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SCIENTIFIC SECTION

Orthodontic tooth movement in cholestatic and cirrhotic rats

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Objective: To investigate whether cirrhosis and cholestasis could influence orthodontic tooth movement.

Design: Basic science, animal experimental study.

Setting: This study was conducted in the Department of Pharmacology, School of Medicine at Tehran University of Medical Sciences in 2007.

Participants: A total of 40 male Sprague–Dawley rats (150–200 g) were divided into five experimental groups: non-operated, cholestatic-sham, cirrhotic-sham, cholestatic and cirrhotic groups.

Methods: An orthodontic appliance, consisting of a 5 mm nickel titanium closed coil spring, was ligated between the maxillary right incisor and first molar of each rat to deliver an initial force of 60 g. The cholestatic and cirrhotic groups underwent a bile duct ligation operation and received an orthodontic appliance for 7 days (cholestatic group) and 28 days (cirrhotic group) after surgery. Two other groups underwent a sham operation and had an orthodontic appliance inserted after 7 (cholestatic-sham) and 28 days (cirrhotic-sham). A fifth control group underwent neither bile duct ligation operation nor sham operation.

Results: The cirrhotic group showed significantly increased orthodontic tooth movement (OTM), compared to all other study groups ($P<0.001$). The mean OTM in the cholestatic group was significantly higher than in the other three groups (two sham groups and unoperated one) ($P<0.01$). Bone density was also significantly decreased in the bile duct ligated (cirrhotic and cholestatic) groups ($P<0.01$).

Conclusion: Our data demonstrated that biliary cirrhosis could cause a significant increase in the OTM and decrease in the bone density in rats, though there was no significant alteration in bone resorption or osteoclasts detected in such animals.

Key words: Orthodontic tooth movement, densitometry, resorption, cholestasis, cirrhosis

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Introduction

Orthodontic tooth movement (OTM) evokes a cascade of cellular responses within the enveloping alveolar bone. Bone remodelling and metabolism associated with this tooth movement occur through the activation of specific signalling pathways.

Cholestasis is impaired bile secretion and results in several systemic complications such as metabolic bone disorders.[1] Bone loss and reduced osteoblastic function

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have been reported in patients with cholestatic liver disease.[2] Reduced bone mass is a common finding in patients with cirrhosis, especially in those with cholestatic liver disease. Moreover, earlier studies conducted in humans revealed various patterns of bone abnormalities in cirrhotic patients, including osteomalacia (normal bone quantity, but poorly mineralized)[3–5] as well as various forms of osteoporosis (reduced bone quantity and quality) such as decreased bone formation (which is also called low bone turnover osteoporosis),[1,2,6,7] increased formation/resorption rates (high bone turnover osteoporosis).[8,9]

Despite the well-known effects of cholestasis and cirrhosis on skeletal tissue, little is known about their effect on orthodontic treatment. On the other hand, it is noteworthy that cirrhotic liver disease, especially alcohol-induced, and its complications is still one of the most frequent causes of mortality and morbidity in the Western world, though its pathophysiology has not been completely elucidated.[10] In a previous study, we found that OTM increases in cholestatic rats,[11] thus the aim of the present study was to examine the rate of OTM in subsequent cirrhosis, as well as the amount of bone (using densitometry and osteoclast counts) and root resorption in cholestatic and cirrhotic rats.

Methods and materials

Animals

A total of 40 male Sprague–Dawley rats (Pasteur Institute, Paris, France), weighing 150–200 g, were used in this study. The animals were housed in groups of four in polycarbonate cages in a temperature-controlled ($22 \pm 3^{\circ}C$) colony room. They were maintained in a 12-hour on and 12-hour off light/dark schedule with *ad libitum* food and water. Each rat was used only once throughout the study. The study conformed to the Guidelines for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH publication No. 85–23 revised, 1985).

The rats were randomly divided up into five experimental groups. Each group consisted of eight rats which were randomly housed in two cages (four rats in each cage). The sample size of eight animals/group was based on the numbers used in previous studies.[11,12] Experimental groups were: (1) control group, (2) and (3) sham-operated groups that underwent sham operation without bile duct ligation, (4) cholestatic group, and (5) cirrhotic group. An orthodontic appliance was placed 7 days after the surgery in groups (1), (2) and (4) and 28 days after surgery in groups (3) and (5).

