

Effect of combined oral contraceptives on orthodontic tooth movement in a female rat model

Jinghong Yu,^{*#} Yifei Zhou,^{†#} Tao Zhang,⁺ Jialing Lv,^{*} Fiona Firth,[±] Xian Zhao,^{*} Xiaomei Xu^{*} and Li Mei[±]

Department of Orthodontics, Hospital of Stomatology, Orofacial Reconstruction and Regeneration Laboratory, Southwest Medical University, Luzhou, People's Republic of China,^{*} Department of Stomatology, People's Hospital of Langzhong City, Nanchong, People's Republic of China,[†] Department of Stomatology, The Third People's Hospital of Dongguan City, Dongguan, People's Republic of China⁺ and Discipline of Orthodontics, Department of Oral Science, Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago, Dunedin, New Zealand[±]

[#]Both authors contributed equally to the work.

Background: To investigate the effect of combined oral contraceptives (COC) on orthodontic tooth movement (OTM) and periodontal remodelling in a female rat model.

Methods: A total of 80 three-month-old female, Sprague-Dawley rats were randomly divided into experimental and control groups. The maxillary first molars were moved mesially using nickel-titanium coil springs (50 g force). The experimental group (N = 40) ingested 1.5 ml COC (Marvelon, 0.12 mg/d, N.V. Organon, Oss, The Netherlands) daily. The control group (N = 40) ingested 1.5 ml saline (0.9% sodium chloride) daily. After 7, 14, 21 and 28 days of force application, 10 rats in each group were euthanased and a vernier calliper was used to measure the orthodontic movement of the first molar. Root resorption at pressure areas was assessed by H and E staining. Micro-CT was used to detect alveolar bone mineral density.

Results and conclusion: The amount of OTM in the experimental group (0.46 ± 0.16 mm) was significantly less than in the control group (0.85 ± 0.25 mm; $p = 0.003$) during the 28 days of observation. There were significantly smaller ($p = 0.002$) root resorption lacunae in the experimental group (111710 ± 4037 pixels) compared with the control group (204962 ± 21318 pixels) after 28 days. There was no statistically significant difference in the bone mineral density between the experimental and control groups throughout the study period ($p > 0.05$ at each time point). The short-term administration of COC may retard tooth movement and reduce the level of root resorption during OTM in female rats. (Aust Orthod J 2019; 35: 127-133)

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Jinghong Yu: 1585179920@qq.com; Yifei Zhou: 421346157@qq.com; Tao Zhang: 570538684@qq.com; Jialing Lv: 1241940221@qq.com; Fiona Firth: fiona.firth@otago.ac.nz; Xian Zhao: 895572171@qq.com; Xiaomei Xu: xuxiaomei@hotmail.com; Li Mei: li.mei@otago.ac.nz

Introduction

Orthodontic tooth movement (OTM), a result of tissue remodelling within the periodontium and alveolar bone, is mediated by a localised inflammatory response.¹ The process of alveolar bone remodelling usually involves resorption of existing bone by osteoclasts in the pressure zone, and bone formation

by osteoblasts in the tension zone. The rate of OTM depends on the rate of bone resorption and formation during this physiological alveolar bone remodelling process.²

Sex hormones, such as oestrogen, androgen and progesterone, have been confirmed to have important physiological roles in OTM through their effects on

bone remodelling.^{3,4} For example, oestrogen plays a role in the levels of bone metabolism mediators, including interleukin-17, tumor necrosis factor- α , osteoprotegerin and transforming growth factor- β , and oestrogen deficiency has been found to lead to increased osteoclast formation and enhanced bone resorption.⁵⁻⁷ Additionally, a lack of oestrogen has been reported to accelerate tooth movement in rat models.^{4,8} Androgens have been shown to affect OTM through the inhibition of bone resorption and modulation of the muscular system.^{4,9} Progesterone has been found to affect periodontal regeneration during OTM in pregnant rats, and long-term administration of progesterone has been shown to decrease the rate of OTM in rabbits.^{10,11} In addition, the hormonal changes during pregnancy have been found to influence the amount of OTM, the duration of orthodontic treatment, and the side effects of tooth movement.^{12,13}

