Alcohol Induced Osteopenia Can Cause Accelerated Orthodontic Tooth Movement in Male Wistar Rats

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Abstract

Background and Aim: Considering the effect of alcohol on bones, consuming alcohol may have some consequences on orthodontic tooth movement. The present study was aimed to evaluate the effect of alcohol consumption on bone density and orthodontic tooth movement in rats.

Materials and Methods: In this experimental study, thirty rats were divided into three groups and underwent 3 weeks of different injection regimen. Group A received no injection while rats in group B and C were infused with saline and a mixture of alcohol and saline respectively. Tooth movement at day 21 was measured by leaf gauge. Optical density was measured using a digital densitometer at the beginning and the end of the experiment around four lateral cephalometric landmarks. One-way analysis of variance (ANOVA) was used to determine the differences in tooth movement. Changes in bone density were analyzed using paired T-test after evaluation of interactions.

Results: Mean tooth movement in group C was $(0.4\pm 0.06 \text{ mm})$ was significantly higher than no injection $(0.26\pm 0.04 \text{ mm})$ and saline infusion $(0.29\pm 0.04 \text{ mm})$ groups (P=0.001). Significant decrease in bone density were observed in alcohol injected group in skull (P=0.005) and mandible(P=0.004) after three weeks of experiment.

Conclusion: Alcohol infusion could induce decreased bone density which might be a reason for faster tooth movement in alcohol-treated rats.

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Introduction

Alcohol consumption has increased in different societies particularly in young populations in recent decades. Although some deterministic effects of daily alcohol intake on different organs have been reported, considerable controversial concepts about the actions on bone were

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acknowledged. Increased bone resorption in patients with moderate to severe alcohol consumption seems to be a profound finding [1,2]. Some studies claimed that osteoporosis and increased risk of bone fractures may be the result of chronic alcohol administration [2,3]. Direct effects on function and transformation of the bone cells, [4] inhibition of osteoblast proliferation and impaired DNA synthesis [5] are the proposed mechanism of ethanol–induced osteoporosis.

Contrary to these findings, it has been stated that light to moderate consumption of alcohol has some positive effects with the U-shaped curve on bone mineral density and it is beneficial to the bone health of men and possibly to that of postmenopausal women [6,7]. Decreased bone remodeling may be the result of higher estrogen concentrations or decreased serum PTH [6].

Today many invasive and non-invasive ways of evaluation of bone mass have been developed. Dual-energy X-ray absorptiometry, quantitative computed tomography, magnetic resonance imaging, histologic assessment of biopsy are all proposed to be useful in the evaluation of bone quality. In addition, some studies declared that mandibular bone densitometry in intra-oral radiographs would be an effective way of detecting osteoporosis [8,9]

In orthodontics treatments, teeth move within the bone so orthodontic tooth movement (OTM) is affected by endogenous and exogenous factors that interact with mandibular bone. With regard to the ethanol actions on bone, chronic alcoholism may exert some effects on OTM.

To our knowledge, no study has assessed the impact of alcohol intakes on orthodontic tooth movement, therefore the objectives of the present study were to evaluate the effect of chronic alcohol consumption on bone density in rat models and to determine whether this change would affect the orthodontic tooth movement.

Materials and Methods

In the present experiment, thirty male Wistar rats with the initial weight of 220-250 gm were maintained under controlled conditions of the constant temperature of 24-25°C and humidity of $55\pm5\%$ with an artificial 12-hour light-dark cycle and were fed with powdered food and water. All the experiments were in accordance with US National Institute of Health guidelines (publication 85-23 revised 1985) [10].

After weighing the rats, they were anesthetized by intraperitoneal injection of 0.9mg/kg of xylazine and 70mg/kg of ketamine. The technique of spring anchoring and applied force was similar to previous studies [11-15]. A 9-mm NiTi closed-coil spring (Hiek, 0.006×0.022 in, 3M Unitek, Monrovia, California, USA) was tied between the left maxillary first molars and central incisors using a 0.010" ligature wire to exert a force of 60 N. The ligature was fixed in a shallow groove which was prepared in gingival one-third of the incisor (Figure 1) with a light cure composite (Transbond XT, 3M Unitek, Monrovia, California, USA).

