Original Article

Effect of Atorvastatin on Orthodontic Tooth Movement in Male Wistar Rats

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Abstract

Objectives: Statins are used as cholesterol-lowering drugs by many patients and have been recently shown to affect bone metabolism. The aim of this study was to determine the effect of atorvastatinon on orthodontic tooth movement (OTM) in rats.

Materials and Methods: Thirty-six adult male Sprague-Dawley rats were randomly divided into three groups of 12 samples each. Group A, served as control with no medication while groups B and C received a daily gavage of carboxymethyl cellulose (CMC) as vehicle and atorvastatin (5 mg/kg) as test substance, respectively. In all three groups, 6mm nickel-titanium closed-coil springs were ligated between the maxillary incisors and first left molars to deliver an initial force of 60g. Tooth movement was measured following sacrifice, 21 days after appliance insertion**.** Root resorption, PDL width and osteoclast number were histologically evaluated and compared between the groups.

Results: The mean amount of tooth movement was 0.62 mm in group A, 0.59 mm in group B and 0.38 mm in group C. OTM reduction following administration of atorvastatin was statistically significant ($p<0.05$), but there was no significant difference in the studied histologic variables among the three groups ($p>0.05$).

Conclusion: According to the results obtained in the current study, atorvastatin appears to reduce tooth movement in rats; however its effect on osteoclasts, especially osteoclastic function, requires further investigation.

Keywords: Atorvastatin, Tooth movement, Rats

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INTRODUCTION

Cholesterol biosynthesis is regulated by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in hepatic tissues and its reduction has been successfully achieved by a class of drugs known as statins or HMG-CoA reductase inhibitors. Atorvastatin is a member of this group which in addition to

lowering cholesterol, also affects endothelial and vascular smooth muscle cells, and processes like apoptosis, angiogenesis and plaque stabilization, resulting in decreased cardiovascular events and death [1].

Modulation of bone formation and inflammation are considered among the various functions of statins [2-9] and are both key factors in orthodontic tooth movement (OTM) [10]. Therefore the hypothetical effect of these agents on OTM can be an interesting topic for further research. Previous studies have shown anabolic effects of simvastatin and lovostatin on bone formation both in vitro and in rodents [7]. Maeda et al [11] demonstrated osteoblastic differentiation and enhanced osteogenesis in MC3T3-E1 cells following simvastatin treatment and suggested angiogenesis to be involved in this process. Inhibition of the mevalonate pathway by simvastatin has been claimed to be responsible for the induction of proliferation and differentiation of human periodontal ligament cells [12]. Moreover this drug can promote osteoblastic function in culture and stimulate alveolar bone formation in rats with experimentally-induceed periodontitis [13].

Additional bone-related features of statins include osteoconductivity in calvarial defects of rabbits [14] and bone formation around titanium implants following intraperitoneal injection in rat tibiae [15]. In a recent study, simvastatin was shown to enhance alveolar osteogenesis and PDL remodeling subsequent to OTM in rats. Furthermore it inhibited bone resorption by osteoclasts and was proposed as a suitable agent to promote retention [16]. Considering the osteogenic and anti-inflammatory effects of simvastatin, which are both known to influence orthodontic treatments; the aim of the present study was to investigate whether systemic administration of this drug can affect the rate of orthodontic tooth movement in rats. Similar research in the English literature dealing with this issue is sparse.

MATERIALS AND METHODS

All experiments were performed according to the US National Institute of Health (publication 85-23; revised: 1985) and approved by the ethics committee of Tehran University of Medical Sciences.

Thirty-six male Sprague-Dawley rats weighing 220±20g were housed in plastic cages at 22°c±2°C temperature and a humidity of 55% with a standard 12-hour light/dark photoperiod.

The animals had free access to basic rat chow and water before the experiments. All rats were randomly divided into three groups of 12 and received orthodontic treatment followed by vehicle or drug administration or no further manipulation.

Group A consisted of control animals with no medication. Groups B and C received a daily gavage of carboxymethyl cellulose (CMC) as vehicle and 5 mg/kg of atorvastatin in CMC, respectively. The rats were weighed at the beginning of the study and daily, after appliance insertion.All animals were anesthetized by intra-peritoneal injection of 0.9mg/kg xylazine HCL and 70mg/kg ketamine. Orthodontic appliances consisted of 6-mm 0.006 ×0.022 inch NiTi closed-coil springs wich were tied between the left maxillary first molars and central incisors [17] using a 0.010" ligature wire (stainless steel, Dentaurum, Ipsringen, Germany) to deliver a force of 60g at 2 mm activation . A cervical notch was prepared in the palatal concavity of the incisors in the cervical third just above the gingival margin with a 0.8 mm diamond bur.

To prevent slipping of the ligature wires, they were secured on the incisors by application of light-cure composite (Transbond XT,3M Unitek, Monrovia, Calif).

The incisal edges of the incisors were reduced (1.5 mm) once a week in order to preclude any possible disruption of the appliances by continuous eruption of these teeth. During orthodontic treatment, the animals were fed ground rat chow and water ad libitum.

