sciendo

DOI: 10.2478/aoj-2010-0019

The effect of morphine on orthodontic tooth movement in rats

Mohammad S.A. Akhoundi,* Ahmad Reza Dehpour,† Mahsa Rashidpour,+ Mojgan Alaeddini,+ Mohammad Javad Kharazifard+ and Hassan Noroozi+

Departments of Orthodontics* and Pharmacology,† and the Dental Research Center,+ Tehran University of Medical Sciences, Tehran, Iran

Objectives: To investigate the effect of morphine as an exogenous opioid on orthodontic tooth movement. Naltrexone will be used as an opioid antagonist to confirm the results.
Methods: Forty rats were randomly divided into four equal groups. The first group received no injection; the second group received daily injections of morphine; the third group received daily naltrexone-morphine injections and the fourth group daily injections of naltrexone-normal saline. The left first maxillary molar in each rat was tipped mesially with a NiTi closed coil spring. The rats were sacrificed after 14 days and the maxillae fixed, sectioned serially and examined histologically.
Results: The greatest amount of tooth movement occurred in the Control group and the least amount of tooth movement in the Morphine group. Tooth movement in the Morphine group was significantly different from the other three groups ($p < 0.05$). The differences in tooth movement in the Control, Morphine-naltrexone and Naltrexone-saline groups were not statistically significant ($p > 0.05$). No statistically significant histological differences were found.
Conclusions: Morphine reduced orthodontic tooth movement in rats. This effect was reversed by the opioid antagonist, naltrexone, which had no effect on tooth movement.
(Aust Orthod J 2010; 26: 113–118)

Received for publication: April 2009
Accepted: February 2010

Mohammad S.A. Akhoundi: ahmadakh@tums.ac.ir
Ahmad Reza Dehpour: dehpour@yahoo.com
Mahsa Rashidpour: mahsa_rp@yahoo.com
Mojgan Alaeddini: malaeddini@tums.ac.ir
Mohammad Javad Kharazifard: mj_khf@yahoo.com
Hassan Noroozi: noroozih@yahoo.com

Introduction

During orthodontic tooth movement a series of complex changes occur in the alveolar bone cells and periodontal ligament. These changes are mainly controlled by osteoblasts and osteoclasts in a process called 'coupling'.[1] Factors such as certain drugs/medication taken during orthodontic treatment can interfere with the remodelling mechanisms and affect tooth movement.[2–4] The same drugs can be used in experimental studies to explore some of the mechanisms controlling tooth movement.

Non-steroidal anti-inflammatory drugs (NSAIDs), which are taken for pain control during orthodontic treatment, prevent the conversion of arachidonic acid to prostaglandins and so reduce the amount of tooth movement.[5,6] Opioids, which are categorised as exogenous and endogenous, can also interfere with bone metabolism.[7,8] Opioid receptors (mu, delta and kappa) can be found in the cells of the central nervous system and in other cells,[4,7,9,10] and opioid receptors in osteoblast-like cells are connected with the structure and metabolism of bone.[4,11]

Opioids may either increase or reduce the rate of tooth movement. For example, endogenous opioids interact with nitric oxide in cholestatic rats and increase the rate of tooth movement, but exogenous opioids taken for pain relief (e.g. acetaminophen codeine) may have an entirely different effect.[12–14] A recent study of Iranian high school and university students reported that 91 per cent resorted to self-medication to relieve pain and acetaminophen

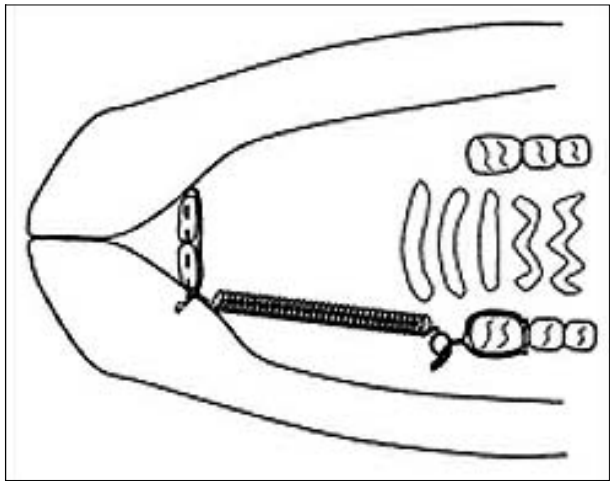

Figure 1. Schematic view of the appliance.