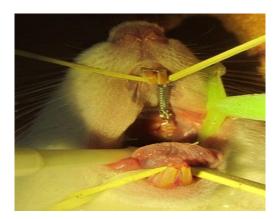


Figure 1. Intra-oral view of the tooth movement device used in the present experiment.

Rats randomly assigned into three groups and submitted to 3 weeks of injections as follows:

- Group A received no injection.

- Group B was treated with 0.2 ml of the saline injection per day.

- Group C received an injection of a mixture of 0.2 mlethanol 96 % and 0.2ml saline per day.

Lateral cephalometric radiographs were obtained on Kodak (no.2 size) E-speed (Eastman Kodak, Rochester, NY) at 70 kV and 8 mA and exposure time of 0.3 seconds on days 1 and 21. The focus–film distance was controlled using a box in which rats' necks were fixed. Radiographs were processed by an automatic film processor (Velopex Extrax, Medivance, London, UK) and a digital densitometer (Tobias TBX, Ivyland, PA, USA) was used for optical density measurements within 1 mm of four anatomical landmarks (Figure 2) including:

1-BU point which located on the maxillary alveolar bone between the jaw bone and the lingual surface of the upper incisors.



Figure 2. Four anatomical landmarks which used to measure optical density

2- MU point which positioned on the hard palate at the intersection between the maxillary bone and the mesial surface of the upper first molar.

3-PO point which situated on the skull in the most posterior part of the cranium.

4- K Point was placed on the mandibular bone at the intersection between the Go-Mn (Gonion to Menton) line and the perpendicular to the Go-Mn (Gonion to Menton) line through GN (Gnathion) [8].

At the end of the experiment, rats were weighed again. The distance between the first and second upper left molars, which indicates the amount of tooth movement was measured using a feeler gauge (Mitutoyo co, Kawasaki-shi, Japan).

One way analysis of variance (ANOVA)was used to determine the differences in tooth movement followed by Tukey post hoc test for multiple comparisons utilizing SPSS software version 20 (SPSS). Collected data from density measurements were analyzed using paired t-test after evaluation of interactions.

Results

Descriptive statistics result of tooth movements in each group after the injections are presented in Table 1. The highest amount of OTM was observed in group C (P-value=0.001) whereas group A was the lowest. However, Post hoc test revealed

no statistical differences between group A and B (Table 1).

Results of initial and final optical density

measurement of each group are presented in Table 2. Paired t-test showed significant differences between initial and final bone density in PO point (P=0.005) and Kpoint (P=0.004) in alcohol group only.

Discussion

The present study aimed to investigate the effect of alcohol consumption on bone density and its potential effect on the orthodontic tooth movement. All of the rats in this experiment showed some weight loss which might be due to the impaired oral function in presence of intra-oral orthodontic appliance and shaving of lower teeth. Although the optical density has not significantly increased in saline and no medication groups, the body weight loss may be a reason for this insignificant increase of optical bone density in these experimental groups.

In the group C that experienced alcohol injection, the OTM was increased compared to the group A which received no injection. In order to assess whether the alcohol induced OTM is related to bone density, optical densitometric evaluation was performed. Collected data showed a significant decrease in mandibular bone density of alcohol treated group compared to the other groups. Findings of the present study are in agreement with those of Talaeipour et al, [8] who reported that radiograph of rat's mandible can be utilized for bone density evaluation since it is more susceptible to density changes compared to other cranial bones such as hard palate, skull and alveolar bone [8]. Horner et al, [9] also have shown that bone densitometry of oral radiographs of mandibular bone is a reliable approach for detecting osteoporosis.

Several studies that examined the effect of alcohol intake on bones have discovered alcohol reduces cortical and cancellous bone mass in rats [16,17] which maintain throughout the life. Dual-photon X-ray absorptiometry in rats also has shown that alcohol consumption lessens mineral density of both trabecular and cortical bones [18]. Additionally, alcohol consumption can disturb the bone metabolism and it decreases bone mechanical properties [19].

