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The effect of caffeine on orthodontic tooth movement in rats

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ABSTRACT

Objective(s): to determine the effect of different doses of caffeine on orthodontic tooth movement (OTM) in rats.

Materials and Methods: Forty male 250-300 g Sprague-Dawley rats were randomly divided into four groups of ten animals each and received 0 (control), 1 g/l, 2 g/l and 3 g/l caffeine in tap water for 3 days. Orthodontic appliances were ligated between the maxillary first molars and incisors on the 4th day of the study period. All rats were sacrificed after 2 weeks of treatment after which OTM was measured. Hematoxylin/eosin-stained sections of the molars were prepared and the mesial roots were examined for resorption-lacunae depth and osteoclast number. ANOVA was used for statistical analysis (P<0.05).

Results: A significant decrease in OTM was observed only in the 2 g/l (P=0.043) and 3 g/l (P<0.01) caffeine-receiving rats compared to the control animals. Osteoclast counts and resorption-lacunae depths demonstrated significant differences between each of the caffeine groups and control rats (P<0.05). None of the variables showed significant differences between the caffeine groups (P>0.05).

Conclusion: According to our findings, one of the effects of caffeine consumption during orthodontic treatment in rats was decreased root resorption. Additionally, concentrations of 2 g/l and 3 g/l inhibited OTM which seems to be due to its influence on osteoclast numbers.

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Introduction

Tooth movement is the basis of orthodontic treatments and involves active alveolar bone remodeling. Mechanical forces stimulate local cellular responses in the periodontal ligament (PDL) and osseous tissues, which enable the teeth to relocate. The exact molecular mechanisms of this process are unclear (1) and considering that an enhanced comprehension of elements affecting orthodontic tooth movement (OTM) may enable better treatment planning and patient management, numerous items including hormones (2, 3), vitamins (4), nitric oxide (5), opioids (6), prostaglandins (7, 8), calcium, and bisphosphonates (9) have been investigated and proposed to influence this phenomenon.

Caffeine is a methylxanthine derivative found in various dietary sources and drugs, which has been regarded as one of the most commonly consumed substances worldwide. Considering its wide distribution in tea, coffee, and chocolate and carbonated soft drinks (10) it can affect individuals of all age groups, among which could be patients seeking orthodontic treatments. At the cellular and molecular level, caffeine has multiple actions involving phosphodiesterases (PDE) (11), adenosine receptors (12), prostaglandins (13) and inflammatory mediators (14). The biochemical functions of this substance influence numerous organ specific events including bone formation and resorption which are the foundation of OTM.

The impact of caffeine consumption on bone metabolism, density and healing has been studied with conflicting results. Some investigators have suggested that caffeine promoted osteoporosis and periodontal disease (15-17), while others found no correlation between this substance and bone loss (18), bone density (19) or disease (20). Moreover, it has been demonstrated that at specific doses, caffeine could positively influence the mineralization, strength and mechanical features of osseous tissues (21).

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The number of researches investigating the impact of caffeine on orthodontic treatments is limited in the English literature. Considering the widespread use of this substance in different doses and the lack of information on its numerous effects, we aimed to study a broad range of concentrations of caffeine on OTM in rats.

Materials and Methods

Animals and experimental design

The present investigation was approved by the Ethics Committee of Tehran University of Medical Sciences (code no. 4237) which operates according to Good Clinical Practice (GCP) and follows the national research council's guide for the care and use of laboratory animal's ethical guidelines, laws and regulations. A total of 40 male, Sprague-Dawley rats weighing 250-300 g were placed in eight plastic cages and acclimated to a 12-h light/dark cycle for 7 days with free access to water and standard laboratory rat chow. The rats were randomly assigned to 4 groups of 10 animals each and 1 g/l, 2 g/l and 3 g/l caffeine was added to their drinking water, 3 days before appliance insertion. The controls ingested plain water with no added substance. Water supply was restored and prepared every other day until the end of the study period.

Application of orthodontic appliances

Orthodontic treatments were administered using the method described by Shirazi *et al* (2) Briefly, each animal was anaesthetized by intra-peritoneal injection of 25 mg/kg ketamine hydrochloride (Rotexmedica, Trittau, Germany) and 8 mg/kg xylazine (Rotexmedica, Trittau, Germany) followed by insertion of a nickel-titanium closed coil spring (Sentaloy®, GAC, NY). All animals were in stage 3 (surgical stage) of anesthesia before orthodontic treatment. This was controlled by observation of reflex loss (corneal, pedal and pinnae), relaxation of muscles and rhythmic deep breathing. Rats showed no response to tail or abdominal skin pinching and there was no vocalization. The subjects were kept warm by wrapping them with a drape and their vital signs were monitored until full recovery. Appliances were ligated between the maxillary right first molars and incisors and activated to deliver a force of 60g, as measured by a dial push–pull gauge according to former investigations (2). Composite resin was used to bind the upper incisors, therefore limiting their distal dislocation and enforcing anterior anchorage. To prevent appliance damage, the lower incisors were reduced every 4 days by means of a diamond bur (Dentsply Maillefer, Ballaigues, Switzerland) using a high speed handpiece. Care was taken not to induce pulp exposure. In order to minimize animal discomfort and protect their appliances, standard chow was finely ground and moistened with tap water. The animals had access to food and caffeinated or plain water *ad libitum* (2). Animal weight and water intake was monitored every other day and accordingly, the amount of added caffeine (to tap water) was adjusted to supply doses of 1 g/l/day, 2 g/l/day and 3 g/l/day in each experimental group. Each rat consumed approximately 34-36 ml water in 24 hr which was in accordance with that measured previously (22).