Surgery

Midline laparotomy was performed under general anaesthesia induced by intraperitoneal (IP) injection of ketamine HCl (50 mg/kg) and promazine HCl (10 mg/kg).[10] In bile duct ligated animals (groups (4) and (5)), the bile duct was isolated, doubly ligated and resected between the ligatures.[12] In the sham groups, the bile duct was identified, manipulated and left *in situ*.[13–15] Bile duct ligation is a standard method that is extensively used as an animal model of cholestasis and biliary cirrhosis.[12] One day after surgery, bile duct-ligated rats showed signs of overt cholestasis such as jaundice, dark urine and steatorrhoea. These manifestations were not seen in any of the sham-operated animals. In the biliary cirrhotic rats (28 days after bile duct ligation), there were manifestations of cholestasis and also increased body weight gain, ascites (as evidenced by visible pools of fluid in the lateral abdomen) and postmortem histological examination.[14,15]

Orthodontic treatment and OTM measurements

All five study groups received orthodontic treatment. Each rat was anesthetized with an IP injection of ketamine HCl (50 mg/kg) and promazine HCl (10 mg/kg). The orthodontic appliance used was a replication of the appliance presented by King and Fischlschweiger.[16]

An orthodontic force of 60 g was applied with a 5 mm long nickel titanium closed-coil spring (NiTi, 3M Unitek, Monrovia, CA, US Hitek, 0.006×0.022-inch) running between the right upper first molar and incisor. The spring was connected to the first molar and central incisor via ligature wires (Dentaurum Steel Ligature Wire 0.010-inch, Dentaurum Group, Ispringen, Germany). Due to a lack of undercuts in the incisor area, a cervical groove was prepared on the tooth where the ligature wire could be seated in and secured by composite resin (Self-cure Degufill, Degussa AG, Frankfurt Germany) on both incisors. Each spring was activated once to produce 60 g of force which was measured by spring gauge device.

Two weeks after appliance insertion, the animals were killed using cervical dislocation. Orthodontic tooth movements (OTM) were then measured using a filler gauge (Mitutoyo Corp., Kawasaki-Shi, Japan) directly in the mouth to measure the distance between the first and second right molars. This distance was initially zero in the samples.

Bone densitometry

Digital lateral skull images at the end of the experiment were obtained. The investigators were unaware of which

experimental group each rat belonged to. Pixel intensity analysis is a measure of the blackness or whiteness in a digital image. Pixel intensity analysis could be a simple and useful method to measure mandibular bone mass, and it has been suggested as a method to detect the presence of osteoporosis. The square of 50×50 pixels was located immediately above the maxillary molars.[17,18]

Histologic analysis

Histomorphometric analysis of root resorption on the mesial and distal surfaces of the mesial root of the first molar was performed. All micrographs were taken under $\times 40$, and all were stained by haematoxylin and eosin. The investigators were unaware of which experimental groups the rats belonged to. The estimation of root resorption for the purpose of this experiment was defined as any clear interruption of the cemental or dentinal surface. The following measurements (which served as root resorption parameters) were recorded and evaluated statistically for differences between groups at a significance level of $P<0.05$.

- The summed amount of linear surface resorption and the summed amount of the maximum depth of the resorption per lacuna on the mesial and distal surfaces of the mesial root divided by the length of their respective root length multiplied by 100 and the number of osteoclasts in the lamina dura of mesial and distal surfaces of the mesial root.[19]

Statistical analysis

As all groups of data proved to be normally distributed ANOVA (analysis of variance) followed by Tukey's *post hoc* test was used to detect and locate statistically significant differences. $P<0.05$ was considered statistically significant.

Results

Orthodontic tooth movement measurements

Figure 1 illustrates the values obtained for OTM in the five groups with an orthodontic appliance. The mean OTM in the cholestatic ($P<0.01$) and cirrhotic ($P<0.001$) groups were significantly higher than the other groups (Table 1). The increased amount of overall tooth movement observed in the cirrhotic group showed statistical significance as compared with the cholestatic rats ($P<0.001$).