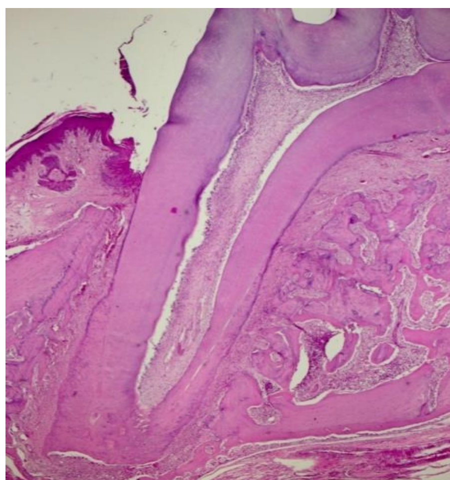

Fig 1. Representative section of the mesio-buccal root of a rat upper first molar (hematoxylin/eosin staining; Original magnification ×40)

Orthodontic tooth movement measurements

Three weeks after orthodontic force application, the rats were weighed for the last time and sacrificed by ether overdose.

The interproximal distance between the left upper first and second molars represented the amount of tooth movement and was measured by a filler gauge (Mitutoyo co, Kawasaki-shi, Japan), before appliance removal.

All measurements were performed twice, by a blinded operator and their mean value was recorded as the final OTM. Intra-class correlation coefficient between the two measurements was estimated to be 0.985.

Histologic analysis

After tooth movement measurements, the maxillae were separated and immersed in 10% formalin for 5 days and decalcified in formic acid (5%) for approximately 7 days. The specimens underwent routine processing and 5µm sections were cut for hematoxylin and eosin staining. The mesio-buccal roots of the first molars were histomorphometrically analyzed on six sections with the greatest root areas and the mean of the six measurements were representative of that specimen (Figure 1).

All calculations were performed by two observers under a double-headed light microscope (Bx-51, Olympus CO, Tokyo, Japan) equipped with a digital camera (DP25 Olympus) and analysis software (DP2-BSW, Olympus). Conflicts were resolved by consensus. The level of root resorption was determined by the number and size of the resoption lacunae. The length of the lacunae were assessed by measuring the length of a virtual line joining the edges of the defect, while the distance between this line and the deepest point of the crater was considered as "lacunae depth" [18]. Osteoclastic count as an indicator of bone resorption, was quantified by counting the large multinucleated cells with slightly basophilic cytoplasms at a magnification of ×200.

PDL width was estimated at the most coronal and apical parts of the bone-root interface [19], on both mesial and distal root aspects.

Statistical analysis

One-way ANOVA followed by Tukey post-hoc test were performed to clarify the differences between the groups. Probability values of less than 0.05 were considered significant.

RESULTS

Table 1 shows the amount of tooth movement in all groups. After 3 weeks of atorvastatin administration, group A showed the most and group C showed the least amount of tooth movement.

The reduction observed in the experimental group compared to the two control groups was statistically significant ($P<0.05$).

However according to the post-hoc test, the difference between groups A and B did not reach a significant level ($P=0.89$).

Descriptive histologic data are shown in tables 2 and 3. Osteoclast counts in the bone adjacent to the mesiobuccal roots of rat molars demonstrated no significant difference among the three groups ($P=0.17$).

Periodontal ligament width was analyzed separately for each of the locations including mesiocoronal (P=0.706), mesioapical (P=0.84), distocoronal (P=0.927) and distoapical (P=0.8) surfaces and none of them exhibited a significant difference between the groups.

Neither length nor depth of the resorptive lacunae was different among the groups as measured in both mesial (P=0.832 and P=0.843, respectively) and distal (P =0.398 and P=0.499, respectively) root aspects.

DISCUSSION

Statins, including atorvastatin, have been suggested to affect bone metabolism at the cellular level using different pathways. The function and formation of osteoclasts, one of the target cells of these drugs, are impeded through interference of statins with the mevalonate pathway. This action is upstream of where nitrogen-containing bisphosphonates inhibit conversion of mevalonate to farnesyl-PP, which was an initial step to understanding

Table 1. Description of tooth movement in 3 experimental groups(mm)

Groups	N	Mean	SD	Min	Max
Group A	12	0.62	0.22	0.48	0.76
Group B	12	0.58	0.22	0.45	0.73
Group C	12	0.37	0.16	0.27	0.49

Table 2. Descriptive data of lacunae length and depth (micrometer)

	Depth	Length
Group A mesial	19.95(29.56)	51.4(79.12)
Distal	19.29(27.7)	69.14(107.9)
Group B mesial	19.15(29.91)	75.2(110.9)
Distal	17.2(27.01)	18.27(29.61)
Group C mesial	18.34(25.17)	30.08(54.35)
Distal	9.02(16.66)	20.14(36.71)

Table 3. Descriptive data of PDL Width (micrometer)