OTM measurements

At the experimental end-point (2 weeks) all rats were weighed and sacrificed under anesthesia by sodium pentobarbital overdose prior to decapitation. Maxillae were separated and the distance between the 1st and 2nd molars was measured using a standard millimeter inter-proximal gauge before appliance removal.

Histological investigation

Histologic processing was carried out based on former studies (23, 24). Right hemimaxillae were dissected, fixed in 10% buffered formalin for 2 days and then immersed in 5% formic acid for approximately 7 days or until complete decalcification. All specimens underwent routine histologic processing and were embedded in paraffin followed by mesio-distally-directed cutting to obtain 5 µm sections for hematoxylin and eosin staining. Histopathologic examination was performed on the mesiobuccal root of the maxillary first molar as described previously (24). For this purpose, six sections containing the largest root area and including the entire length of the molar root were selected and osteoclast number along with maximum resorptive lacunae depths were assessed under an Olympus BX41 light microscope. The latter was determined by measuring the distance between the deepest points of the lacuna perpendicular to a line connecting its two edges. In cases with more than one resorptive lacuna the mean of lacunar depths was considered to represent root resorption and means of the six sections' measurements were used for data analysis (24).

Statistical analysis

All findings were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey test were used for statistical analysis and $P<0.05$ was considered significant.

Results

All rats showed a healthy behavior and survived to the end of the experiment period. There was no significant difference in weight among the study samples ($P>0.05$). The appliances remained intact and induced tooth movement in all animals. Mean changes in OTM, osteoclast count and root resorption in the 4 studied groups are shown in Table 1.

Table 1. Mean changes in the studied variables among the groups

Groups	Orthodontic tooth movement (mm) (mean±SD)	Osteoclast count (mean±SEM)	Maximum resorptive lacuna depth (mm) (mean±SEM)
Control	.485 ± .059	7.300 ± 1.337	.462 ± .004
Caffeine 1 g/l	.430 ± .032 (†)	5.400 ± 1.074*	.035 ± .006*
Caffeine 2 g/l	.427 ± .042*	4.400 ± .843#	.038 ± .007*
Caffeine 3 g/l	.388 ± .050#	4.200 ± .919#	.031 ± .009#

†: Not significant compared with control group; * P<0.05 compared with control group; # P<0.01 compared with control group

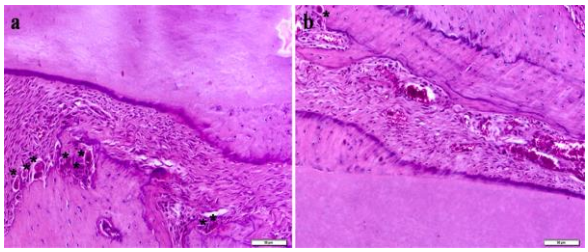

Figure 1. Photomicrograph depicting representative samples of (a) control animals with high osteoclastic count and (b) rats receiving 3 g/l caffeine demonstrating low osteoclast number. Osteoclasts are indicated by asterisks (Hematoxylin and Eosin staining; Original magnification ×200; scale bar for both images: 50 µm)

Tooth Movement

Comparison among groups revealed a significant difference between the control animals and those receiving 2 g/l (P=0.043) and 3 g/l (P<0.001) but not 1 g/l (P=0.059) caffeine. No significant differences were found between the caffeine groups (P>0.05), but a gradual decrease in OTM was observed with increasing caffeine dosage.

Histologic findings

The decrease in osteoclast numbers was more pronounced in groups receiving higher concentrations of caffeine (Figure 1). Significant differences in osteoclast numbers were observed between the controls and 1 g/l (P=0.002), 2 g/l (P<0.001) and 3 g/l (P<0.001) caffeine groups. There was no significant difference in this variable among the caffeine treated groups (P>0.05). Root resorption lacunae were more conspicuous in the mesial aspects of the mesial molar roots compared to the distal sides. Similar to osteoclastic counts, resorption lacunae demonstrated significant differences between the controls and 1 g/l (P=0.005), 2 g/l (P=0.042) and 3 g/l (P<0.001) caffeine-receiving animals (Figure 2). Root resorption was lowest in the 3 g/l followed by the 1 g/l and 2 g/l caffeine groups, with control animals showing the highest amount of root resorption (Table 1). There were no significant differences among the caffeine groups regarding resorption lacunae depths.