codeine was the most commonly used analgesic.[15] Since orthodontic treatment can be painful and patients may resort to self-medication we decided to investigate the effect of morphine, an exogenous opioid, on tooth movement in the rat. We used naltrexone, an opioid antagonist, which binds opioid receptors in a non-selective manner, to confirm the action of morphine on tooth movement.[16]

Materials and methods

Animals

Forty male Wistar rats between 200 and 250 g body weight were used. The animals were housed in plastic cages, with a 12/12 hour light-dark cycle. They were fed soft laboratory food to minimise any discomfort from the appliances and to reduce the risk of an appliance being dislodged or deformed.[12] The experimental protocol was approved by the Ethics Committee of Tehran University of Medical Sciences.

The animals were weighed on the day the appliances were placed and immediately before death. All animals had an orthodontic appliance consisting of a NiTi closed coil spring ligated to the left maxillary first molar and both upper incisors. The rats were randomly divided into four equal groups. The first group received no injection, the second group received a daily injection of morphine (5mg/kg body weight), the third group received a daily injection of naltrexone (20 mg/kg body weight)-morphine (5 mg/kg body weight) and the fourth group received a daily injection of naltrexone (20 mg/kg body weight)-normal saline. All injections were administered intra-peritoneally at 24-hour intervals for 14 days.[17]

Orthodontic appliance and measurement of tooth movement

Each rat was anaesthetised with an intra-peritoneal injection of chlorpromasine (30 mg/kg body weight) and ketamine (50 mg/kg body weight). The maxillary left first molars were tipped mesially using 6 mm 0.006 x 0.022 inch NiTi closed coil springs for 14 days.[12] The springs were anchored at the ends with 0.010 inch stainless steel ligature wires tied to the left maxillary first molars and the upper incisors (Figure 1). The molar ligature wires were passed between the first and second molars and tied around the cervical margins of the first molars. The anterior ligature was tied around the incisors and secured in shallow grooves cut into the labial and distal surfaces of the incisors, close to the gingival margins. A small amount of light-cure composite resin was placed over the ligature wires to protect the wires from damage. Each spring was activated about 1 mm to deliver a 60 g mesial tipping force and it was not reactivated during the course of the study.[18,19] After insertion of the appliance, the crowns of the lower incisors were reduced 1.5 mm to prevent the incisors from cutting the ligature wires.

Mesial movement of the first molar was measured at the start and end of the experiment with a filler gauge inserted between the first and second maxillary left molars. The initial distance between the molars was zero in all animals. The final measurement was carried out following decapitation, but before the appliances were removed and potential relapse of the first molar into the space between the molars. All measurements were repeated twice by the same operator, who was blinded to the treatment each rat had received. The intraclass correlation coefficient (ICC) between the two sets of measurements was .984.

Histological evaluation

The maxillae were fixed in 10 per cent formalin for 10 days and decalcified in 5 per cent formic acid for 4 days. The decalcified maxillae were embedded in paraffin and parasagittal serial sections of the mesial roots of first molars cut and stained with haematoxylin and eosin.[20]

