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Effect of tramadol at different doses on orthodontic tooth movement and bone resorption in rats

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ABSTRACT

Background: Tramadol is an opioid agonist that has the potential of being abused. The aim of this study was to compare the effect of different doses of tramadol on orthodontic tooth movement (OTM) and bone resorption in rats.

Materials and Methods: Forty-two male rats were assigned randomly to two experimental groups and one control group. Nickel–titanium coil springs were used to exert orthodontic force. The rats in the control group and experimental group-1, respectively, received a daily injection of 0.1 ml of normal saline and 10 mg/kg of tramadol for 14 days. The rats in experimental group-2 received 10 mg/kg of the same drug on days 1-4, 20 mg/kg on days 5-8, 40 mg/kg on days 9-12 and 60 mg/kg on days 13 and 14. OTM was measured on days 4, 8, 12, and 14. At the end of the experimental period, the rats were sacrificed. Histological analysis was also performed to evaluate the number of osteoclasts, osteoblasts, and Howship's lacunae.

Results: Statistical analysis with analysis of variance tests showed that the rats in experimental group-2 had significantly decreased OTM compared with the other two groups ($P < 0.05$), whereas OTM for the rats in experimental group-1 was comparable to that in the control group ($P > 0.05$). The histological evaluations did not show any significant difference among the groups ($P > 0.05$).

Conclusion: The effect of tramadol hydrochloride on OTM depends on the dosage used. High doses of the drug reduce the extent of OTM significantly.

Key Words: Orthodontic tooth movement, rat, tramadol

INTRODUCTION

Teenagers comprise the main group of orthodontic patients.[1] During the teenage years, young people often experience significant psychological, social, and physical crises, which may increase their engagement in risky behavior, such as drug abuse.[2]

The prevalence of illicit drug use has been reported to be 21.5% in eighth graders and 39.8% in tenth graders in the United States.[2] If these statistics are extrapolated to all adolescents, it is reasonable to assume that considerable numbers of orthodontic patients in every practice have taken at least one illicit substance.[2] Despite the young age profile of drug abusers in the United States.[2] such abuse is more restricted in Iran due to cultural issues and parents' monitoring. However, some drugs that are not generally known for their illicit use potential, such as tramadol, may be abused, that is, used for other than analgesic effect, by adolescents without their parents' knowledge.

Tramadol is a narcotic-like pain-relieving drug that increasingly is being taken illicitly in Iran, especially among youngsters.[3] Explanations for this may include the ease with which this drug can be acquired compared with other opioids and the anticipation that it has a low potential for abuse. In Iran, tramadol has been classified as a controlled substance because of

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its side effects and because of the increased abuse of the drug since 2007.[4] The drug is a centrally acting analgesic that has a weak agonistic effect on μ-opioid receptors and that inhibits the re-uptake of serotonin and norepinephrine.[5,6] Commonly, it is manufactured as a hydrochloride salt (tramadol hydrochloride). The therapeutic use of tramadol is to manage moderate-to-severe pain. Depending on the treatment protocol, it is prescribed at dosages of 50-100 mg every 4-6 h for pain relief, up to a maximum dose of 400 mg/day. The habituating nature of tramadol emanates from its μ-opioid agonism as well as serotonergic and noradrenergic effects.[7,8]

Numerous studies have indicated the effect of opioids on the metabolism of bone.[9,10] Opioids exert their effect through opioid receptors that are located on the cells of the central nervous system and other tissues.[9,11,12] Recent studies have shown the expression of opioid receptors in the human osteoblast-like cell line MG-63.[9] Opioids may modify orthodontic tooth movement (OTM) through their effect on the metabolism of bone since OTM requires alveolar bone remodeling.[13,14]

Bartzela, *et al*.[15] conducted a systematic review and found no research meeting their inclusion criteria on the effects of opioids on OTM. However, later studies showed that endogenous opioids increased OTM in cholestatic rats by interacting with nitric oxide.[16] whereas morphine decreased OTM.[17] Recently, it was reported that therapeutic doses of tramadol had no significant effect on OTM.[18]

Since therapeutic use of tramadol usually does not occur in orthodontic practice, the objective of this study was to elucidate whether high doses of tramadol, as a result of the increasing tendency for this drug to be abused by young people, have a significant effect on OTM.

