

## Effect of Tramadol ( $\mu$ -opioid receptor agonist) on orthodontic tooth movements in a rat model

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### Abstract

**Objective:** Tramadol is a synthetic analgesic of opioids which has more flexible mechanisms of action than typical opioids. Since it has been reported in previous study that typical opioids like morphine can affect the bone homeostasis, it is worthwhile to examine the effects of tramadol on tooth movement. In this study we investigated effects of tramadol on orthodontic tooth movement in rats.

**Materials and Methods:** 30 male wistar rats were selected and received orthodontic appliance. 3 groups were designed based on the substance that they received daily injections of during a 2-week orthodontic treatment. 1. Control group with no injection. 2. Control group with normal saline injection. 3. the tramadol group. After the two-week treatment period the amount of tooth movement were measured in all the groups. Also the histological analysis was performed assessing the root resorption, osteoclasts numbers and bone resorption.

**Results:** The amount of tooth movement was not significant in the tramadol group comparing to the other groups ( $P > 0.05$ ). The results of 3 histological parameters (amount of root resorption, osteoclastic numbers and bone resorption) were statistically insignificant ( $P > 0.05$ ).

**Conclusion:** Tramadol as an atypical opioid does not interfere with the process of bone remodeling and tooth movement in rat. Tramadol does not affect osteoclastic activity and bone resorption and it does not cause to change the resulted root resorption either.

**Keywords:** Tramadol, opioids, orthodontic tooth movement, rats

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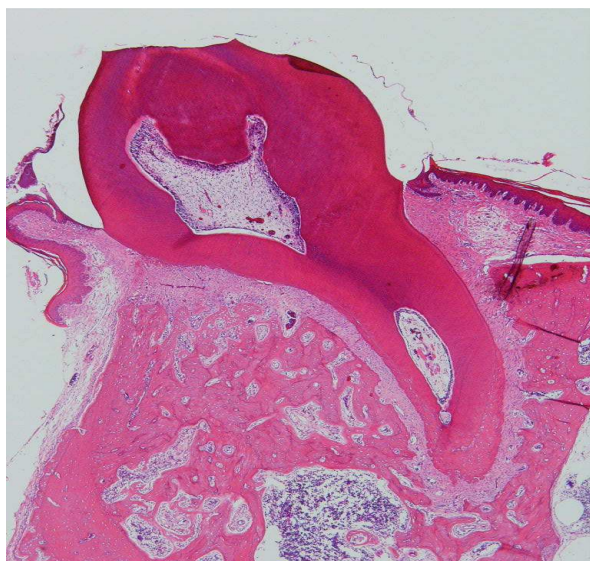
### INTRODUCTION

The orthodontic treatments are based on the inflammatory response of the periodontium to the balanced and continuous pressure on the

teeth. Orthodontic forces shift the balance and organization of the periodontal cells in favor of alveolar bone remodeling and as a result the teeth move to new positions [1-3].

Bone remodeling is a phenomenon which occurs frequently in response to external stimuli as well as biochemical changes. Resistance to outside forces, wound healings, fractures and homeostasis of calcium and phosphate in the body all involve bone remodeling. Orthodontic tooth movements are also dependent on bone remodeling. Osteoblasts and osteoclasts are two main cells that carry out this process. [1-3] Biologically the process of bone remodeling is regulated by systemic and immunological mediators. In orthodontic tooth movement the process of bone resorption is coupled simultaneous bone apposition [2]. The amount and the type of orthodontic tooth movement changes according to the quantitative and qualitative features of the force and also the bone metabolism. It is well documented that different drugs and chemical substances can affect the bone metabolism and tooth movement. Consequently the rate of orthodontic tooth movement may be altered. Analgesics are one of the drugs that are widely used in orthodontics and their potential effect on bone metabolism is worth studying. The effects of different groups of analgesics (namely NSAIDs and opioids) on inflammatory processes have been demonstrated. NSAIDs inhibit the synthesis of prostaglandin and this way they can reduce the degree of pain felt by patients. Indometacin has been shown to modulate the synthesis of prostaglandin E. This way the rate of differentiation of osteoblastic and osteoclastic cells that occurs as a result of inflammatory stimulation by PGE [1-3] and other inflammatory mediators is decreased and the process of bone remodeling slows down as a result [4,5] so we have less amount of tooth movement in the time period of application of orthodontic appliance. Also studies on Ibuprofen and aspirin showed that both lead to lowered number of osteoclasts [6]. Moreover it has been reported that Rofecoxib and aspirin have inhibitory effect on PGE<sub>2</sub> although this effect is milder in Rofecoxib [7]. Hall et al reported that Bupre-