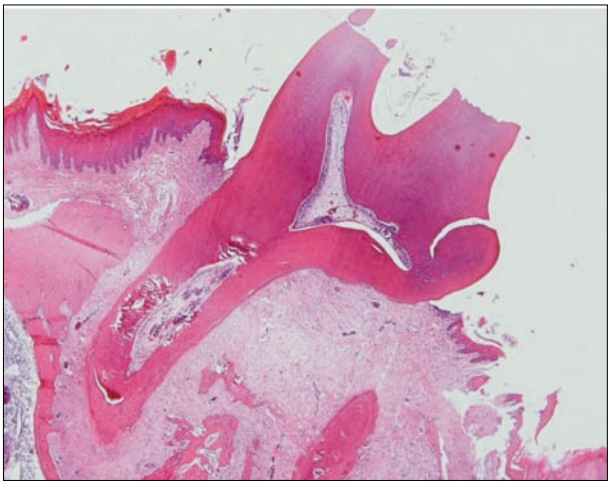

Figure 2. Parasagittal section of the mesio-buccal root of an upper first molar in the Morphine group. The tooth was moved from right to left. Original magnification x40.

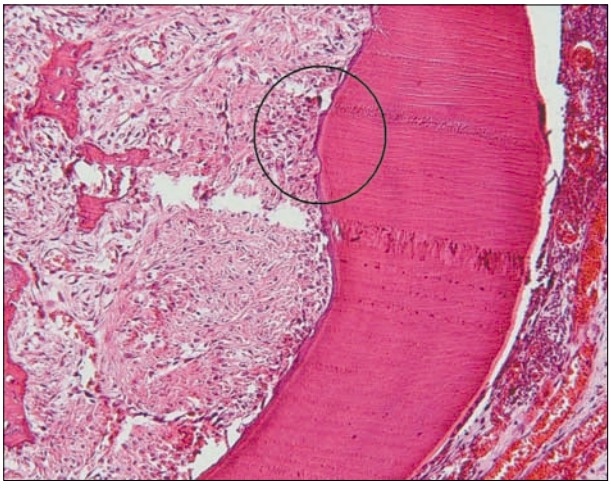

Figure 3. Resorption lacunae on the mesial surface of the mesio-buccal root of a first molar in the Morphine group. Original magnification x100.

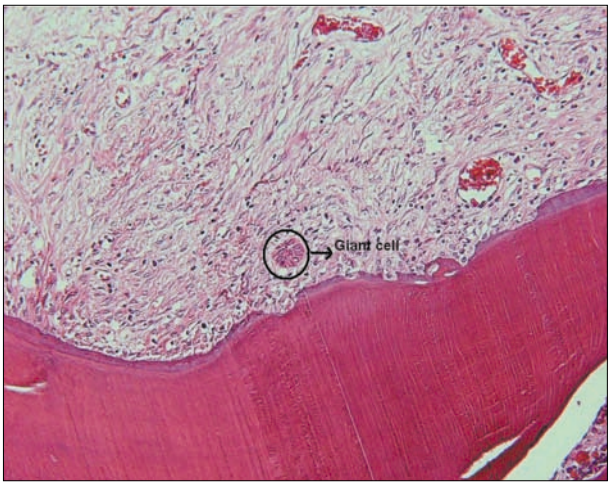

Figure 4. An osteoclast (circled) on the mesio-buccal root of a specimen in the Morphine group. Original magnification x200.

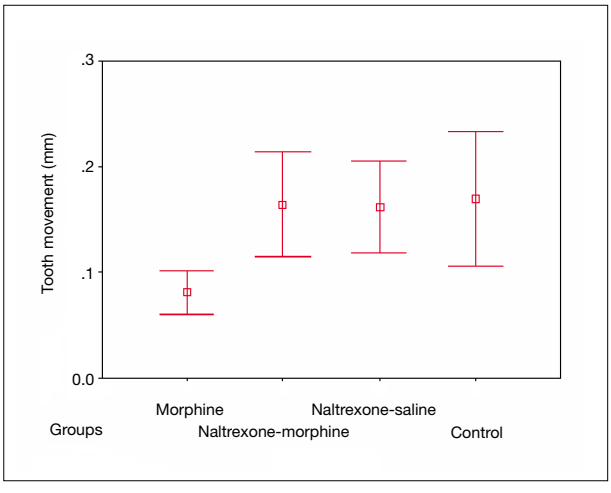

Figure 5. Tooth movement in the groups. Means and SD bars are shown.