In the present study, the administration of intraperitoneal alcohol caused a significant loss in

Groups	Mean	\mathbf{SD}^{\dagger}	Min	Max
Groups A	0.26	0.04	0.2	0.33
Groups B	0.29	0.04	0.25	0.38
Groups C	0.4	0.06	0.33	0.55

Table 1. Descriptive statistics of tooth movement (mm) in the experimental groups

 \dagger = Standard Deviation

 Table 2. Results of the optical bone density measurements in 3 experimental groups. Data are represented as mean±standard deviation. BU=alveolar bone, MU=hard palate, PO=skull, K=mandible

-	Initial Bone Optical Density				Final Bone Optical Density			
Groups	BU	MU	PO	K	BU	MU	PO	K
Group A	1.74 ± 0.27	1.48 ± 0.17	1.83±0.17	1.87 ± 0.17	1.91±0.17	1.53±0.13	1.92 ± 017	1.96±0.18
Group B	1.81 ± 0.32	1.51 ± 0.15	1.92±0.22	1.9±0.12	$1.90 \pm .16$	$1.54{\pm}0.1$	1.95±0.11	2.01±0.07
Group C	1.55 ± 0.31	$1.28{\pm}0.2$	1.6 ± 0.28	1.62±0.29	1.80 ± 0.26	1.54±0.23	1.87 ± 0.26	1.90 ± 0.28

body weight which was similar to Iwaniecet al, [20] study. This could be contributed to the systemic bone loss due to the alcohol-induced systemic and local inflammations [21,22]. Histomorphometric studies on cortical and cancellous bone in rats revealed that unlike oral administration of alcohol, intraperitoneal injection of alcohol would result in suppressed bone formation and decreased mRNA levels in distal femur.²¹Increased bone marrow adiposity and decreased fat mass after alcohol intake also could be related to lower bone density [23].

Several studies in human have also shown that alcohol has some effects on bone metabolism. alcohol consumption might affect bone density through several mechanisms; alcohol seems to decrease osteoblast activity and differentiation [24,25] and increase osteocyte apoptosis [26]. Considering findings of the present study and recognizing the effects of alcohol on bone density in human, alcohol consumption may exert some influence on orthodontic tooth movement in the human which could be evaluated in future studies.

Conclusion

Considering the findings of the present study, it can be concluded that increased tooth movement in

the alcohol-treated group may be related to decreased bone density which needs to be addressed during orthodontic treatments. Alcohol-induced osteoporosis has a positive impact on the rate of orthodontic tooth movement however the negative impacts on the bone density should also be considered.

References

1. Marrone JA, Maddalozzo GF, Branscum AJ, Hardin K, Cialdella-Kam L, Philbrick KA, et al. Moderate alcohol intake lowers biochemical markers of bone turnover in postmenopausal women. Menopause. 2012 Sep; 19(9):974-9.

2. Johnson TL, Gaddini G, Branscum AJ, Olson DA, Caroline-Westerlind K, Turner RT, et al. Effects of chronic heavy alcohol consumption and endurance exercise on cancellous and cortical bone microarchitecture in adult male rats. Alcohol Clin Exp Res. 2014 May;38(5):1365-72.

3. GaddiniGW, Grant KA, Woodall A, Stull C, Maddalozzo GF, Zhang B, et al. Twelve months of voluntary heavy alcohol consumption in male rhesus macaques suppresses intracortical bone remodeling. Bone. 2015 Feb;71:227-36.

4. Suh KT, Kim SW, Roh HL, Youn MS, Jung JS. Decreased osteogenic differentiation of mesencymal stem cells in alcohol-induced osteonecrosis. Clin Orthop Relat Res. 2005 Feb; (431):220-5.

5. Klein RF, Fausti KA, Carlos AS. Ethanol inhibits human osteoblastic cell proliferation. Alcohol Clin Exp Res. 1996 May;20(3):572-8.

6. Mikosch P. Alcohol and bone. Wien Med Wochenschr. 2014 Jan;164(1-2):15-24.

7. Tucker KL1, Jugdaohsingh R, Powell JJ, Qiao N, Hannan MT, Sripanyakorn S, et al. Effects of beer, wine, and liquor intakes on bone mineral density in older men and women. Am J Clin Nutr. 2009 Apr;89(4):1188-96.

8. Talaeipour AR, Shirazi M, Kheirandish Y, Delrobaie A, Jafari F, Dehpour AR. Densitometric evaluation of skull and jaw bones after administration of thyroid hormones in rats. Dentomaxillofac Radiol. 2005 Nov;34(6):332-6.

9. Horner K, Devlin H, Alsop CW, Hodgkinson IM, Adams JE. Mandibular bone mineral density as a predictor of skeletal osteoporosis. Br J Radiol. 1996 Nov;69(827):1019-25.