MATERIALS AND METHODS

Preparation and grouping of rats

Forty-two male Sprague–Dawley rats with initial weight of 250 ± 20 g and similar ages were obtained from the Razi Institute (Tehran, Iran). The rats were housed in a standard environment, with alternating 12-h cycles of light and darkness, a temperature of 21°C ± 2°C, and a relative humidity of 55%. The rats had a standard diet that consisted of 0.8-1.2% calcium, 0.7-0.9% phosphorus, 3060-kg units of vitamin D, and sufficient water. All the processes were conducted in accordance with the U.S. National Institutes of Health's (NIH's) Guide for the Care and Use of Laboratory Animals.[19]

The experimental protocol was approved by the Ethics Committee (no: 130786) of Shahid Sadoughi University of Medical Sciences (Yazd, Iran). The rats were weighed at the beginning of the study and daily thereafter to ensure their health and for use in calculating the dosages of the drug to be administered. The rats were divided randomly into two experimental groups and one control group (14 rats for each group). Injections of tramadol were administered intra-peritoneally daily for 14 days. The rats in experimental group-1 received a constant dose of 10 mg/kg/day of tramadol hydrochloride (100 mg; Amp German Grunenthal Company, Aachen Germany) and the rats in experimental group-2 were injected with increasing doses of tramadol hydrochloride, beginning with 10 mg/kg/day on days 1-4, followed by 20 mg/kg/day on days 5-8, 40 mg/kg/day on days 9-12, and 60 mg/kg/day on days 13 and 14. The control group received an injection of 0.1 ml of normal saline solution daily. To the extent possible, all injections were done at the same time.

Orthodontic appliance

To insert the orthodontic appliance the rats were anesthetized by intra-peritoneal injection of a mixture of 44 mg/kg body weight ketamine hydrochloride (Gedeon Richter Ltd., Budapest, Hungary) and 2 mg/kg body weight xylazine (Rompoun; Bayer, Leverkuzen, Germany).

Orthodontic force was exerted similarly in all cases by using a 5.0-mm length of a closed-coil spring (Niti, 3M Unitek, Monrovia, California, USA; Hitek, 0.010 × 0.030 inches) tightened with a ligature wire (Dentaurum steel ligature wire, 0.010 inches; Dentaurum Group, Ispringen, Germany) to the maxillary right first molar and the maxillary right central incisor [Figure 1]. No problems were encountered in ligating to the molar tooth because of a prominence on the mesiopalatal surface. To prevent the ligature wire from sliding on the cone-shaped incisor, we made a groove using a 0.8-mm diamond bur at the cervical third of the crown and etched the groove with 37% phosphoric acid for 40 s. After washing, we ligated the wire in the groove and made it immobile with composite resin (self-cured, Degufill, Degussa, Frankfurt, Germany). Sixty grams of force were exerted by activating this orthodontic appliance for 2-5 mm.

Tooth movement measurement

Tooth movement was measured on days 4, 8, 12, and 14. The measurements were made directly in the mouth on days 4, 8, and 12 after the rats were anesthetized and before injections were done. The rats were given an overdose of ether on day 14, after which they were decapitated. Following decapitation, to prevent incorrect measurements due to relapse, tooth movement was measured in each rat before removing the appliance. Tooth movement was measured as the distance between the first and second molars using a standard feeler gauge (Mitutyo Co., Kawasaki-shi, Japan) that was calibrated in increments of 0.01 mm. To measure OTM on day 14 and inhibit the wedging effect of inserting the measuring device, we used the same method that Sekhavat *et al*. used.[20] In this method, the distance between the mesial surface of the maxillary right first molar and the distal surface of the maxillary right third molar was measured using a digital caliper (resolution: 0.01 mm; code no. 500-320, model CD4; Mitutyo Co.). Every effort was made to prevent any increase in this distance due to the insertion of the feeler gauge. The measurements were taken by an operator blinded to the method of study, and all measurements were recorded twice. The mean value of the two measurements was considered to be the OTM in each case.