From each tooth, six sections that showed the full width of the root from the cemento-enamel junction (CEJ) to the apex were examined under an Olympus Bx-41 light microscope (Figure 2). Root resorption and the width of the periodontal ligament (PDL) surrounding the mesio-buccal root were evaluated with the aid of an eyepiece graticule with the accuracy of 10 µm. The number of resorption lacunae and their maximum depths were used to determine the amount of root resorption (Figures 3 and 4). The latter were measured using the method described by Sekhavat et al.[21] Apparent 'roughness' of the dentine or cementum was considered as resorption. Thus, each section produced a number representing resorption and the mean of the six representative sections determined the amount of resorption affecting a tooth. The number of osteoclasts was also used to determine bone resorption.

The width of the PDL was measured on the mesial and distal surfaces of the root in the most coronal and apical regions.[22] All sections were measured twice by the same operator and the mean of the two measurements used in all subsequent calculations.

Table I. Molar separation over 14 days.

Group	N	Mean	SD	SE	95% CI		Minimum	Maximum
		(mm)			Lower	Upper		
Morphine	10	0.081	0.029	0.009	0.060	0.102	0.05	0.15
Naltrexone - morphine	10	0.164	0.069	0.022	0.114	0.214	0.10	0.30
Naltrexone - saline	10	0.162	0.061	0.019	0.119	0.205	0.08	0.25
Control	10	0.170	0.089	0.028	0.106	0.233	0.10	0.35

Statistical analysis

Differences between the groups were analysed by the one-way ANOVA followed by Tukey post-hoc tests for multiple comparisons. The statistics were analysed with SPSS 11.5 software. Probability values < 0.05 were considered as statistically significant.

Results

There were no statistically significant differences in the mean overall weights of the groups. All first molars showed evidence of tooth movement (Table I). Among the groups, the Control group showed greatest mesial movement (Mean: 0.170 mm) and the Morphine group (Mean: 0.081 mm) the least mesial movement (Figure 5). The first and second molars in the Morphine group were significantly less separated than the molars in the other three groups ($p < 0.05$). The differences between the Control, Morphine-naltrexone and Naltrexone-saline groups were not statistically significant ($p > 0.05$).

Osteoclasts were found lining the bone on the mesial surfaces of the roots in all groups, but there were no statistically significant group differences in the number of osteoclasts ($p > 0.05$). Although the resorption lacunae in mesial roots in the Morphine group were fewer and shallower than the lacunae in the other groups, the differences were not statistically significant ($p > 0.05$). There were no significant differences in the widths of the PDL on mesio-apical, disto-apical, mesio-coronal and disto-coronal areas of the mesio-buccal root ($p > 0.05$).

Discussion

We determined the magnitude of tooth movement in rats administered morphine, an exogenous opioid, by measuring separation of the maxillary first and second molars following mesial movement of the first molar with a simple orthodontic appliance. We found that morphine (an exogenous opioid) reduced the amount of tooth movement and this effect was reversed by naltrexone. We found no differences in tooth movement between the groups that received naltrexone either with morphine or with saline, or the Control group, which suggests that the naltrexone did not affect tooth movement. Our histological methods failed to disclose supporting differences in the number and depths of resorption lacunae or the width of the PDL.

Previous studies have shown that opioids affect osteoblasts and bone remodelling.[4,7] Since there are three opioid receptors (mu, delta and kappa) on osteoblast-like cells (MG-63), the high concentration of morphine, as an agonist of mu receptor sites, prevented the synthesis of osteocalcin, which is a marker of osteoblastic activity.[7] Rosen et al. suggested the existence of opioid receptors in osteoblasts and confirmed the presence of specific mRNA of kappa receptors in rat osteoblasts.[4,23]

