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The effect of sialoprotein local injection on dental anchorages for orthodontic tooth retraction in dogs

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Abstract

Background and objective: Bone sialoprotein is a mineralized tissue-specific protein expressed in differentiated osteoblasts that appear to function in the initial mineralization of bone. The aim of this study was to investigate the effect of local bone sialoprotein on increasing the rate of anchorage during orthodontic tooth movement.

Methods: This study used 14 dogs wearing orthodontic appliance for 40 days. They were divided equally into two groups; experimental group that injected with 0.1 µg /10µL sialoprotein around the anchoring tooth in three different time intervals, while the other control group received normal saline injection. Different clinical measurements including loss of anchorage, space closure, rotation, tipping and extrusion were done on the stone casts of each dog before and after tooth retraction.

Results: Clinical measurements revealed a highly significant difference between experimental and control group regarding loss of anchorage and space closure. The sialoprotein injected group showed less loss of anchorage than control group and the space closure was higher in experimental group than in the control group.

Conclusion: This study showed that the local injection of sialoiprotein reduced movement of the anchoring tooth during orthodontic treatment and provided higher stability for the anchoring tooth.

Keywords: Sialoprotein, Mini-implant, Dental Anchorage.

Introduction

Anchorage loss is a reciprocal reaction that could obstruct the success of orthodontic treatment by complicating the antero-posterior correction of the malocclusion and possibly detracting from facial esthetics.[1] Many attempts have been done to increase anchorage by studying the effects of different systemic or local application of medications or the intake of dietary supplements, such as vitamins,[2] minerals,[3] hormones,[4] proteins[5] and immunomodulators[6] on the rate of tooth movement during orthodontic treatment. Kohno et al[7] injected anti-vascular endothelial growth factor antibody into the buccal gingival groove of experimentally moved teeth in mice. Clinically there was reduction in the amount of tooth movement and in the relapse, and the histological section demonstrated a significant decrease in the number of osteoclasts. Liu et al[8] used Clodronate solution as a local injection into the subperiosteal area adjacent to the left upper molar of rats in a dose of 2.5, 10 and 40 mM which subjected to orthodontic movement with a standardized expansion spring. Clinically there was a significant and dose dependent reduction in tooth movement in the experimental rats. The local injection of epidermal growth factor in liposome in a dose of 2 ng/µl into the region of root furcation of the left first molar of Wister rats after elastic band insertion led to increase in the rate of osteoclast recruitment, producing faster bone resorption and tooth movement.[9] Bone sialoprotein (BSP) is a highly sulfated, phosphorylated, and glycosylated protein that is expressed almost in

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mineralized tissue, polyglutamic acid sequences.[10,11] Primarily BSP expressed in osteoblasts, osteoclasts and hypertrophic chondroblasts but it's also expressed in breast and lung cancers.[12,13] In a study done by Goldberg et al,[14] real-time PCR, western blot, enzyme analysis and mineral deposition were used to determine the potency of BSP in promoting mineralization. The over expression of bone sialoprotein increased the osteoblast-related gene expression, as well as, nodule formation and calcium incorporation by osteoblastic cells in culture. Similarly, the osteoblastic culture increase several markers of osteogenic differentiation when supplied with recombinant bone sialoprotien. Conversely, suppression of bone sialoprotein expression by small hairpin RNA-encoding plasmids inhibited expression of osteoblast markers and nodule formation. The deposition of BSP represents the first step of bone formation in ectopic transplantation systems in vivo.[15] Although its functional specificities are unknown, it has been proven that bone sialoprotein deficiency impairs bone growth and mineralization; concomitant with dramatically reduced bone formation, conversely its presence may induce bone formation.[16] All previous studies concerning the effect of BSP on bone homeostasis were done on cell culture.[11,14,15,17] This study aims to investigate the effect of local BSP supplementation on bone homeostasis in vivo, and to investigate the effect of series of this injection on increasing the rate of anchorage stability during orthodontic tooth movement.