10. Guide for the care and use of laboratory animals. Available at: https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf/Accessed March 10, 2016.

11. Akhoundi MS, Dehpour AR, Rashidpour M, Alaeddini M, Kharazifard MJ, Noroozi H. The effect of morphine on orthodontic tooth movement in rats. Aust Orthod J. 2010 Nov;26(2):113-8.

12. Rashidpour M, Ahmad Akhoundi MS, Nik TH, Dehpour A, Alaeddini M, Javadi E, et al. Effect of Tramadol (μ -opioid receptor agonist) on orthodontic tooth movements in a rat model. J Dent (Tehran). 2012 Spring;9(2):83-9.

13. Mir Hashemi AH, Afshari M, Alaeddini M, Etemad-Moghadam S, Dehpour A, Sheikhzade S, et al. Effect of atorvastatin on orthodontic tooth movement in male Wistar rats. J Dent (Tehran). 2013 Nov;10(6):532-9.

14. Mirhashemi AH, Ahmad Akhoundi MS, Sheikhzadeh S, Momeni N, Dehpour A, Alaeddini M, et al. Effect of fluoxetine consumption on orthodontic tooth movement in rats. J Dent (Tehran). 2015 Dec;12(12):882-9.

15. Ahmad Akhoundi MS, Ghazanfari R, Etemad-Moghadam S, Alaeddini M, Khorshidian A, Rabbani S, et al. Effect of supplementary zinc

on orthodontic tooth movement in a rat model. Dental Press J Orthod. 2016 Mar-Apr; 21(2):45-50. 16. Sampson HW, Hebert VA, Booe HL, Champney TH. Effect of alcohol consumption on adult and aged bone: composition, morphology, and hormone levels of a rat animal model. Alcohol Clin Exp Res. 1998 Nov;22(8):1746-53.

17. Sampson HW, Perks N, Champney TH, DeFee B 2nd. Alcohol consumption inhibits bone growth and development in young actively growing rats. Alcohol Clin Exp Res. 1996 Nov;20(8):1375-84.

18. Nishiguchi S, Shiomi S, Tamori A, Habu D, Takeda T, Tanaka T,et al. Effect of ethanol on bone mineral density of rats evaluated by dual-photon X-ray absorptiometry. J Bone Miner Metab. 2000;18(6):317-20.

19. Nyquist F, Halvorsen V, Madsen JE, Nordsletten L, Obrant KJ. Ethanol and its effects on fracture healing and bone mass in male rats. Acta Orthop Scand. 1999 Apr;70(2):212-6.

20. Iwaniec UT, Turner RT. Intraperitoneal injection of ethanol results in drastic changes in bone metabolism not observed when ethanol is administered by oral gavage. Alcohol Clin Exp Res. 2013 Aug;37(8):1271-7.

21. Desimone DP, Greene VS, Hannon KS, Turner RT, Bell NH. Prostaglandin E2 administered by subcutaneous pellets causes local inflammation and systemic bone loss: a model for inflammation-induced bone disease. J Bone Miner Res. 1993 May; 8(5):625-34.

22. Boé DM, Richens TR, Horstmann SA, Burnham EL, Janssen WJ, Henson PM, et al. Acute and chronic alcohol exposure impair the phagocytosis of apoptotic cells and enhance the pulmonary inflammatory response. Alcohol Clin Exp Res. 2010 Oct;34(10):1723-32.

23. Maddalozzo GF, Turner RT, Edwards CH, Howe KS, WidrickJJ, Rosen CJ, et al. Alcohol alters whole body composition, inhibits bone formation, and increases bone marrow adiposity in rats. Osteoporos Int. 2009 Sep;20(9):1529-38.

24. Chavassieux P, Serre CM, Vergnaud P, Delmas PD, Meunier PJ. In vitro evaluation of dose-effects of ethanol on human osteoblastic cells. Bone Miner. 1993 Aug;22(2):95-103.

25. Sun T, Deng Z, Zhu Y. Effects of insulin-like growth factor 1 on inhibition of osteoblastic

proliferation and function by ethanol. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi. 2007 Dec;21(12):1338-41.

26. Maurel DB, Jaffre C, Rochefort GY, Aveline

PC, Boisseau N, Uzbekov R, et al. Low bone accrual is associated with osteocyte apoptosis in alcohol-induced osteopenia. Bone. 2011 Sep; 49 (3):543-52.