Our results can be compared with a study of the role of opioid systems on orthodontic tooth movement in cholestatic rats.[12] Endogenous opioids in cholestasic animals may interfere with bone remodelling by affecting osteoblast-like cells and, in turn, increasing the rate of orthodontic tooth movement.[12] In these conditions, endogenous opioids interacting with nitric oxide were thought to be responsible for the increased rate of orthodontic tooth movement.[24,25] In these studies, naltrexone blocked the increase in endogenous plasma opioids and inhibited the rate of orthodontic tooth movement, whereas we found it had no effect on the rate of tooth movement. It appears that endogenous and exogenous opioids have quite different effects: endogenous opioids increase tooth movement and exogenous opioids reduce it. Part of the answer may lie with the strength and/or

dosage of the opioid used. For instance, codeine is a relatively weak opioid, but morphine and heroin are strong opioid agonists, and pentazosine is an agonist-antagonist opioid. The latter appears to work as a kappa agonist and mu antagonist.[9]

We measured tooth movement 14 days after appliance activation, because the period for completion of the bone remodelling cycle is 10–14 days.[12,18,26] Although we found a difference in macroscopic tooth movement between the groups there was no statistically significant group difference in the number of osteoclasts or the maximum depths of the resorption lacunae. One view of the difference in tooth movement is that morphine reduces the activity of osteoclasts rather than reducing the number of osteoclasts. Our methods did not enable us to confirm or refute this notion. Future studies should investigate the histological changes over longer periods of time, use histological methods able to detect smaller changes in cell activity and investigate different dosages of morphine.[27,28]

Our results, which demonstrated that morphine reduced tooth movement in a small laboratory animal, cannot be extrapolated to humans. The results suggest that a clinical trial of tooth movement in subjects using opiate-based analgesics should be initiated to determine if small pharmacological doses affect orthodontic tooth movement.

Conclusions

1. Morphine reduces the rate of tooth movement in rats.

2. This effect was reversed by administering the opioid antagonist, naltrexone, which had no effect on orthodontic tooth movement.

3. Further studies are needed to investigate the effects and modes of action of morphine and other opiates on tooth movement in rats and man.

Corresponding author

Dr Hassan Noroozi
Dental Research Center
Faculty of Dentistry
Tehran University of Medical Sciences
Tehran
Iran
Tel: +98 21 88986677
Fax: +98 21 88986688
Email: noroozih@yahoo.com

References

1. Newman M, Takei H, Carranza F. Carranza's clinical periodontology. 9th ed. 2002;47.
2. Adachi H, Igarashi K, Mitani H, Shinoda H. Effects of topical administration of a bisphosphonate (risedronate) on orthodontic tooth movements in rats. J Dent Res 1994;73: 1478–86.
3. Yamasaki K, Miura F, Suda T. Prostaglandin as a mediator of bone resorption induced by experimental tooth movement in rats. J Dent Res 1980;59:1635–42.
4. Rosen H, Metzer E, Benzakine S, Bar-Shavit Z. Functional opioid receptors on skeletal cells. J Bone Miner Res 1997; 12:(suppl).411.
5. Zhou D, Hughes B, King GJ. Histomorphometric and biochemical study of osteoclasts at orthodontic compression sites in the rat during indomethacin inhibition. Arch Oral Biol 1997;42(10–11):717–26.
6. Chumbley AB, Tuncay OC. The effect of indomethacin (an aspirin-like drug) on the rate of orthodontic tooth movement. Am J Orthod 1986;89:312–14.
7. Peréz-Costrillón JL, Olmos JM, Gomez JJ, Barrallo A, Riancho JA, Perera L et al. Expression of opioid receptors in osteoblast-like MG-63 cells, and effects of different opioid agonists on alkaline phosphatase and osteocalcin secretion by these cells. Neuroendocrinology 2000;72:187–94.
8. Hall TJ, Jagher B, Schaeublin M, Wiesenberg I. The analgesic drug buprenorphine inhibits osteoclastic bone resorption in vitro, but is proinflammatory in rat adjuvant arthritis. Inflamm Res 1996;45:299–302.
9. Pleuvry B J. Opioid mechanisms and opioid drugs. Anaesthesia and intensive care medicine 2005;6:30–4.
10. Brownstein MJ. A brief history of opiates, opioid peptides, and opioid receptors. Proc Natl Acad Sci U S A 1993;90: 5391–3.
11. Rico H, Costales C, Cabranes JA, Escudero M. Lower serum osteocalcin levels in pregnant drug users and their newborns at the time of delivery. Obstet Gynecol 1990;75:998–1000.
12. Nilforoushan D, Shirazi M, Dehpour AR. The role of opioid systems on orthodontic tooth movement in cholestatic rats. Angle Orthod 2002;476–80.
13. Saper JR, Lake AE. Continuous opioid therapy (COT) is rarely advisable for refractory chronic daily headache: limited efficacy, risk, and proposed guidelines. Headache 2008; 48:838–49.
14. Minai-Tehrani D, Minoui S, Sepehre M. Inhibitory effect of codeine on sucrase activity. Drug Metab Lett 2009;3: 58–60.
15. Sedighi B, Ghaderi-Sohi S, Emami S. Evaluation of self-medication prevalence, diagnosis and prescription in migraine in Kerman, Iran. Saudi Med J 2006;27:377–80.
16. Farid W.O, Dunlop S.A, Tait R.J, Hulse G.K. The effects of maternally administered methadone, buprenorphine and naltrexone on offspring: review of human and animal data. Curr Neuropharmacol 2008;6:125–50.
17. Brudvik P, Rygh P. The repair of orthodontic root resorption: an ultrastructural study. Eur J Orthod 1995;17: 189–98.
18. King GJ, Fischlschweiger W. The effect of force magnitude on extractable bone resorptive activity and cemental cratering in orthodontic tooth movement. J Dent Res 1982;61: 775–9.
19. Igarashi K, Woo JT, Paula H. Effect of a selective cyclo oxygenase-2 inhibitor on bone resorption and osteoclastogenesis in vitro. Biochem Pharmacology 2001;63:523–32.