	Mean(SD)		
	Group A	**Group B**	**Group C**
Mesiocoronal	182.58(102.23)	134.8(43.58)	170.31(88.49)
Distocoronal	242.08(134.97)	227.32(143.22)	225.67(113.51)
Mesioapical	207.07(98.99)	128.74(61.35)	152.39(46.83)
Distoapical	127.84(58.23)	83.01(38.14)	134.11(61.14)

the osseous effects of statins [20]. In order to further evaluate the impact of these drugs on bone remodeling, we measured OTM in a rat model following atorvastatin administration using 0.006× 0.022 NiTi closed coil springs.Previous studies have also indicated that nickel-titanium springs produce more persistent forces compared to steel springs [21] and that there is no difference in rat OTM between application of 40 or 60 g forces [22]. Despite the fact that 14 days has been reported to be adequate for completion of bone remodeling, our study period was 3 weeks to assure the long term effect of atorvastatin on osseous tissues [23]. Male rats were utilized in this experiment to reduce the effect of sex hormones on bone remodeling. According to the results obtained in the current investigation, atorvastatin decreases tooth movement in rats. Several recent studies have shown that different medications can interfere with various cell signaling pathways and as a result may affect orthodontic tooth movement [24,25]. Such information is essential to clinicians, since the expected duration of treatment might be influenced by drug usage. Considering that statins are among the most commonly prescribed pharmaceutical agents for prevention of hyperlipidemia [26] and cardiovascular diseases [27], their high intake [28] along with their effects on tooth movement might be a great concern to some clinicians. Numerous attempts have been made to explain the cellular events that take place following statin administration. This drug class is proposed to act through several mechanisms including blockade of the mevalonate pathway. Coxon and Rogers [29] showed that due to mevalovate pathway suppression, development of several isoprenoid groups are obstructed leading to disrupted vesicular fusion and impaired establishment of ruffled borders which are the resorbing unit of osteoclasts. On the other hand, some forms of statin have the ability to impede the development and function of osteoclasts which can be reversed by mevalonate and geranylgeraniol treatment [30]. In addition to their bone resorbing characteristics, statins have been shown to stimulate bone formation by increasing osteoblastic number and differentiation and also through induction of mineralization and new bone formation [7,30]. These effects may be due to increased bone morphogenic protein-2 (BMP-2) transcription and mRNA/protein expression in bone cells, which have been demonstrated to occur after administration of most statins, except pravastatin [5,8]. BMP-2 stimulates differentiation of stem cells to osteoblast-like cells [31] and promotes differentiation of mesenchymal cells to chondrocytes and osteoblasts [32]. In confirmation, Maeda et al [33] found that simvastatin augmented the expression of BMP-2, vascular endothelial growth factor (VEGF) and alkaline phosphatase mRNA among other molecules [33]. It is noteworthy that alkaline phosphatase plays an important role in mineralization of osteoid by osteoblasts [11]. Another anabolic effect of statins on bone metabolism may be the suppression of receptor activator of nuclear factor kappa-B ligand (RANKL) expression by mediating repression of the mevalonate pathway. RANKL promotes osteoclastic differentiation and activation [34], while osteoprotegerin (OPG) inhibits RANKL, hence blocking osteoclastogenesis leading to decreased bone resorption. Atorvastatin has been shown to activate OPG generation by human osteoblasts [35]. Wong and Rabie [14] suggested employing "statin collagen grafts" to replace lost bone. When implanted into rabbit calvarial defects, these grafts induced significantly larger amounts of new bone, compared to defects filled with control material. Similarly, gingival injection [13] of simvastatin and oral gavage of atorvastatin [36] was demonstrated to augment alveolar bone formation in rats. In addition Han et al [16] reported enhanced PDL remodeling and alveolar osteogenesis following simvastatin administra-

tion which led to reduced relapse of OTM in rats. Simvastatin was proposed to be responsible for both increased bone development and decreased bone resorption by regulation of RANKL and OPG.

Our findings regarding reduced rat tooth movement following treatment with atorvastatin, support the osteogenic potential of this drug group along with its preventive effect on bone resorption. In contrast to the clinical results obtained in the present study, the histologic data were inconclusive. Osteoclast number did not differ among the groups, which may be related to technical issues, low overall counts or its actual inefficacy in this process. Increased osteoclastic number is not the sole factor responsible for bone resorption, since function is regarded to be similarly important in this process. Moreover, statins have been shown to affect osteoclast activity and the formation of ruffled borders in these cells [29,30]. It could be postulated that the dose of atorvastatin used in our experiment might have affected osteoclast function and not its number. Therefore further investigation using more sophisticated methods is proposed to clarify the effect of atrovastatin on different aspects of osteoclasts.

CONCLUSION

According to the results obtained in the current investigation, atorvastatin decreases the rate of experimentally-induced tooth movement in rats. If future studies support this finding in human subjects, reduced OTM should be considered in the active phase of orthodontic treatment and the patient should be informed of the effects of this drug on treatment duration.

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