Combined oral contraceptive (COC) drugs, such as Marvelon (main components: ethinyl estradiol and desogestrel), have been considered a highly efficacious contraception method and are widely used throughout the world.¹⁴ Oral contraceptives can inhibit ovulation by down-regulating the production of pituitary gonadotropins, follicle-stimulating hormone and luteinizing hormone.¹⁵ A prospective randomised controlled trial has compared the effects of two oral contraceptives (Belara and Marvelon) on hormone levels and identified a significant reduction in the levels of follicle-stimulating hormone, luteinizing hormone, estradiol and progesterone during the treatment period.¹⁶ The potential influence of the use of COC on OTM has been highlighted, with ethinyl estradiol/norgestrel decreasing the amount of OTM in Wistar rats during a 14-day study.¹⁷ The association of oral contraceptives and hormonal changes, as well as the relation between the hormonal changes and OTM, have been extensively studied; however, the effect of oral contraceptives on OTM, especially on root resorption and bone remodelling, is still poorly understood.

The aim of the present study was to investigate the effect of combined oral contraceptives on the rate of OTM, root resorption and bone mineral density in a rat model.

Materials and methods

Grouping and treatment of experimental animals

A total of 80 adult female Sprague-Dawley rats were included in the study (three months of age, average weight 250 ± 30 g, provided by the Experimental Animal Centre of Southwest Medical University, Luzhou, China).¹⁸ Ethics approval was gained from the Ethics Committee, Southwest Medical University Stomatological Hospital, China (2014SW0073) and all experiments were performed according to the National Institute of Health Guide for the Care and Use of Laboratory Animals.

The rats were initially acclimatised for seven days in cages at a temperature of 22.5 ± 0.5 °C, with a 12-hour light/dark cycle with ad libitum access to a normal diet and water. The rats were randomly divided into a control group (N = 40) and an experimental group (N = 40). Vaginal smears were used to determine the oestrous cycle of each rat.¹⁹ Rats in the same oestrous cycle within each group were housed in the same cage, and gavage was used during the oestrus interval of the rats.

The experimental group was given 1.5 mL COC (Marvelon, N.V. Organon, Oss, The Netherlands) (0.12 mg/d, 20 tablets of Marvelon [30 mcg ethinyl oestradiol and 0.15 mg desogestrel] dissolved in 50 mL of 0.9% saline), and the control group was given 1.5 mL 0.9% saline, at a set time each day for the period of the study (28 days).

Tooth movement distance measurement

A 50 g force to induce tooth movement was generated using nickel-titanium coil springs (0.12 inch diameter, Innovative Material and Devices Inc., Shanghai, China).²⁰ Following ketamine anaesthesia (100 mg/kg, intra-muscular), the coil springs were ligated unilaterally between the first molars and central incisors with a stainless steel ligature wire (0.008 inch, Hangzhou Westlake Biomaterial, China), and bonded using a light-cured composite resin (Enamel Adhesive Resin, Hangzhou Westlake Biomaterial, China) to enhance anchorage and prevent the appliance from detaching.^{21,22} At 7, 14, 21 and 28 days of force application, 10 rats in each group were euthanased and OTM measurements were taken.²³ The distance between the central fossae of the maxillary first

molar to the maxillary second molar was measured thrice using a vernier calliper (accuracy of 0.01 mm, Shanghai Shen Korea Measuring Tool, China) by an operator who was blind to the grouping. The intra-class correlation coefficient revealed an excellent correlation (0.95). Mean values were calculated based on the triplicated measurements.

Myocardial perfusion was implemented at days 7, 14, 21 and 28 on 10 rats in each group to allow assessments of root resorption and bone mineral density.