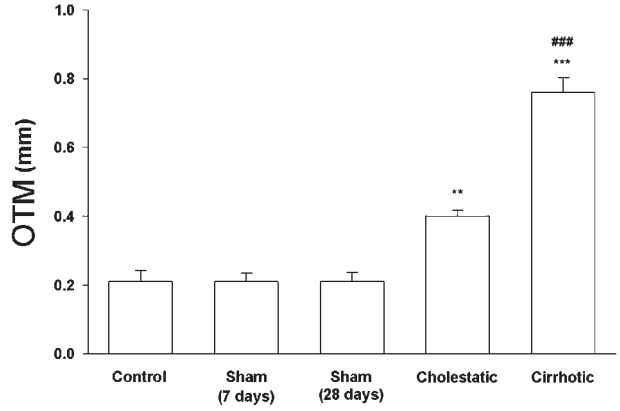

Figure 1 Orthodontic tooth movement (OTM) measurements (mm) in the 5 study groups. Data are expressed as mean ±SEM. **$P<0.01$ for cholestatic group compared to control and sham (7 days) and sham (28 days). ***$P<0.001$ for cirrhotic group compared to control and sham groups. ###$P<0.001$ for cirrhotic group measurements compared to cholestatic group

Densitometry

The results of bone densitometry are shown in Figure 2. Compared to control and sham groups, there was a statistically significant decrease in the bone density of cholestatic ($P<0.01$) and cirrhotic ($P<0.001$) groups (Table 2). Mean bone density was lower in the cirrhotic group when compared with cholestatic group but the result was not significant (Figure 2).

Histological results

As shown in Table 3, compared to control and sham groups, more osteoclasts and bone resorption were seen in the cholestatic and cirrhotic groups but it was not significant ($P>0.05$; Figure 3).

Table 1 The orthodontic tooth movement (OTM) measurements (mm) in five study groups. Data are expressed as mean ±SEM.

	Orthodontic tooth movement (mm)	
Groups	Mean	SEM
Control	0.215	0.030
Sham (7 days)	0.211	0.028
Sham (28 days)	0.210	0.029
Cholestatic	0.401*	0.182
Cirrhotic	0.765†‡	0.451

*$P<0.01$ for cholestatic group compared to control and sham (7 days) and sham (28 days).
†$P<0.001$ for cirrhotic group compared to control and sham groups.
‡$P<0.001$ for cirrhotic group measurements compared to cholestatic group.

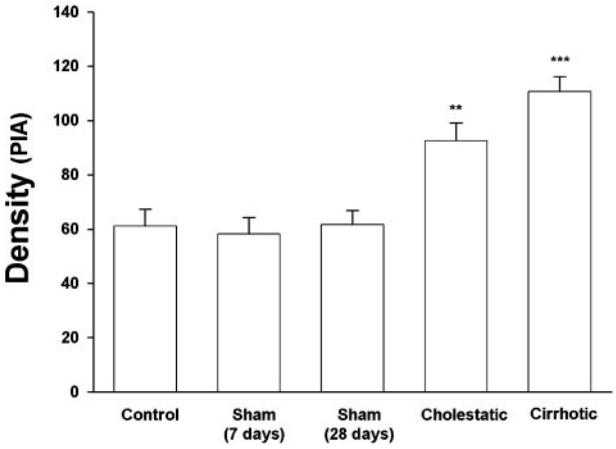

Figure 2 Bone density measurements (based on pixel intensity analysis; PIA) in the five study groups. Data are expressed as mean ±SEM. $^{**}P<0.01$ for cholestatic and $^{***}P<0.001$ for cirrhotic groups' measurement compared to control and sham groups

Table 2 The bone density measurements (based on pixel intensity analysis; PIA) in five study groups. Data are expressed as mean ±SEM.

	Bone density measurements (PIA)	
Groups	Mean	SEM
Control	61.34	6.40
Sham (7 days)	58.66	5.60
Sham (28 days)	62.41	5.21
Cholestatic	93.34*	6.53
Cirrhotic	111.32†	5.46

*$P<0.01$ for cholestatic group compared to control and sham (7 days) and sham (28 days).
†$P<0.001$ for cirrhotic group compared to control and sham (7 days) and sham (28 days).

