher, 2005). Another critical factor that mediates osteoclastogenesis is the ratio of receptor activator of nuclear factor kappa B ligand (RANKL) to osteoprotegerin (OPG). RANKL induces osteoclast formation and activity by interacting with its receptor, RANK, which is expressed on the surface of osteoclasts, while OPG competitively binds to RANK to abolish this effect (Pacios et al., 2012). Importantly, the RANKL/OPG ratio in subjects with periodontitis and diabetes was demonstrated to be unfavourably influenced by poor glycaemic control (Santos, Lima, & Goncalves, 2010) leading to increased osteoclastogenesis. In addition, diabetes has been shown to enhance the accumulation of advanced glycation end products (AGEs) and their receptor (RAGE) in the periodontium (Brownlee, Cerami, & Vlassara, 1988). The accumulation of AGEs and their interaction with RAGE contribute to osteoclastogenesis (Lamster, Bittner, & Lorber, 2014). Reactive oxygen species (ROS) also play a part in this process. It has been demonstrated that ROS can activate osteoclasts and promote their maturation (Garrett, Boyce, & Oreffo, 1990). Neutrophils in diabetic subjects have been shown to produce more ROS than neutrophils in normal subjects (Thomas, Ramesh, & Suresh, 2013), thus increasing osteoclast formation. Diabetes can lead to osteoblast apoptosis by up-regulating pro-apoptotic factors, including TNF-α, AGEs and ROS (Lalla & Papapanou, 2011). (Pacios, Andriankaja, and Kang, 2013 have demonstrated that diabetes-related enhancement of inflammation accelerates the loss of osteoblasts and thus induces alveolar bone loss. The abovementioned evidence demonstrates that diabetes can enhance osteoclastogenesis and osteoblast apoptosis and reduce bone formation, which eventually accelerates alveolar bone loss in periodontitis.

Parathyroid hormone (PTH), a protein composed of 84 amino acids, is a product of the chief cells of the parathyroid glands. PTH is a major regulator of bone remodelling and calcium homeostasis. Intermittent administration of PTH has been shown to effectively stimulate osteoblastic bone formation not only in vitro (Ishizuya, Yokose, & Hori, 1997) but also in vivo (Okimoto, Tsurukami, & Okazaki, 1998), and clinical studies have shown that treatment with PTH has a positive effect on patients with osteoporosis (Fujita, Inoue, & Moril, 1999; Neer, Arnaud, & Zanchetta, 2001). As periodontitis is a bone-destructive disease, the application of PTH may be effective for reversing alveolar bone loss. Barros et al. (2003) reported that intermittent administration of PTH (1–34) in rat models subjected to periodontal disease significantly reduced the amount of periodontitis-associated bone loss and the number of inflammatory cells in the marginal gingival area, and Hamann, Picke, and Campbell (2014) reported that PTH partially reversed the adverse effects of type 2 DM on bone mass, bone strength, and bone defect repair in rats but did not affect energy metabolism.

Based on these findings, we have hypothesized that PTH is a potent suppressor of periodontal inflammation and alveolar bone loss in diabetic rats. Therefore, the aim of the present study was to examine the validity of this hypothesis and to validate whether the effect occurs through bone remodelling, regulation of inflammation or both.

2. Materials and methods

2.1. Animals

Fifty male Sprague Dawley rats aged between 6 and 8 weeks and weighing 180–220 g were purchased from the Laboratory Animal Centre of Sichuan University. All rats were housed at a constant temperature (22 °C), with humidity of 45–55%, under a 12-h/12-h light/dark cycle and had free access to water. The rats were weighed every week. All animal protocols were approved by the Institutional Ethics Committee of the State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University.

2.2. Group assignment and administration of PTH

The rats were randomly divided into the following five groups (n = 10 animals/group) after one week of acclimatization to their new circumstances: (1) a control group (saline placebo without ligature and STZ injection), (2) a PTH group (PTH injection without ligature and STZ injection), (3) an L group (saline placebo with ligature), (4) an L + STZ group (saline placebo with ligature and STZ injection), and (5) an L + STZ + PTH group (PTH injection with ligature and STZ injection). The treatments (saline or PTH injection) were given via subcutaneous injection 4 times per week for the entire experimental period, beginning 1 day after ligature attachment, and all the rats were sacrificed 1 day after the last injection.

To induce diabetes, we intraperitoneally injected the rats with a 65 mg/kg dose of the diabetogenic agent streptozotocin (STZ) diluted in 0.3 mL of 0.1 mol/L citrate buffer (pH 4.4) once per week for three weeks. Glycaemia levels were measured weekly after the third dose of STZ. Animals were considered diabetic if their fasting glycaemia measurement was greater than 11.1 mmol/L (> 200 mg/dL). Control animals were intraperitoneally injected with equivalent volumes of citrate buffer and subjected to the same tests. To induce periodontitis, we placed a 4-0 silk suture submarginally around the right maxillary first molar under general anaesthesia (40 mg/kg of ketamine) 1 day after confirmation of diabetes. The sutures were checked weekly after application, and any lost or loose sutures were replaced. All ligatures were placed by the same operator. The animals were kept in individual cages and received water and food ad libitum. A powdered form of PTH (1–34) dissolved in PBS containing 0.1% rat serum albumin was used. PTH was subcutaneously administered at a dose of 75 μg/kg 4 times a week for 4 weeks.

2.3. Determination of fasting blood glucose

Following a 10-h fast, the tail veins of the rats were pierced with a needle. Blood was collected from the tails and subsequently used to determine fasting blood glucose levels using a glucometer (OneTouch Glucometer, LifeScan, Milpitas, CA, USA). Measurements were obtained every week, and blood glucose levels were expressed in units of mmol/L.

2.4. Micro-computed tomography analysis

At the time the rats were sacrificed, the left maxillae were harvested, fixed in 10% formalin and then sent for micro-computed tomography (micro-CT) scanning. The maxillae were oriented vertically in a sample holder to keep them in the correct longitudinal position. Scans were performed by determining the angle based on the positions of the buccal and palatal cusps of the first molar. The micro-CT apparatus was set as follows: pixel size, 1024 × 1024; slice thickness, 12 μm; magnification, 10; voltage, 50 kV; and electrical current, 0.1 mA. Three-dimensional images were produced using the computer software TRI/3D-BON. The distance from the buccal cemento-enamel junction to the alveolar bone crest of the first molar was measured as a