Root resorption measurement

The right hemi-maxilla of each animal was removed, dissected, and fixed in 4% paraformaldehyde for 24 hours and prepared for histological evaluations. The specimens were decalcified using 10% EDTA for four weeks, embedded in paraffin blocks and serially sectioned in the frontal plane at 5 μ m intervals.²⁴ The samples were dehydrated and stained with haematoxylin and eosin (H&E).²⁵ Light microscopy was used to assess the periodontal tissues and root resorption of the first molar (mesial root pressure area) by a pathologist who was blind to the grouping.^{26,27} Three different regions were selected from each slice. The area of the resorption lacuna was measured thrice using the Image-Pro Plus 6.0 image analysis system (Media Cybernetics System, MD, USA). The intra-class correlation coefficient revealed an excellent correlation (0.96). Mean values were calculated based on the triplicated measurements.

Bone mineral density measurement

The hemi-maxillae were placed side by side in the loading container of a microcomputed tomography scanner (micro-CT) (SIEMENS Inveron MM GantystDCT, Munich, Germany), with energy settings of 80 kV and 500 μ A. The sagittal axes of the maxillae were parallel to the examination bed. Under the root furcation of the hemi-maxillary molars, one point was randomly selected, and Hounsfield unit (Hu) scale values were assessed in triplicate to calculate the mean values.^{22,28}

Statistical analysis

Statistical analysis was performed using SPSS 20.0 software (SPSS, IL, USA). The Kolmogorov–Smirnov test was applied to test the normal distribution of

the data. An independent sample *t*-test was used to compare the distance of OTM, root resorption area and bone mineral density between the experimental and control groups. The level of statistical significance was set at $p < 0.05$.

Results

Distance of tooth movement

After 28 days of observation, the distance of OTM in the experimental group (0.46 ± 0.16 mm) was significantly less than in the control group (0.85 ± 0.25 mm) ($p < 0.001$) (Table I). There were statistically significant differences in the amount of OTM between the experimental and control groups after 7, 14, and 21 days. The distance of OTM in both groups increased with time during the study, but the amount of OTM in the experimental group was approximately half of that in the control group at each of the assessed time points (0.04 ± 0.04 mm vs. 0.09 ± 0.04 mm on the 7th day, 0.11 ± 0.03 mm vs. 0.20 ± 0.07 mm on the 14th day, 0.32 ± 0.13 mm vs. 0.60 ± 0.16 mm on the 21st day; $p < 0.05$ at each time point; Table I).

Root resorption

The identified root resorption mainly occurred at the apical and root bifurcation areas in both groups (Figure 1). There were statistically significant differences in the amount of root resorption between the experimental and control groups after 7, 14, and 21 days. The root resorption lacunae in the experimental group were significantly smaller than in the control group (111710 ± 4037 pixels vs. 204962 ± 21318 pixels) after 28 days of tooth movement ($p = 0.002$), as well as at all the other time points during the study (Table II).

Table I. Distance of tooth movement of the first molars in the experimental and control groups.

Group	Distance (mm)	
	Control	Experiment
7 days	0.09 ± 0.04	0.04 ± 0.04 *
14 days	0.20 ± 0.07	0.11 ± 0.03 **
21 days	0.60 ± 0.16	0.32 ± 0.13 **
28 days	0.85 ± 0.25	0.46 ± 0.16 **

* represents $p < 0.05$; ** represents $p < 0.01$.

Bone mineral density

The micro-CT analysis of the bone mineral density indicated that there was no significant difference between the experimental and control groups throughout the study ($p > 0.05$ for all) (Figure 2 and Table III).

Discussion

The present study investigated the effect of COC on OTM and the related periodontal remodelling in female rats. The findings indicate that the experimental group, provided with COC, displayed a reduced rate of OTM and a lesser level of root resorption, while there was no difference in alveolar bone density during the 28 days of observation.

One of the limitations of the study was the short-term nature of the study design. The clinical administration of oral contraceptives in female human adults is usually ongoing, over months or years;²⁹ however, the

length of the present study was compatible with the short oestrus cycle of the female rat. Clinical trials with long-term follow-up are still needed for a more substantial understanding about the effect of oral contraceptives on OTM.

