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Caffeine may enhance orthodontic tooth movement through increasing osteoclastogenesis induced by periodontal ligament cells under compression

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ABSTRACT

Objective: Caffeine is the kernel component of coffee and has multiple effects on bone metabolism. Here we aimed to investigate the effects of caffeine intake on orthodontic tooth movement (OTM).
Design: (1) In the *in vivo* study, two groups comprising 15 randomly assigned rats each underwent orthodontic treatment. One group ingested caffeine at 25 mg/kg body weight per day and the other, plain water. After 3 weeks, the degree of tooth movement and effect on the periodontium were assessed. (2) In the *in vitro* study, we established a model mimicking the essential bioprocess of OTM, which contained a periodontal ligament tissue model (PDLtm), and a co-culture system of osteoblasts (OBs) and osteoclast precursors (pre-OCs). After being subjected to static compressive force with or without caffeine administration, the conditioned media from the PDLtm were used for the OB/pre-OC co-cultures to induce osteoclastogenesis.
Results: (1) *In vivo*, the caffeine group displayed a significantly greater rate of tooth movement than the control. The alveolar bone mineral density and bone volume fraction were similar between the two groups; however, immunohistochemical staining showed that the caffeine group had significantly more $TRAP^+$ osteoclasts and higher RANKL expression in the compressed periodontium. (2) *In vitro*, caffeine at 0.01 mM significantly enhanced the compression-induced expression of RANKL and COX-2, as well as prostaglandin E2 production in the PDLtm. Furthermore, the "caffeine + compression"-conditioned media induced significantly more $TRAP^+$ OC formation when compared with compression alone.
Conclusions: Daily intake of caffeine, at least at some specific dosage, may enhance OTM through increasing osteoclastogenesis.

1. Introduction

Coffee is one of the most popular beverages worldwide. An investigation reported by the International Coffee Organization in 2008 indicated that daily coffee consumption throughout the world exceeds 75 million liters (Kim, Nam, Kim, Choi, & Won, 2012). The effects of coffee on human health are profound (Higdon & Frei, 2006). Previous studies have revealed the impacts of coffee intake on various aspects of human physiology, including cardiovascular health, diabetes, Parkinson's disease and osteoporosis (Hasling, Sondergaard, Charles, & Mosekilde, 1992; James, 2004; Muley, Muley, & Shah, 2012; Ross et al., 2000).

The kernel and functional component of coffee is caffeine. Although investigated extensively, the effects of caffeine on bone metabolism remain elusive. Though still under debate, it may decrease bone mineral density (BMD) through the disruption of calcium balance by facilitating urinary calcium excretion and reducing intestinal calcium resorption (Massey & Whiting, 1993; Sakamoto et al., 2001). Moreover, it has also shown controversial effects on the proliferation and function of osteoblasts (Liu et al., 2011; Tsuang, Sun, Chen, Sun, & Chen, 2006;). Caffeine may enhance osteoclastogenesis *via* the COX-2–prostaglandin E2 (PGE2) pathway (Liu et al., 2011). Activation of P38 mitogen-activated protein kinase and subsequent cathepsin K and TRAP enhancement are also involved in caffeine-induced osteoclastogenesis (Choi et al., 2013).

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In addition, high doses of caffeine increase alveolar bone loss in ligature-induced periodontitis in rats (Bezerra, da Silva, de Alvarenga Lemos, Duarte, & Bastos, 2008). However, a recent clinical study demonstrated that coffee ingestion may not induce further tissue destruction even in patients with periodontitis (Ng, Kaye, & Garcia, 2013). In short, the effects of caffeine on bone metabolism are varied and complex.

Orthodontic tooth movement (OTM) is characterized by bone resorption in areas of compression and bone apposition in areas under tension (Wise & King, 2008). Recent studies have highlighted that OTM can be affected by various systemic factors. Daily injection of nicotine accelerates OTM, possibly because of enhancement of bone resorption (Sodagar, Donyavi, Arab, & Kharrazifard, 2011). Induced allergic asthma appears to exacerbate OTM (Machado, Nojima Mda, Rodrigues e Silva, & Mandarim-de-Lacerda, 2012). In addition, it has long been suggested that non-steroidal anti-inflammatory drugs may slow down OTM because of the inhibition of prostaglandin synthesis (Karthi et al., 2012). As caffeine plays multiple roles in bone metabolism, it may also affect OTM, especially when ingested daily.

Empirically, we have found that the coffeeholic patients seem to have relatively faster tooth movement in orthodontic treatment compared with ordinary patients. Therefore, we hypothesized that caffeine intake may enhance OTM. In this study, first we employed a conventional rat OTM model to investigate the actual effects of coffee drinking on bone remodeling in OTM. Second, we combined our newly established periodontal ligament tissue model (PDLtm) with a co-culture system of osteoblasts (OB) and osteoclast precursors (pre-OC) (Li et al., 2011; Li, Li, Tan et al., 2013), and further investigated the effects of caffeine on the osteoclastogenesis induced by PDL cells under static compression.