20. Leiker BJ, Nanda RS, Currier GF, Howes RI, Sinha PK. The effects of exogenous prostaglandins on orthodontic tooth movement in rats. Am J Orthod Dentofacial Orthop 1995; 108:380–8.
21. Sekhavat AR, Mousavizadeh K, Pakshir HR, Aslani FS. Effect of misoprostol, a prostaglandin E1 analog, on orthodontic tooth movement in rats. Am J Orthod Dentofacial Orthop 2002;122:542–7.
22. Tengku BS, Joseph BK, Harbrow D, Taverne AA, Symons AL. Effect of a static magnetic field on orthodontic tooth movement in the rat. Eur J Orthod 2000;22:475–87.
23. Rosen H, Polakiewiez RD, Benzakine S, Bar-Shavit ZB. Proenkephaline A in bone-derived cells. Proc Natl Acad Sci USA 1991;88:3705–9.
24. Namiranian K, Samini M, Mehr SE, Gaskari SA, Rastegar H, Homayoun H et al. Mesenteric vascular bed responsiveness in bile duct-ligated rats: roles of opioid and nitric oxide systems. Eur J Pharmacol 2001;423:185–93.
25. Nahavandi A, Mani AR, Homayounfar H, Akbari MR, Dehpour AR. The role of the interaction between endogenous opioids and nitric oxide in the pathophysiology of ethanol-induced gastric damage in cholestatic rats. Fundam Clin Pharmacol 2001;15:181–7.
26. Gameiro GH, Nouer DF, Pereira-Neto JS, Urtado MB, Novaes PD, de Castro M et al. The effects of systemic stress on orthodontic tooth movement. Aust Orthod J 2008;24: 121–8.
27. Rozisky JR, Dantas G, Adachi LS, Alves VS, Ferreira MB, Sarkis JJ, Torres IL. Long-term effect of morphine administration in young rats on the analgesic opioid response in adult life. Int J Dev Neurosci 2008;26:561–5.
28. Zarrindast MR, Ebrahimi-Ghiri M, Rostami P, Rezayof A. Repeated pre-exposure to morphine into the ventral pallidum enhances morphine-induced place preference: involvement of dopaminergic and opioidergic mechanisms. Behav Brain Res 2007;181:35–41.