Histological evaluation

After sacrificing the rats on day 14, the premaxilla of the rats were removed and placed in 10% formalin. Following fixation, the samples were decalcified with 5% formic acid and embedded in paraffin. The paraffin blocks were sectioned serially at a thickness of 4-6 μm in the parasagittal plane from the level of the first molar mesio-buccal root. The sections were mounted on microscope slides and stained with hematoxylin and eosin. The numbers of osteoclasts and Howship's lacunae were counted by a pathologist who was blinded to the study.

Statistical analysis

Descriptive statistics, including means and standard deviations, were calculated using the Statistical Package for Social Sciences (SPSS) version 18 for Windows (SPSS Inc., Chicago, Illinois, USA). One-way analysis of variance and Tukey tests were used for multiple comparisons of the extents of OTM and counts of osteoclasts and Howship's lacunae between the groups. Statistical significance was considered for $P \leq 0.05$.

RESULTS

The amounts of tooth movement were measured 14 days following the application of force [Table 1]. One-way analysis of variance test showed a significant difference in the amount of tooth movement between the groups ($P < 0.05$). The results of Tukey test showed that the amount of tooth movement in experimental group-1 (0.20 mm) was comparable to that in the control group ($P > 0.05$), whereas the amount of tooth movement in experimental group-2 (0.15 mm) was significantly less than that in experimental group-1 and the control group ($P < 0.05$) [Table 2].When the injected dosage of the drug exceeded 40 mg/kg, OTM in experimental group-2 decreased significantly [Figure 2]. Figure 2 shows that the three study groups had essentially the same amount of OTM until day 8. After that, there was no further increase in the amount of OTM in experimental group-2, whereas the amount of OTM in the other two groups continued to increase until the experiment ended. Histological analyses did not show any significant differences in the numbers of

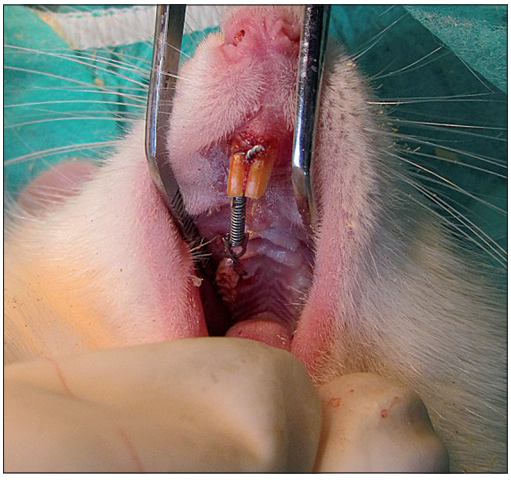

Figure 1: Application of nickel–titanium closed-coil spring

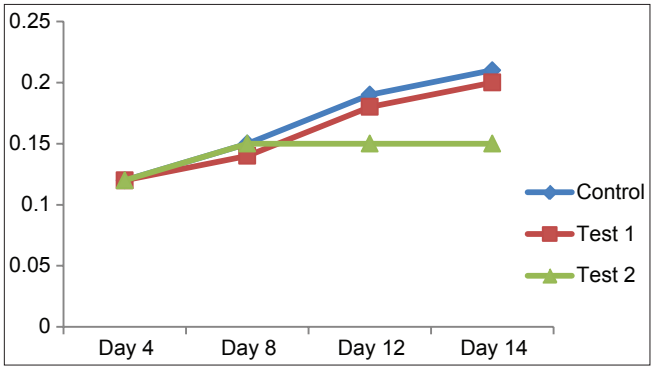

Figure 2: The OTM–time curves of the study groups

Table 1: Descriptive statistics of OTM after 14 days

Group	*N*	Mean (mm)	SD*	SE*	95% confidence interval for mean		Minimum	Maximum
					Lower bound	Upper bound		
Control	14	0.2121	0.01424	0.00381	0.2039	0.2204	0.19	0.23
Experimental 1	14	0.2007	0.01141	0.00305	0.1941	0.2073	0.18	0.22
Experimental 2	14	0.1507	0.01439	0.00385	0.1424	0.1590	0.13	0.17
Total	42	0.1879	0.03000	0.00463	0.1785	0.1972	0.13	0.23