Methods

No study was found in literature on sialoprotein local injection around anchoring tooth to calculate the sample proportion, while similar studies on dogs used 5-6 dogs as a maximum. Therefore, 14 dogs with age ranged between (16-17 months) were used in this study. They wear an orthodontic appliance to retract the 3rd incisor against the canine in order to close the space which already existed between these two teeth. The appliance consist of Ni-Titanium closed coil spring by banding both teeth through which rectangular straight stainless steel wire (17*25 mil) pass in two tubes to slide the 3rd incisor along this arch wire. A force of 150 g was applied and measured using pressure-tension gauge. The dogs were divided equally into two groups (seven dogs for each), an experimental group received 0.1 µg /10µL subperiosteal injection of sialoiprotien around the anchoring tooth (canine), while the remaining seven dogs were considered as control group which received normal saline injection in the same dose and site as in the experimental group. The local injection of both BSP and normal saline was given in three different time intervals (7th, 20th and 33rd day) following insertion of the appliance. To record the clinical measurements and changes that took place during the studying period, two impressions were taken for each dog; one impression was taken before appliance insertion and the other impression at day 40. Both impressions were poured with stone to form stone casts and the measurements were done on photographs of these casts using the auto CAD software (2012, 64bit).

Measuring the clinical changes:

*Loss of anchorage (L.O.A): The loss of anchorage was estimated by measuring the distance between two fixed points (the midpoint between the 1st incisors, and the midpoint of the distal side of canine cervical area) (Figure 1a). *Space closure (S.C): Space from the cervical area of the 3rd incisor (midpoint of its distal side) to the cervical area of the canine (midpoint of its distal side) was measured (Figure 1b). The absolute space closure was calculated by subtracting the loss of anchorage from the amount of closed space in order to get the pure space closure.

*Degree of rotation (D.O.R): From the occlusal view a straight line was drawn from the incisal tip through the cingulum of the 3rd incisor form an angle with the inter-palatal line, the value of this angle

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indicating the degree of rotation (Figure 1c).

*Degree of tipping (D.O.T): From the buccal view of the cast a straight line was drawn from the incisal tip of the 3rd incisor to the midpoint of the labial gingival margin formed an angle with the horizontal line connecting the deepest point of the gingival margins of 1st premolar, 2nd premolar and 3rd premolar (Figure 2a) which was measured before and after tooth retraction to calculate the amount of tipping.

*Extrusion: Extrusion was estimated by measuring the vertical distance of 3rd incisors tip in relation to a fixed line connecting the incisal edge of the 1st and 2nd incisors before and after orthodontic treatment (Figure 2b).

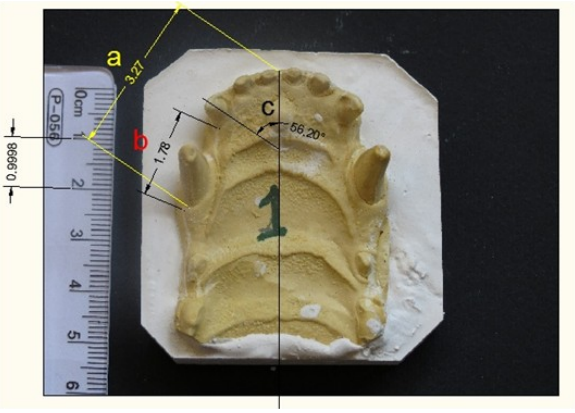

Figure 1: Oclussal view of the stone cast with clinical measurements.

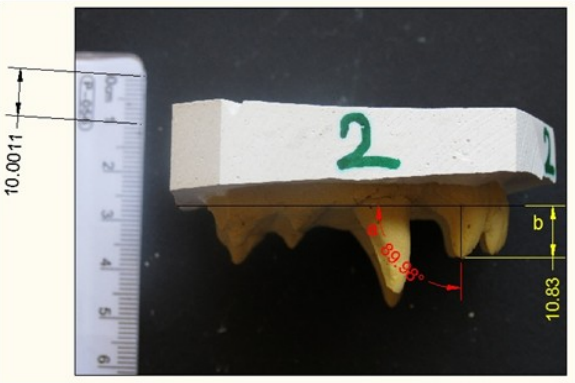

Figure 2: Buccal view of the stone cast with clinical measurements.

Statistical analysis:

Statistical analyses of the collected clinical measurements were performed with the Statistical Package for the Social Sciences (version 17.0). The two groups were compared by using independent-samples t-test.

Ethical permission:

The study was approved by the Scientific and Ethics Committee of the College of Dentistry, Hawler Medical University.

Results

The injection of bone sialoprotein around the anchoring tooth resulted in many clinical changes which were measured and recorded on the stone cast. As shown in Table 1 the statistical analysis of the clinical measurements revealed obvious differences between experimental and control group. Regarding loss of anchorage, there was a highly significant difference between saline injected group and sialoprotein injected group (P <0.001). The sialoprotein group demonstrated less loss of anchorage (0.234 mm) while the control group demonstrated (1.396 mm). Concerning space closure, there was also a highly significant difference between the two studied groups (P <0.001). The sialoprotein group revealed higher rate of space closure (1.814 mm) compared to the saline group (0.850 mm). About the degree of rotation, a significant difference was also recorded (P = 0.03). The saline group revealed a higher D.O.R of the 3rd incisor (2.013°), while the sialoprotein group showed less D.O.R (1.080°). There was no significant difference between the two groups (P = 0.113) in the degree of extrusion and the same for tipping which also revealed no significant difference (P = 0.465).