A number of previous animal studies have presented findings on the influence of sex hormones on the rate of OTM. The amount of OTM in Wistar rats provided with ethinyl oestradiol/norgestrel was significantly less than the OTM in a control group over 14 days.¹⁷ The results are in agreement with the present findings. The rate of OTM in rats following an ovariectomy was more rapid than in a control group over 28 days.³⁰ In cats, OTM during the oestrus phase was significantly less than in anoestrous and ovariectomised groups.³¹

Although the association between oral contraceptives and hormonal change, as well as the relationship between hormonal change and OTM, have been well established, the direct effect of oral contraceptives on

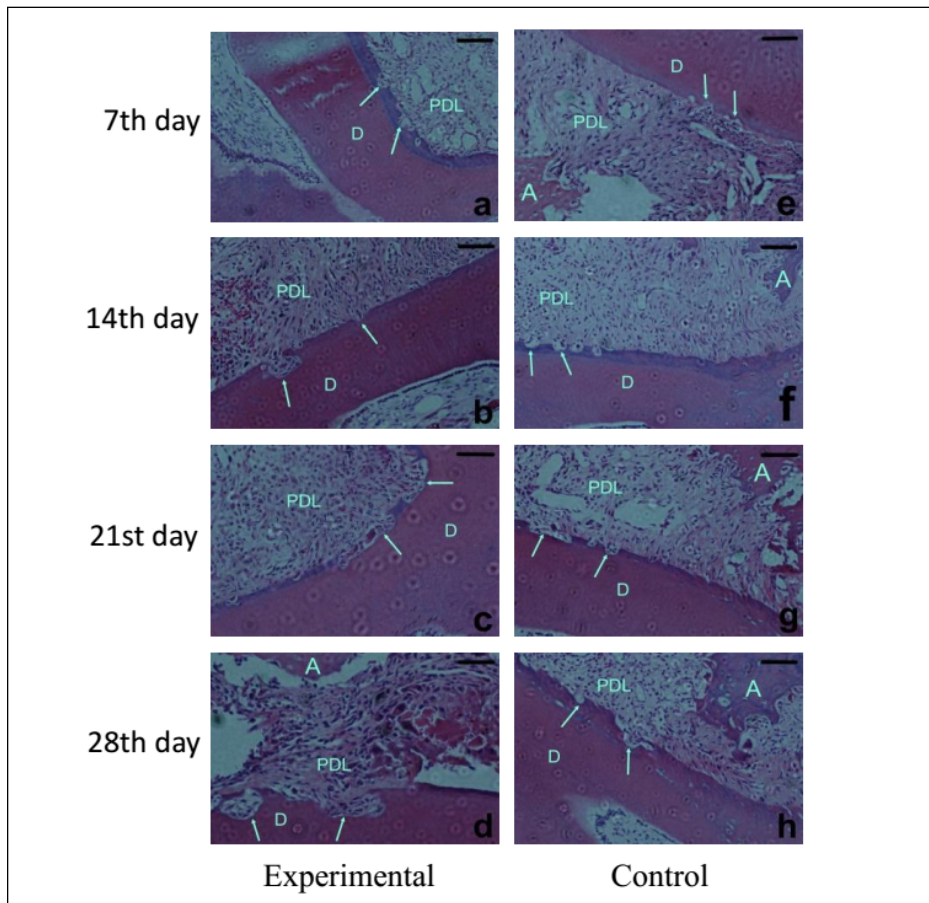


Figure 1. Photomicrographs of hemi-maxillae sections stained with H&E. The mesial side of the periodontal ligament and buccal root of the first molar are visible. a,b,c,d represent the resorption lacunae of the control group on days 7, 14, 21, 28; e,f,g,h represent the resorption lacunae of the experimental group on days 7, 14, 21, 28. PDL: periodontal ligament; D: dentin; A: alveolar bone. arrows: resorption lacunae (magnification $\times 200$).