OTM: Orthodontic tooth movement; *SD: Standard deviation; SE: Standard error

Table 2: Multiple comparisons of amounts of OTM between groups using Tukey test

(I) group	(J) group	Mean difference (I-J)	SE	Sig.	95% confidence interval	
					Lower bound	Upper bound
Control	Experimental 1	0.01143	0.00507	0.075	−0.0009	0.0238
	Experimental 2	0.06143*	0.00507	0.000	0.0491	0.0738
Experimental 1	Experimental 2	0.05000*	0.00507	0.000	0.0376	0.0624

OTM: Orthodontic tooth movement, *Mean difference is significant at the 0.05 level

osteoclasts and Howship's lacunae between the study groups ($P > 0.05$). Figure 3 shows a histopathological section of an upper first molar tooth that contains osteoclasts, osteobalsts, and Howship's lacunae.

DISCUSSION

Tooth movement induced by orthodontic force is associated with bone remodeling, which is affected by numerous local and systemic factors.[15] Opioids intervene in bone metabolism through three basic opioid receptors, that is, μ, κ, and δ receptors.[9,10,12] Tramadol is an opioid that has dual modes of action, that is, μ-opioid receptor agonism and inhibition of mono-amine re-uptake. The neutral effect of this drug on OTM in rats at the therapeutic dose of 20 mg/kg has been attributed to its dual mechanism of action.[18] We hypothesized that the higher doses of tramadol that are used by drug abusers may affect OTM in the same way that morphine does.[17] Therefore, we decided to evaluate the effect of different doses of tramadol on OTM in rats.

As mentioned before, the therapeutic dose of tramadol for humans varies from 50-100 mg every 4-6 h, not exceeding 400 mg per day. As stated earlier, for accurate translation of the human dose to an equivalent dose for a rat, we used the method based on body surface area, as suggested by the U.S. Food and Drug Administration.[21] Dose conversion based on body surface area is done by using conversion factors that are different for different species and accounting for several parameters of mammalian biology, such as blood volume, plasma proteins in the blood, and renal

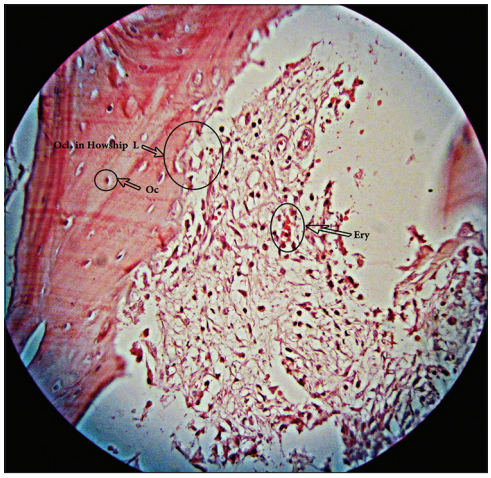

Figure 3: A histopathological section of the upper first molar tooth in a rat (×100) showing osteocytes, osteoclasts, Howship's lacunae, and erythrocytes

function.[22] Translation of dose from different species is best done by using this method rather than simple conversion based on body weight.[22] To convert the dose of tramadol used in this study to a dose based on the surface area of a person's body, one should multiply the rat dose by a conversion factor of 6 for rats and then divide by a conversion factor of 37 for humans. Based on this calculation, doses of 10, 20, 40, and 60 mg/kg in rat are equivalent to 1.62, 3.24, 6.48, and 9.72 mg/kg, respectively, in a person, which are equal to 105.3, 210.6, 421.2, and 631.8 mg for an adult who weighs 65 kg. According to the above calculation, the applied dose in experimental group-1 was comparable to the therapeutic dose used in humans, and the increasing doses in experimental group-2 could be viewed as a simulation of cases of drug abuse.