Discussion

In the present study, we demonstrated that tooth movement in the bile duct ligation (BDL) groups (either cholestatic or cirrhotic rats) was significantly higher than in control and sham-operated groups and was also significantly increased in cirrhotic rats compared to cholestatic ones. The difference between the amount of OTM in the sham groups compared to the control animals was not significant ($P>0.05$). Since there was no significant difference between the OTM in the control and sham groups, we can conclude that the operation itself does not affect OTM and cholestatic and cirrhotic conditions could enhance OTM in rats.

Previous studies have shown that different factors might influence the rate of OTM via various biomediators. It has been reported that intracellular second messengers (Ca^{2+}, cGMP and cAMP) play an important role in the differentiation of osteoclasts from monocytes in bone resorption during mechanical orthodontic force application.[20,21] Orthodontic mechanical stress may induce localized cells to synthesize prostaglandins, which stimulate osteoclastic bone resorption.[22,23]

In BDL rats, alkaline phosphatase (ALP) elevates and is obviously suppressed by prior administration of indomethacin. Prostaglandin is a possible ALP inducer in BDL rats, probably working by elevating the cAMP level.[24] It has been shown that, in cirrhosis, mean trabecular bone volume is significantly reduced and mean osteoclastic per surface length and total bone resorption surfaces are considerably increased.[8,9,11] The histological data in the present study revealed that more osteoclasts and bone resorption were seen in the cholestatic and cirrhotic groups compared to control and sham groups. However, it was not significant. This discrepancy in our results might be due the small number of animals in each experimental group in our study which warrants further studies with larger sample sizes and based on appropriate sample size calculations.

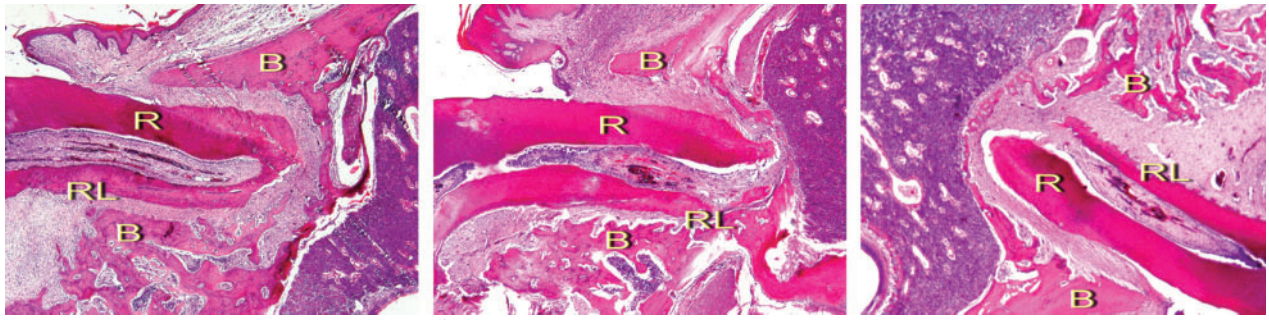

Figure 3 Histological sections of the first molar root on compression side from (a) unoperated control, (b) sham-operated, and (c) cirrhotic rats. B, bone; R, root; RL, resorption lacuna (×40)

Parathyroid hormone (PTH) increases serum calcium by direct and indirect vitamin D-mediated effects. Vitamin D_3 is important in maintaining the integrity of bone collagen.[25] It has also been shown that the rate of synthesis of soft tissue mature collagen decreases with calcium and vitamin D_3 deficiency. Therefore, a decreased synthesis of vitamin D may partly be responsible for the increase of OTM. It is established that vitamin $25(OH)D_3$ decreases in bile duct ligated cirrhotic rats.[4,5] Lower levels of $25(OH)D_3$ in the BDL rats could have resulted from vitamin D malabsorption caused by intraluminal bile salt deficiency,[26] inhibition of hepatic vitamin D25-hydroxylase by bile acid accumulation in BDL rats,[27] increased urinary clearance of vitamin D,[28] and lower levels of albumin and vitamin D binding protein in the BDL rats as previously reported.[29] The elevation of total opioid activity in BDL rats[30,31] also can contribute to changes of bone metabolism. Human osteoblast-like cells, MG-63, present immunohistochemical reactivity of mu, delta and kappa; three main specific cell surface opioid receptors.[32,33] High concentrations of morphine inhibit the $1,25(OH)_2$ D_3-induced osteocalcin secretion.[34] The role of the opioid system on OTM in BDL rats is established.[11] Taken together and according to the results of the present study, more detailed studies are clearly needed for demonstrating the exact mechanisms by which the OTM is increased in biliary cirrhotic condition.