OTM is still poorly understood. Oral contraceptives have been shown to suppress the level of oestrogen and progesterone by mediating the gonadal axis.¹⁶ Additionally, alterations in plasma levels of oestrogen, progesterone and testosterone hormones have been found to influence the rate of OTM.^{4,30} The application of orthodontic force during the ovulation period, when the most active oestrogen is present, would result in less OTM, whereas orthodontic force during the menstrual period would promote OTM.^{32,33} Another limitation of the study was that the exact mechanism of the negative effect of oral contraceptives on the rate of OTM was not investigated.^{4,12,16,17,31} Future studies with long-term follow-up on the association between oral contraceptives and OTM are needed for a better understanding of the related mechanisms.

Root resorption during OTM has been considered to be an inevitable sequela and is a multifactorial phenomenon, involving root morphology or abnormalities, orthodontic force, malocclusion type, and sex hormone deficiencies.^{4,34-36} The rate of root resorption

Table II. The area of resorption on the first molar root in the experimental and control groups.

Group	Area (pixels)	
	Control	Experiment
7 days	57388 ± 1513	29674 ± 667 **
14 days	78810 ± 1637	41984 ± 1668 **
21 days	171120 ± 10801	74865 ± 3229 **
28 days	204962 ± 21318	111710 ± 4037 **

** represents $p < 0.01$.

Table III. Bone mineral density in the experimental and control groups.

Group	Hu	
	Control	Experiment
7 days	4681 ± 149	4685 ± 88 NS
14 days	4697 ± 151	4744 ± 89 NS
21 days	4679 ± 112	4782 ± 110 NS
28 days	4735 ± 69	4777 ± 56 NS

Hu: bone mineral density.
Comparison between groups: NS represents $p > 0.05$.