norphine (opioid analgesic) inhibits the osteoclastic resorption of the bone [8]. Furthermore it has been suggested that opioids play a significant role in raising the rate of OTM in cholestatic rats [9]. In previous research conducted by the same team as the present study the rate of OTM was significantly lower in rats injected with morphine (typical opioid) [10]. Tramadol is a synthetic analgesic of opioid class with the chemical formula of  $N_2O_2H_{25}C_{16}$ . It is prescribed to treat moderate to severe pain. Tramadol is known as atypical opioid in that it has some distinguishing features other than general characteristics of opioids [11]. Most of the effects of drugs in opioid class is mediated via receptors. These receptors are G-protein-coupled receptors with subtypes known as kappa, delta, mu. These receptors are present in central nervous system as well as peripheral sensory neurons [12]. Unlike other opioid drugs Tramadol has 2 unique mechanisms of action: 1. Tramadol has low affinity for mu receptors and even lower affinity for delta and kappa receptors. 2. Tramadol is a serotonin releasing agent and also a norepinephrine reuptake inhibitor. Tramadol plays its analgesic role by inhibiting the reuptake of monoamines through mu receptors [13]. Tramadol possesses the distinctive feature of acting via non-opioid ways. This mechanism of action is the reason why the analgesic effects of tramadol is not fully antagonized by mu receptor antagonists (naloxone) a feature that is not seen in other opioids [14]. In a study on rats treatment with morphine showed a significant biochemical and histological osteoporotic changes while treatment with tramadol leads to non-significant osteoporotic effect [15]. According to this, it seems the effect of tramadol on orthodontic tooth movements in a rat may be different from that of morphine. The aim of this study was to examine the effect of tramadol as an analgesic drug on orthodontic tooth movement in a limited period of orthodontic treatment in rats.



**Fig 1.** Section of the whole root of upper first molar in rat tooth. Original magnification x40

Results from effects of tramadol is comparable with the results from the previous study [10] (morphine as a typical opioid) which was conducted with the exact same manner and the same circumstances as this study.

## MATERIALS AND METHODS

40 male wistar rats, 10 weeks old and weighing 200-250 g were used in this study. The animals were provided by and also kept in the animal center of the Pharmacology Department of Tehran University of Medical Sciences.

All the Rats were treated the same and in a 12 h light/dark environment. The food was grinded and softened with water in order to avoid any displacement of the appliance. The rats were kept in special cages that were labeled to be identified by their group, weight, and their number. The rats were acclimated to the cage living conditions one week prior to the experiment.

The adopted protocol was approved by the ethics committee of Tehran University of Medical Sciences. Weight measurement was done again at the time of appliance insertion and also at the day of their sacrifice. 4 groups of 10

rats each were designed: 1. Control group (no injection): This group received the appliance with no injection. 2. Control group (normal saline injection): received the appliance also they were injected with daily dose of 0.2ml. 3. Tramadol group: received the appliance plus daily injection of 20mg/kg. 4. As reported in our previous study [10] (Morphine group): received the appliance, plus daily injection of morphine 5mg/kg. All the injections were done IP (intraperitoneal) with 1 mm syringe and also at a certain time each day.

## Orthodontic treatment and OTM measurements

First the rats were weighed on a scale then they were injected by ketamine (50 mg/kg + Chlorpromazine (30 mg/kg) for general anaesthesia. After that the rats received orthodontic appliance for a two-week treatment period. The orthodontic appliance consisted of a NiTi closed coil spring (0.022 × 0.006 mm) and the initial length of 6mm running between the first molar and the central incisor of the left upper quadrant [9].

The spring was fixed in place with a 0.010" ligature wire.

In order to enhance the retention around the teeth, in regard to the pattern of eruption and the palatal concavity in incisors a cervical notch was prepared on the cervical third and on distal and labial surfaces of 2 central incisors just above the gingival margin.

After fixing the ligature wire, the wire loop around the teeth was secured with light-cure composite.

The reciprocal force of the spring was measured with a gauge, and the spring was fixed to exert approximately 60g of force which corresponded to 1mm of length increase in spring. Since the load-deflection curve of these springs is a straight line there is no need to reactivate them during the experiment. Also the incisal edges of the incisors were reduced by 1/5 mm in order to prevent any possible

disruption of the appliance by continuous eruption of the incisors in rats.

Considering the rate of continuous eruption of the incisors, the reduction needs to be repeated after one week of experiment.

#### Orthodontic tooth movement measurements

After 2 weeks of orthodontic treatment and drug injections, the rats were weighed again by a scale. Then they were sacrificed by chloroform. Before removing the appliance the interproximal distance between the left upper first and second molars were measured by a filler gauge.

The measurement was performed two times each by a person unaware of the groups to which the rats belonged. intra-class correlation coefficient between the two measurements was estimated to be 0.985.

#### Histologic analysis

After the tooth movement measurements, the maxillas of the skull were removed and placed in formalin 10% for fixation. Then the specimens were placed in a formic acid (5%) for decalcification. Finally the specimens were placed in paraffin molds and were ready for sectioning.

Parasagittal sections serially (6 $\mu$ m thick) were prepared by microtome. Then the sections were stained with hematoxylin and eosin.