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Table 1: Independent-samples t-test of the clinical measurements.

Variables	Group	N	Mean	±SD	±SE of mean	p value
loss of anchorage (mm)	Saline (control)	7	1.396	0.241	0.091	< 0.001
	Sialoprotein (exp.)	7	0.234	0.078	0.030	
Space closure (mm)	Saline (control)	7	0.850	0.212	0.080	< 0.001
	Sialoprotein (exp.)	7	1.814	0.419	0.158	
Degree of rotation (degree)	Saline (control)	7	2.013	0.904	0.342	0.030
	Sialoprotein (exp.)	7	1.080	0.439	0.166	
Extrusion (mm)	Saline (control)	7	0.599	0.437	0.165	0.113
	Sialoprotein (exp.)	7	1.086	0.614	0.232	
Degree of tipping (degree)	Saline (control)	7	4.900	1.704	0.644	0.465
	Sialoprotein (exp.)	7	4.276	1.375	0.520	

Discussion

This study revealed that the subperiosteal injection of 0.1 µg /10µL of sialoprotein around the anchoring tooth in three different time intervals, (seven days following the beginning in orthodontic treatment, mid-time of the treatment and seven days before the end of the orthodontic treatment) decreases the rate of movement of the anchoring tooth and increases the rate of space closure while have no effect on the extrusion, intrusion or tipping of the retracted tooth. To our knowledge, there is no clinical study on the effect of bone sialoprotein on tooth movement. Similar results were recorded following the administration of different substances. Kohno et al[7] recorded similar results after the injection of anti-vascular endothelial growth factor antibody in the buccal groove of experimentally moved teeth in mice. The injection led clinically to reduction in the amount of tooth movement and histologically demonstrated a decrease in the number of osteoclasts. The obtained results of this study are similar to the results obtained by Liu et al[8] who injected Clodronate into the sub-periosteum area adjacent to orthodontically moved tooth. The clodronate injection caused a significant and dose dependent reduction in tooth movement in the rats. Madan et al[18] and Gonzales et al[19] studied the effect of human relaxin and fluoride intake systemically on orthodontic tooth movement in rats. They showed that both human relaxin and fluoride led to a decreased tooth movement compared with the control groups. On the other hand, the results of this study differ from the results obtained by Hashimoto et al[20] and Kobayashi et al[21] who studied the effect of local purified osteocalcin injection in three different doses (0.1, 1 and 10 µg) into the palatal bifurcation site of the 1st molar of rats which subjected to experimental tooth movement. The local injection of osteocalcin around the orthodontically moved tooth led to a marked acceleration of tooth movement especially at early experimental period than at the end of the experiment. Soma et al[22] and Siefi et al[23] recorded that the local and chronic application of prostaglandin significantly increases orthodontic movement of maxillary 1st molar in rats. There is considerable evidence accumulating that suggests an emerging “paradox” with respect to the biological functions of BSP in that, it has the capacity to participate in two major and opposing bone biology events, namely bone formation, i.e., an anabolic process, versus resorption, i.e., a catabolic process.[24] In vitro studies have suggested that BSP may function at several steps in

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bone modeling and remodeling. In addition to its ability to nucleate hydroxyapatite crystal formation and promote mineralization,[25,26] BSP was reported to increase osteoclastogenesis and bone resorption.[27,28] Furthermore, BSP expression is coincident with de novo bone formation[29] and ectopic calcification.[30] The butyric acid was used for BSP transcription regulation and revealed that the butyric acid increases the BSP transcription and in turn induces osteoblast activity and bone formation.[31,32] The osteoinductive effect of BSP was also reported after coating femoral implants which inserted into bones subjected to mechanical loading,[33] and the pre-coating of the rough implant surface with BSP enhanced the osteoinductive effect much more than did collagen precoating.[34] Malaval et al[16] reported that BSP deficient mice are viable and breed normally, but their weight and size are lower than normal mice. They suggested that BSP deficiency impaired bone growth and mineralization, concomitant with dramatically reduced bone formation. The clinical results reported in this study may be attributed to a higher rate of bone formation as a result of local application of BSP, since the previous in vitro data suggested that BSP initiate hydroxyapatite crystal formation in bone matrix[9,10,16,35] and the BSP expression is increased in osteoblasts subjected to mechanical stimulation.[36]

Conclusion

The localized use of BSP could be a beneficial therapeutic adjunct for orthodontic treatment. The local injection of sialoiprotein reduced the movement of the anchoring tooth and provides greater stability during orthodontic treatment. Further histological and biochemical studies are needed to confirm the increase in bone formation around the anchoring tooth.