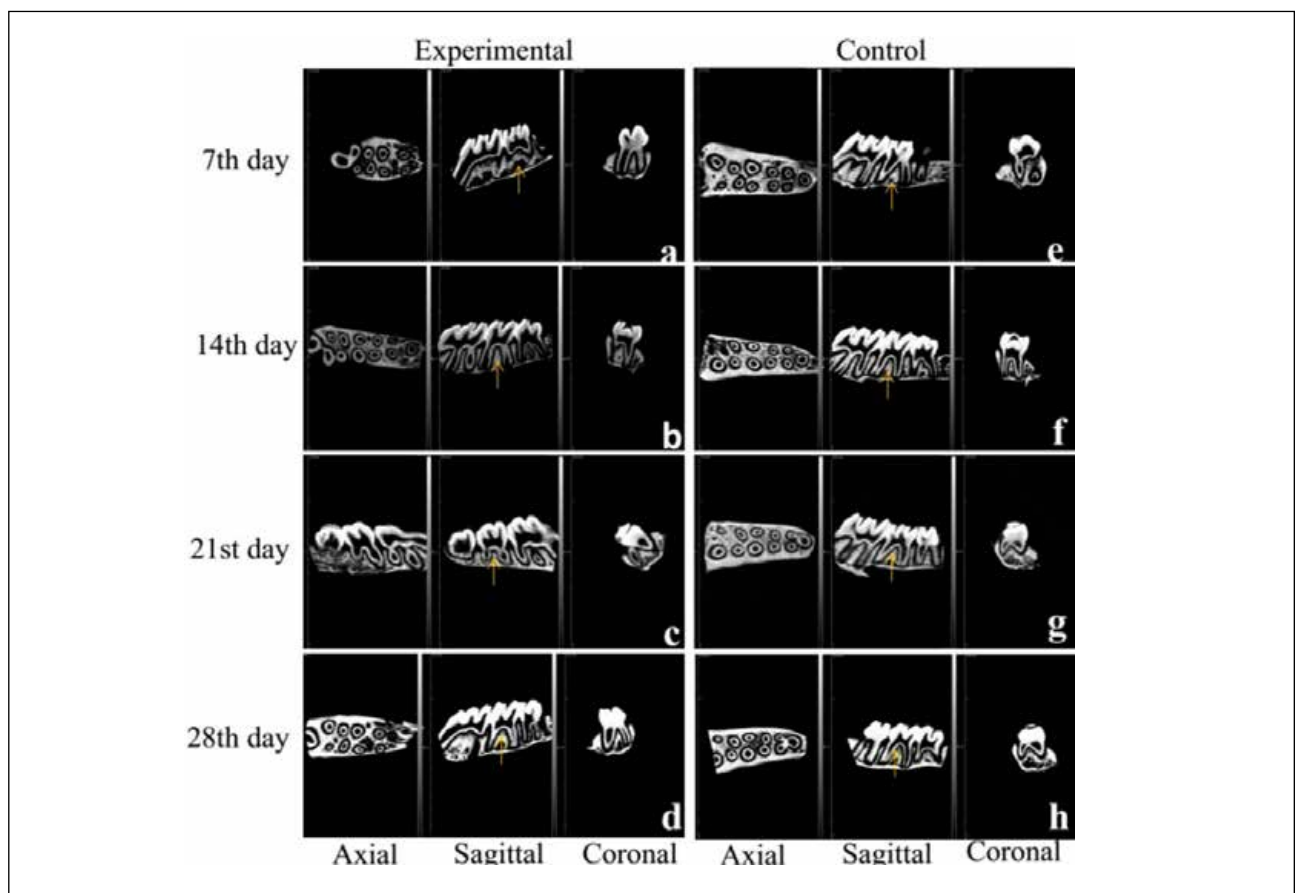


Figure 2. Micro-CT images of the left hemi-maxilla alveolar bones. Arrows: collection of BMD.

of the mesial root in rats was significantly lower in ovariectomised rats than in control animals when a 60 g force was applied.⁴ However, a number of studies have shown that the amount of orthodontically-induced root resorption in the disto-buccal and disto-palatal roots of maxillary first molars in ovariectomised rats was more severe than in non-ovariectomised control groups following the application of a 25g force. This indicates the importance of the extent of orthodontic force on root resorption.^{30,34} To the best of current knowledge, few studies have investigated the direct influence of oral contraceptives on root resorption during tooth movement. In the present study, the area of root resorption in the experimental group receiving oral contraceptives was significantly less than in the control group following an application of 50g of orthodontic force. This may be due to the change of hormone level induced by oral contraceptives, affecting the metabolism of cementum and periodontal tissues.

The sex hormones have a pleomorphic effect on bone physiology.^{37,38} For instance, oestrogens could reduce the formation and activity of osteoclasts, increase osteoclast apoptosis, promote osteoblast proliferation, formation and activity, and suppress osteoblast apoptosis. The role of progesterone and synthetic progestins in bone cell function has not been completely elucidated. The administration over two weeks of low-dose COC in humans reduced bone formation and resorption, and the use of low-dose COC was associated with a lower acquisition of bone mass.^{39,40} In a group of obese women with polycystic ovary syndrome, those using steroidal contraceptives were found to have lower bone mineral density in comparison with the non-users.⁴¹ The current study did not find a significant difference in alveolar bone mineral density between the oral contraceptive group and the control. This may be because the observation time of 28 days was not long enough to observe changes in bone density, or the masticatory force and other stimulating factors may conceal the changes in bone, if there were any, during this time period. Orthodontic treatment with fixed appliances has been found to enhance the expression of oestrogen in periodontal tissues and influence the menstrual cycle of adult females in the first month after bonding.⁴² COC, which may alter the level of sex hormones, could thus affect periodontal remodelling indirectly in response to active fixed orthodontic treatment.

Conclusion

The short-term administration of COC may retard OTM and reduce the level of associated root resorption in female rats.

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Corresponding author

Xiaomei Xu, Ph.D
Associate Professor, Department of Orthodontics
Hospital of Stomatology
Orofacial Reconstruction and Regeneration
Laboratory
Southwest Medical University
2 Jiangyang South Road
Luzhou, Sichuan
PR China, 646000
Email: xuxiaomei@hotmail.com

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