Out of all prepared sections 6 sections which contained the most length and width of the root and pulp canals and the whole length of the root from CEJ to the apex were selected and the rest were excluded from the study (Fig 1). The sections were evaluated under light microscope (Olympus Bx-41). Other measurement were done by a calibrated graticule and with a 10 $\mu$ m. Two criteria were used in evaluating the level of root resorption: 1. counting the resorption lacunae. 2. measuring the depth of lacunae. The deepest resorption lacunae on the mesial root is selected and its depth was measured via measuring the virtual line connecting the depth of lacunae to the virtual intact surface of the root [16]. The Criteria for resorption was presence of clear indentation on the surface of dentin or cement. The mean value of 6 sections measurements was used to represent each tooth. Osteoclastic count was used as the indicator of bone resorption. This was done by counting the eosinophilic multinucleated cells under the light microscope. PDL width was also measured. In mesial and distal side of the root (the area between the alveolus and root surface and in the most coronal and the most apical part of the bone-root interface) PDL width was measured [17].

**Table 1.** The comparison of tooth movement between groups , Tooth movement (Multiple Comparisons)

Group(1)	Group(2)	Mean difference(1-2)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	saline	0.0260	0.682	-0.0367	0.0887
	tramadol	0.0220	0.781	-0.0407	0.0847
	morphine	0.0890*	0.003	0.0263	0.1517
Saline	tramadol	-0.0040	0.998	-0.0667	0.0587
	morphine	0.0630*	0.049	0.0003	0.1257
Tramadol	morphine	0.0670*	0.033	0.0043	0.1297

\*The mean difference is significant at the 0.05 level

### Statistical analysis

Differences between the groups were analyzed by one-way ANOVA followed by Tukey post-hoc test for multiple comparisons. Probability values less than 0.05 were considered as statistically significant.

### DISCUSSION

In the present study effects of two types of opioids (Tramadol as an opioid which has a unique mechanism of action and morphine as a typical opioid) were examined. The unique mechanism of action in tramadol and its alternative metabolic pathways can lead to this hypothesis that it does not interfere with orthodontic tooth movement. In this study it was demonstrated that morphine injection can decrease the rate of orthodontic tooth movement significantly, however, no significant difference was found among the tramadol, normal saline and no- injection group (Table 1). Previous investigators also found osteoporosis risk associated with chronic use of morphine in rats but tramadol therapy could not lead to osteoporosis [15]. The inhibitory effects of morphine can be related to decrease in osteoblastic activity (coupling phenomenon) [10]. When the activity of osteoblasts lowered the whole bone remodeling process is affected and the rate of tooth movement is decreased. The neutral effect of tramadol on tooth movement was predictable given the distinctive features of this drug and its mechanism of action comparing to other drugs of its class.

Considering the fact that most of the pharmacological effects of tramadol are associated with inhibition of reuptake of serotonin and norepinephrine and acting via opioid receptors is not as key factor as it is in other opioids [18]. In osteoclastic count there was no significant difference between the groups. These results show that tramadol did not have any effect on the number of osteoclasts and morphine did not cause to reduce the number of osteoclasts. These results corresponded to the results from Gomez and Pedrazzoni study and others that demonstrated that the metabolic effects of opioids is through affecting the activity of osteoblasts and not their numbers [19-21]. With respect to Sekhavat et al [16], two histological parameters were evaluated: The number of lacunae on root surfaces and also the depth of lacunae. In neither of these histological features significant difference was found between the groups. The results further support the idea that tramadol neither has any effect on tooth movement during the experimental period nor does it affect the orthodontic tooth movement side effects such as root resorption. In the case of morphine (typical exogenous opioid), although it modulates the orthodontic tooth movements, it has no major effect on the process of root resorption. In regard to evaluating the PDL width, four different surface areas according to the bone-root anatomical position were selected to include in sample sections (mesioapical, mesiocoronal, distoapical, distocoronal).

**Table 2.** The width of the PDL

Group	PDL(AD)*			PDL(AM)*			PDL(CD)*			PDL(CM)*		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Mini	Max
Control	0.179	0.10	0.30	0.141	0.07	0.20	0.169	0.10	0.27	0.151	0.06	0.22
Saline	0.142	0.10	0.21	0.170	0.11	0.30	0.158	0.06	0.25	0.161	0.13	0.27
Tramadol	0.174	0.12	0.25	0.175	0.10	0.26	0.210	0.10	0.30	0.186	0.13	0.25
Morphine	0.173	0.9	0.28	0.176	0.10	0.30	0.165	0.10	0.25	0.202	0.13	0.27

\* The width of the PDL was measured on the mesial and distal surfaces of the root in the most coronal and apical regions. (AD= apicodistal/ AM= apicomerial/ CD=coronodistal/ CM= coronomesial)

Lack of significant difference among the groups can be attributed to the similarity of exerted force in all the different surface areas (Table 2). Bartzela and et al [22], designed a systematic literature review on the effects of medications on the rate of experimental tooth movement. Their report showed no experimental data were available on the effect of opioid analgesics in this respect. It is noteworthy that experimental evidence for the effects of many drugs on OTM is still lacking. Furthermore, they concluded there is a need for more studies on the effects of various drugs on OTM [22]. In the present study the two types of opioids (morphine and tramadol) exhibit different effects on orthodontic tooth movements in a rat model.

## CONCLUSION

Tramadol as an atypical opioid does not interfere with the process of bone remodeling and tooth movement in rat. Tramadol does not affect osteoclastic activity and bone resorption and it does not cause to change the resulted root resorption either.

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