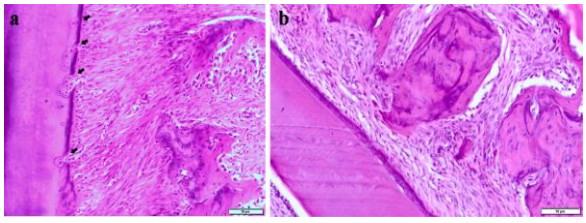

Figure 2. Histopathologic view of root resorption in a representative (a) control animal and (b) 3 g/l caffeine-receiving rat. Arrows show resorptive lacunae. (Hematoxylin and Eosin staining; Original magnification ×200; scale bar for both images: 50 µm)

Discussion

Orthodontic treatments rely on bone turnover which may be affected by consumption of various substances and drugs (9).In the present study we assessed the impact of caffeine on OTM and found it to have a diminishing effect on tooth movement in rats. This clinical finding was corroborated by our histologic results which also showed a decrease in osteoclast numbers and root resorption.

The biochemical mechanisms underlying caffeine function can be used to explain our findings. This substance has been shown to block adenosine receptors (12), inhibit PDEs (11) and counteract the effects of prostaglandins[13]in addition to demonstrating anti-inflammatory characteristics (14) Adenosine, an extracellular purine, exerts its effects through cell-surface receptors, which are widely distributed in various tissues, including bone. The exact function of adenosine in osseous tissues is unclear, but it has been suggested to influence bone metabolism and osteoclast performance/differentiation via different processes. As an adenosine receptor antagonist, caffeine may be able to inhibit the formation and function of osteoclasts and consequently lead to decreased bone resorption (12, 21). It appears that the decreased OTM in caffeine-treated rats observed in the current investigation might have been caused by reduced bone resorption due to similar pharmacological activities.

Caffeine is an inhibitor of PDEs, which are known to elevate intracellular cyclic AMP (c-AMP) levels. The increase of this second-messenger in osteoblasts can aggravate their osteogenic function resulting in increased bone mass (11). Kinoshita *et al* (11) using a PDE inhibitor, suggested a possible role for these group of drugs in the suppression of bone resorption. They also observed a non-significant decrease in osteoclast number and suggested that PDEs may affect the function of these cells. We similarly found reduced osseous resorption reflected by the decreased OTM and osteoclast count in caffeine-treated rats. However our latter findings were significant which could be attributed to the use of different animal species (mice versus rats), routes of administration and drug doses between the studies.

Prostaglandins are significant bone resorption mediators and have been shown to increase OTM through increased osteoclast number and activity (7, 8). Considering that caffeine antagonizes the effect of this mediator (13), it could be expected that bone resorption and osteoclast numbers would decrease following its administration, which confirm the results obtained in the present study.

During osteoclastogenesis in the compression side of orthodontically treated teeth, both TNF- α and IL-1, demonstrate pro-osteoclastic properties which can lead to increased bone resorption (25). Caffeine demonstrates anti-inflammatory effects and has been indicated to suppress TNF-α production (26). Pentoxifylline, similar to caffeine, is a xanthine derivative and was reported to inhibit the production of proinflammatory cytokines including IL-1 by some cell types (27). This feature could also help to explain the reduced OTM, and osteoclast count found in the present study.

Considering the similarities in derivation, function and morphology between osteoclasts and odontoclasts (25), the abovementioned cellular and molecular mechanisms of caffeine can also help clarify the reduced root resorption observed in our investigation.

Previous studies on caffeine and OTM are limited in the English literature. Yi *et al* (28) presented a hypothesis suggesting that caffeine may enhance OTM via reducing bone density. Nonetheless, as stated by the authors, this was only a hypothesis and not based on laboratory investigations. Additionally, in contrast to our findings, Peng and Yong-chun (29) showed increased osteoclastic numbers in orthodontically-treated rat molars receiving caffeine. They employed two doses of high and low caffeine, while we used a wide range of concentrations to examine the effect of this substance. Yi *et al* (30) also showed that intragastric administration of unpurified commercial caffeine accelerated preosteoclast cell synthesis in rats. This contrast could be attributed to concentration, duration and administration method of caffeine in that study. It is noteworthy that the different biochemical mechanisms responsible for the effects of caffeine are efficient only at specific doses; for example inhibition of PDE occurs at higher concentrations compared to those affecting adenosine receptors (10). Furthermore, a biphasic dose-dependent effect of caffeine on bone factors has been demonstrated *in vitro* (31). These facts underline the importance of considering the concentration of caffeine employed in a specific study before drawing conclusions and making comparisons between the results of various investigations.

Conclusion

Efficient tooth movement with minimal side effects is the major goal of orthodontic treatments. According to our findings root resorption decreased following caffeine administration, but a simultaneous reduction in OTM was also noted. Extrapolation of animal findings to human conditions should be practiced with caution; however our observations provide initial evidence that caffeine may not be entirely harmful to bone tissues. If confirmed by future research, orthodontists need to consider the osseous effects of caffeine during orthodontic treatment-planning, especially for patients who consume large amounts of caffeine-containing products.

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