Previous studies have provided evidence in favour of nitric oxide (NO) overproduction in various tissues and systems in animal models of cholestasis.[35–37] It is suggested that orthodontic forces may elevate NO production from periodontal ligament fibroblasts, which then activates guanylyl cyclase in periodontal ligament fibroblasts, leading to an increased level of cGMP.[38,39] NO administration also increases the biosynthesis of prostaglandins by cGMP.[40] cGMP in cell cytoplasm raises lysosome membrane permeability, leading to exocytosis of lysosome content resulting in resorption of organic and mineral element of bone. NO may diffuse to the alveolar bone and influence the function of osteoclastic differentiation.[41] Thus NO plays a role in bone resorption and formation and facilitates these phenomena during force application. It has been reported that NO increases OTM in rats.[42–44]

Other factors may be implicated in generation of increased OTM in cirrhosis and cholestasis. Reduced formation of IGF-1, an important bone growth factor,[45] decreased serum osteocalcin level,[46] accumulation of toxic substances that impair the function of the osteoblasts,[47] were found in cirrhotic rats. These factors may be partly responsible for the increase of OTM. Thus the present study shows that OTM increases in BDL rats and bone density decreases in cholestatic and cirrhotic groups. This suggests that osteopenia and impairment of bone remodelling in BDL subjects might

Table 3 The summed amount of linear surface resorption (μm) and the summed amount of the maximum depth (μm) of the resorption per lacuna on mesial and distal surfaces of the mesial root multiplied by 100 and the number of osteoclasts in the lamina dura of mesial and distal surfaces of the mesial root in control (unoperated), sham-operated (7 or 28 days), cholestatic (7 days) and cirrhotic (28 days) rats. There was no significant alteration in the variables measured among the groups. Each group consisted of eight animals.

		Mesial		Distal	
		Mean	Standard deviation	Mean	Standard deviation
Control	Osteoclasts	2.40	0.89	1.60	0.54
	Surface	5.39	6.81	4.05	5.70
	Depth	1.42	1.38	1.200	1.81
Sham (7 days)	Osteoclasts	2.20	1.09	1.80	0.83
	Surface	5.40	6.27	4.13	5.94
	Depth	1.40	1.66	1.18	1.93
Sham (28 days)	Osteoclasts	2.20	0.83	1.80	0.83
	Surface	5.38	6.81	4.09	4.39
	Depth	1.41	1.342	1.19	1.13
Cholestatic	Osteoclasts	2.80	0.83	3.50	1.29
	Surface	7.76	7.41	5.40	5.00
	Depth	1.91	1.76	1.70	1.54
Cirrhotic	Osteoclasts	4.00	0.81	3.50	1.29
	Surface	9.23	12.50	7.74	5.88
	Depth	2.66	3.61	2.01	1.43

lead to an increased rate of tooth movement and decreased bone density.

Conclusion

Our study demonstrated that the OTM could be enhanced in either cholestatic or biliary cirrhotic rats. We also showed that bone density was significantly decreased in the BDL (cirrhotic and cholestatic) groups while bone resorption and osteoclasts were not significantly altered in such groups compared with control or sham animals. It is notable that although the animal model of biliary cirrhosis was used to examine the effect of this condition on OTM in rodents in the present study, assessment of this effect and the underlying mechanism in humans warrants more detailed studies in cirrhotic patients.

Contributors

Dr Mohsen Shirazi supervised the study. Dr Pouria Motahhary was responsible for laboratory procedure. Dr Aida Ameri was responsible for appliance adjustment for orthodontic tooth movement. Dr Hamed Shafaroodi was responsible for surgical procedure. Dr Mehdi Ghasemi was responsible for technical support and data interpretation; drafting, critical revision, and providing the final revised article. Tawny Saleh was responsible for data interpretation, laboratory procedure, and providing the revised manuscript. Dr A. R. Dehpour is the guarantor.

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