Conflicts of interest

The authors report no conflicts of interest.

References

1. Geron S, Shpack N, Kandos S, Davidovitch M, Vardimon AD. Anchorage loss-a multifactorial response. Angle Orthod 2003; 73:730-7.
2. Esenlik E, Naziroğlu M, Açikalin C , Övey IS. Vitamin E supplementation modulates gingival crevicular fluid lipid peroxidation and antioxidant levels in patients with orthodontic tooth movement. Cell Biochem Funct 2012; 30:376-81.
3. Bartzela T, Türp JC, Motschall E, Maltha JC. Medication effects on the rate of orthodontic tooth movement: a systematic literature review. Am J Orthod Dentofacial Orthop 2009; 135: 16-26.
4. Ong CKL, Joseph BK, Waters MJ, Symons AL. Growth hormone receptor and IGF-I receptor immunoreactivity during orthodontic tooth movement in the prednisolone-treated rat. Angle Orthod 2001; 71:486-93.
5. Kim JY, Kim BI, Jue SS, Park JH , Shin JW. Localization of osteopontin and osterix in periodontal tissue during orthodontic tooth movement in rats. Angle Orthod 2012; 82:107-14.
6. Gameiro GH, Pereira-Neto JS, Magnani MB, Nouer DF. The influence of drugs and systemic factors on orthodontic tooth movement. J Clin Orthod 2007; 41:73-8.
7. Kohno S, Kaku M, Kawata T, Fujita T, Tsutsui K , Ohtani J, et al. Neutralizing effects of an anti-vascular endothelial growth factor antibody on tooth movement. Angle Orthod 2005; 75: 797-804.
8. Liu L, Igarashi K, Haruyama N, Saeki S, Shinoda H, Mitani H. Effects of local administration of clodoronate on orthodontic tooth movement and root resorption in rats. Eur J Orthod 2004; 26:469-73.
9. Saddi KRGC, Alves GD, Paulino TP, Ciancaglini P, Alves JB. Epidermal growth factor in liposomes may enhance osteoclast recruitment during tooth movement in rats. Angle Orthod 2008; 78:604-9.
10. Chaplet M, Detry C, Deroanne C, Fisher LW, Castronovo V, Bellahcene A. Zoledronic acid up-regulates bone sialoprotein expression in osteoblastic cells through Rho GTPases inhibition. Biochem J 2004; 384:591-8.
11. Wang S, Sasaki Y, Ogata Y. Calcium hydroxide regulates bone sialoprotein gene transcription in human osteoblast-like saos 2 cells. J Oral Sci 2011; 53:77-86.
12. Bellahcène A, Castronovo V. Expression of bone matrix proteins in human breast cancer: potential roles in microcalcification formation and in the genesis of bone metastases. Bull Cancer 1997; 84:17-24.
13. Zhang L, Hou X, Lu S, Rao H, Hou J, Luo R, et al. Predictive significance of bone sialoprotein and osteopontin for bone metastases in resected Chinese non-small-cell lung cancer patients:

a large cohort retrospective study. Lung Cancer 2010; 67:114-9.
14. Goldberg HA, Baht G, Gordon J, Pitelka V, Chen H, Holdsworth D, et al. Bone Repair Mediated by Bone Sialoprotein. Eur Cell Mater 2008; 16:47.
15. Riminucci M, Bianco P. Building bone tissue: matrices and scaffolds in physiology and biotechnology. Braz J Med Biol Res 2003; 36:1027-36.
16. Malaval L, Wade-Gueye NM, Boudiffa M, Fei J, Zirngibl R, Chen F, et al. Bone sialoprotein plays a functional role in bone formation and osteoclastogenesis. J Exp Med 2008; 205:1145-53.
17. Hilbig H, Kirsten M, Rupietta R, Graf HL, Thalhammer S, Strasser S, et al. Implant surface coatings with sialoprotein, collagen, and fibronectin and their effects on cells derived from human maxillary bone. Eur J Med Res 2007; 12:6-12.
18. Madan MS, Liu ZJ, Gu GM, King GJ. Effect of human relaxin on orthodontic tooth movement and periodontal ligament in rats. Am J Orthod Dentofacial Orthop 2007; 131:8e1-10.
19. Gonzales C, Hotokezaka H, Karadeniz EI, Miyazaki T, Kobayashi E. Effects of fluoride intake on orthodontic tooth movement and orthodontically induced root resorption. Am J Orthod Dentofacial Orthop 2011; 139:196-205.
20. Hashimoto F, Kobayashi Y, Mataki S, Kobayashi K, Kato Y, Sakai H. Administration of osteocalcin accelerates orthodontic tooth movement induced by closed coil spring in rats. Eur J orthod 2001; 23:535-45.
21. Kobayashi Y, Takagi H, Sakai H, Hashimoto F, Mataki S, Kobayashi K, et al. Effect of local administration of osteoclacin on experimental tooth movement. Angle Orthod 1998; 68(3): 259-66.
22. Soma S, Matsumoto S, Higuchi Y, Takano-Yamamoto T, Yamashita K , Kurisu K, et al. Local and chronic application PTH accelerates tooth movement in rarts. J Dent Res 2000; 79:1717-24.
23. Seifi M, Eslami B , Saffar AS. The effect of prostaglandin E2 and calcium gluconate on orthodontic tooth movement and root resorption in rats. Eur J Orthod 2003; 25:199-204.
24. Curtin P, McHugh KP, Zhou H, Flückiger R, Goldhaber P , Oppenheim FG, et al. Modulation of Bone Resorption by Phosphorylation State of Bone Sialoprotein. J Biochem 2009; 48:6876-86.
25. Tye CE, Rattray KR, Warner KJ, Gordon JA, Sodek J, Hunter GK, et al. Delineation of the hydroxyapatite nucleating domains of bone sialoprotein. J Biol Chem 2003; 278:7949-55.
26. Wang D, Christensen K, Chawla K, Xiao G, Krebsbach PH , Franceschi RT. Isolation and characterization of MC3T3- E1 preosteoblast subclones with distinct in vitro and in vivo differentiation/mineralization potential. J Bone Miner Res 1999; 14:893-903.
27. Valverde P, Tu Q, Chen J. BSP and RANKL induce osteoclastogenesis and bone resorption synergistically. J Bone Miner Res 2005; 20: 1669-79.
28. Razzouk S, Brunn JC, Qin C, Tye CE, Goldberg HA, Butler WT. Osteopontin posttranslational modifications, possibly phosphorylation, are required for in vitro bone resorption but not osteoclast adhesion. Bone 2002; 30:40-7.
29. Chen JK, Shapiro HS, Wrana JL, Reimers S, Heersche JN , Sodek J. Localization of bone sialoprotein (BSP) expression to sites of mineralized tissue formation in fetal rat tissues by in situ hybridization. Matrix 1991; 11:133-43.
30. Contri MB, Boraldi F, Taparelli F, De Paepe A, Ronchetti IP. Matrix proteins with high affinity for calcium ions are associated with mineralization within the elastic fibers of pseudoxanthoma elasticum dermis. Am J Pathol 1996; 148:569-77.
31. Yang L, Li Z, Li X, Wang Z, Wang S, Sasaki Y, et al. Butyric acid stimulates bone sialoiprotien gene transcription. J Oral Sci 2010; 52:231-7.
32. Sasaki Y, Wang S, Ogata Y. Transcriptional regulation of bone sialoprotein gene by CO_2 laser irradiation. J Oral Sci 2011; 53:51-9.
33. O'Toole GC, Salih E, Gallagher C, FitzPatrick D, O'Higgins N, O'Rourke SK. Bone sialoprotein-coated femoral implants are osteoinductive but mechanically compromised. J Orthop Res 2004; 22(3):641-6.
34. Graf HL, Stoeva S, Armbruster FP, Neuhaus J, Hilbig H. Effect of bone sialoprotein and collagen coating on cell attachment to TICER and pure titanium implant surfaces. Int J Oral Maxillofac Surg 2008; 37(7):634-40.
35. Hoshi K, Ozawa H. Matrix Vesicle Calcification in Bones of Adult Rats. Calcif Tissue Int 2000; 66:430-4.
36. Caravlho RS, Bumann A, Schaffer JL, Grestenfeld LC. Predominant integrin ligands expressed by osteoblasts show preferential regulation in response to both cell adhesion and mechanical perturbation. J Cell Biochem 2002; 84:497-508.