2. Materials and methods

2.1. Experimental animals

Thirty 8-week-old male Wistar rats were used. The rats were housed in plastic cages with access to standard rat food and plain water *ad libitum* under a 12 h light/dark photoperiod. After a 1-week acclimatization period to the laboratory environment, the rats were randomly divided into two groups of 15 rats each, and underwent either regular OTM (rO) or caffeine ingestion in combination with OTM (cO). The protocol for this animal study was approved by the West China Stomatology Hospital Institutional Review Board.

2.2. Detection of caffeine concentration in coffee

High performance liquid chromatography (HPLC) was employed to measure caffeine concentration in commercial instant coffee (Nescafe Original 20541545, Nestle, Shanghai, China). Briefly, caffeine standard substance (USP/BP, Amresco, USA) was used to construct the calibration curve. Separation was performed at room temperature using the NH_2 column (250 mm × 4.6 mm, 5 μm, WATERS, USA). The mobile phase consisted of methanol-water (82:18, v/v) and acetonitrile at a flow rate of 1.0 ml/min throughout the analytical run. Wavelength of 270 nm that optimized the absorbance was used to perform the quantification. The mass ratio of caffeine was assessed as 2.06%.

2.3. Caffeine administration and force application

At 9 weeks of age, both groups underwent orthodontic force application as previously reported (Sodagar et al., 2011). Briefly, a 5 mm stainless steel coil spring (Associated Spring Raymond, Shanghai, China) was calibrated to produce an initial force of 15 cN between the incisor and the first molar of the left maxilla. The coil spring was reactivated every week (Fig. 1A). After 3 weeks of force application, the rats were sacrificed by anesthetic overdose. From the first force application until sacrifice, the rats in the cO and rO groups underwent daily intragastric administration of coffee (Nescafe Original 20541545, Nestle, Shanghai, China) solution or plain water respectively. The intragastric administration was performed using a syringe with rounded tip (ZS Dichuang, Beijing, China) which could go into the esophagus without tissue wound.

To simulate the average consumption of human (450 mg caffeine/60 kg body weight/day) (Nawrot et al., 2003), the dosage was calculated as 25 mg caffeine/kg body weight/day for the rats based on metabolic body weight conversion system (Duarte, Marques, Bezerra, & Bastos, 2009; Yeh & Aloia , 1986). The rats were weighed daily throughout the study.

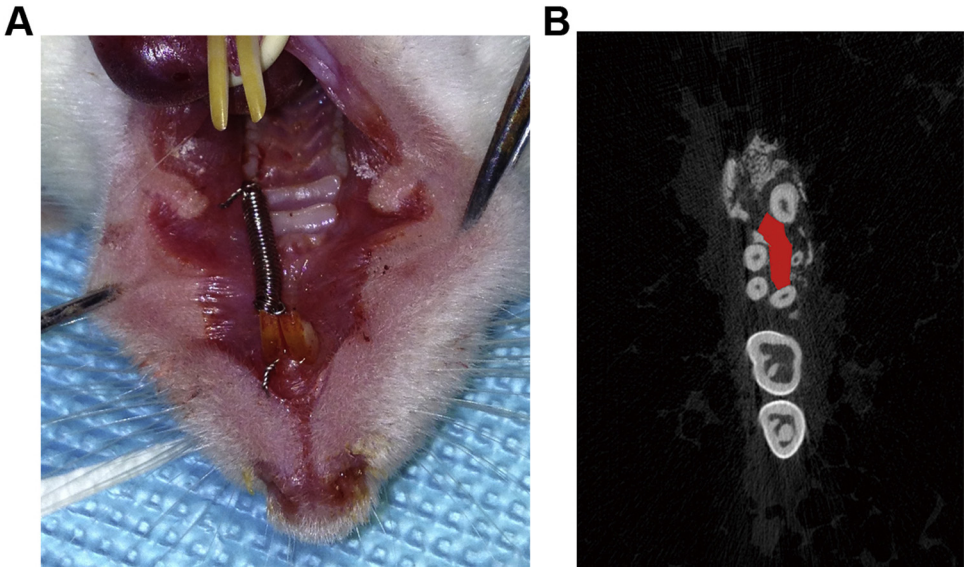

Fig. 1. (A) The animal model of orthodontic tooth movement. The left maxillary first molar was mesialized using a coil spring. (B) A horizontal section of the alveolar bone at the radicular level, scanned with μCT. BMD and BV/TV were analyzed at the furcation area of the maxillary first molar (marked in red).