A closed-coil spring was used to exert orthodontic force. This orthodontic appliance has been found to be more consistent than an elastic module for closing the inter-dental space.[23] It takes 10-14 days to complete the cycle of bone remodeling, so we measured the amount of tooth movement 14 days after activating the appliance.[16]

The results of this study supported previous results regarding the neutral effect of therapeutic doses on OTM in rats.[18] This observation is also consistent with the findings of an earlier investigation that showed that chronic use of 10 mg/kg of tramadol in rats did not lead to osteoporotic changes.[24] An important finding in the present study was that OTM decreased after injection of increasing doses of tramadol in the rats in experimental group-2. This decrease could have resulted from the fact that endogenous opioids, such as proenkephalin-derived peptides, inhibit the activity of alkaline phosphatase (a marker of bone formation) in the murine cell line Ros-17/2.8.[9] However, the level of serum osteocalcin, which is a marker of osteoblastic activity, has been found to be lower in heroin abusers.[9] Local application of osteocalcin has been reported to accelerate the rate of tooth movement.[25] In a study conducted by Perez-Castrillon, *et al*.,[9] the presence of the specific mRNA of three opioid receptors in human osteoblasts, such as cell line MG-63, was identified and it was found that high concentrations of morphine, which is a μ-opioid receptor agonist, inhibited the synthesis of osteocalcin by these cells.[9] Since tramadol hydrochloride also is a μ-opioid receptor agonist,[5,6] it may affect bone metabolism and, consequently, OTM. Although the affinity of this drug for μ-opioid receptors is 400 times less than that of morphine, its major metabolite, *O*-desmethyltramadol, shows a remarkable affinity for μ-opioid receptors (10 times less than morphine and may have a role in the reduced OTM in this study).[26] The different effects of tramadol at different doses can be explained by the fact that the effect of tramadol as a μ-opioid receptor agonist depends on the dosage used. Based on the conversion calculations of opioids, parenteral tramadol is approximately equipotent to parenteral morphine in a 10:1 (tramadol:morphine) ratio.[27] Considering this calculation, tramadol at a dosage of 50 mg/kg or higher is equipotent to morphine at 5 mg/kg, and its affinity for μ-opioid receptors is approximately the same as that of morphine. Morphine (5 mg/kg) has been shown to reduce OTM in rats.[17]

It has been demonstrated that *O*-desmethyltramadol inhibits the function of substance *P* receptors in *Xenopus* oocytes. Substance *P* receptors mediate nociceptive transmission in the spinal cord.[28] The compound *O*-desmethyltramadol inhibits the current of substance *P* receptor-induced chlorine ions at pharmacological concentrations.[28] Substance *P* is one of the initial triggers of the biomechanical cascade that includes activation of different periodontal ligament cells.[29] This neurotransmitter is involved in the remodeling of PDL and alveolar bone during OTM.[29] The numbers of nerve fibers that show substance P-like immunoreactivity in dental pulp, PDL, and marginal gingiva are increased during and after OTM.[30] Therefore, while the inhibitory effect of tramadol hydrochloride's major metabolite (*O*-desmethyltramadol) on the function of substance *P* receptors can be a possible explanation for the findings in this study, more investigation is needed before this can be stated conclusively.

Histological analyses failed to show any significant difference in the number of osteoclasts and Howship's lacunae. These observations are consistent with those of other researchers.[17,18] A possible explanation for these findings may be the fact that opioids affect the activity of bone cells, and the count of these cells is not influenced by the opioids.[9,31]

A routine orthodontic treatment requires monthly visits over several years. For this reason, orthodontists have a special opportunity to monitor the effects of drug abuse on adolescent and young adult patients. An orthodontist, by careful examination of physical changes and observation of behavioral changes, may be able to identify the likelihood of drug abuse before the habit is formed. Although orthodontists are not responsible for treating drug abusers, they can provide valuable service to patients and society by informing patients about the unpleasant effects of their behavior on general health and on the outcome of their orthodontic treatment. They also can refer patients to appropriate healthcare providers early in the formative stages of addiction.

This study was performed on small laboratory animals; therefore, the findings may not be extrapolated directly to humans without further clinical trials. The results of this study should be investigated further in clinical studies with humans to elucidate whether consumption of tramadol at high doses affects OTM.

CONCLUSION

The effects of tramadol on OTM depend on the dosage used. At therapeutic doses, it has no effect on OTM, whereas higher doses reduce OTM. Additional studies are required to clarify the exact underlying processes.

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