Saudi Dental Journal (2022) 34, 282–287

King Saud University

Saudi Dental Journal

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ORIGINAL ARTICLE

Utility of injectable bisphosphonates in enhancing orthodontic retention in a goat model: A split-mouth study

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Received 24 March 2022; revised 25 March 2022; accepted 26 March 2022
Available online 30 March 2022

KEYWORDS

Bisphosphonates;
Orthodontic tooth movement;
Relapse;
Periodontal ligament;
Root resorption

Abstract *Objective:* The aim of this study was to evaluate the utility of bisphosphonates in reducing relapse after orthodontic tooth movement when injected intra-periodontally in a goat model.

Methods: Right and left second incisors of four goats were extracted and the first and third incisors were approximated orthodontically and retained. A bisphosphonate gel was injected into the mesial and distal periodontal spaces of first and third incisors on one side, while the first and third incisors of the other side received normal saline as a control. After 12 weeks of retention, the orthodontic appliance was removed and teeth were relieved from any active force. Following 6 weeks from appliance removal, millimetric measurements were performed to calculate the amount of relapse of orthodontically moved incisors. Histological and microcomputed tomographic examination was performed to assess the periodontal space and surrounding alveolar bone of the study and control incisors.

Results: The millimetric measurements and microcomputed tomographic scanning revealed that the orthodontically moved incisors in the side of bisphosphonate injection had significantly less relapse ($p \leq 0.05$) and significantly narrower periodontal ligament width ($p \leq 0.05$) than in the control side, respectively. Histologically, newly formed bone projecting into the periodontal ligament was observed in the side of bisphosphonate injection with newly formed cementum overlying areas of resorbed cementum.

Conclusion: The results suggest that bisphosphonate injection has the potential to enhance post-orthodontic stability and repair of root resorption following orthodontic treatment.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sdentj.2022.03.007
1013-9052

1. Introduction

The retention of tooth position following orthodontic treatment is a major concern in orthodontics (Littlewood et al., 2017). Several methods of orthodontic retention are in clinical use today; these are mainly mechanical in nature, such as the use of removable Hawley retainers, fixed lingual retainers, and transparent removable retainers (Rinchuse et al., 2007). However, these methods are not usually preferred by patients due to the need for continued compliance after the conclusion of orthodontic treatment and the necessity to perform extra hygiene measures to prevent deterioration of oral health (Sadowsky and Sakols, 1982; Schütz-Fransson et al., 1998). Non-mechanical adjunctive procedures, such as interproximal reduction, overcorrection of rotated teeth, and supracrestal fiberotomy were also reported to enhance retention following orthodontic treatment (Melrose and Millett, 1998).

Establishing a biological method to retain teeth following orthodontic treatment continues to be a high demand in orthodontics. In general, biological agents utilized to increase retention can either block bone resorption or enhance bone formation (Riedel and Brandt, 1976). Manipulating bone remodeling to minimize post-orthodontic relapse with topical application of different materials, such as simvastatin, prostaglandin E2, recombinant human bone morphogenetic protein-2, and bisphosphonates were reported (Han et al., 2010; Okamoto et al., 2009; Hassan et al., 2010; Adachi et al., 1994).

Bisphosphonates are widely used for treatment of skeletal disorders, such as osteoporosis (Liberman et al., 1995). They attach to bone because of their phosphate-carbon-phosphate core structure and they can enhance bone's resistance to enzymatic degradation (Grey and Reid, 2006). They bind to hydroxyapatite crystals and inhibit their breakdown, thereby reducing bone resorption (Ganapathy et al., 2012). Beside their inhibitory effect on osteoclasts, bisphosphonates were reported to directly induce osteoblasts to enhance bone formation (Giuliani et al, 1998). These fundamental properties of bisphosphonates led to the suggestion that they may be used to prevent orthodontic relapse (Adachi et al., 1994). Alendronate is a type of bisphosphonate (4-Amino-I-hydroxybutylidene-1, 1-biphosphonic acid) that inhibit bone resorption and has been used in patients with Paget's disease (Pedrazzoni et al., 1989). In addition, alendronate has been used in the field of periodontics with reported positive effect in treatment of periodontal intraosseous defects (Dutra et al., 2017). The systemic use of alendronate inhibited the orthodontic tooth movement in rats (Kaipatur et al., 2013). In another study, Adachi et al. (1994) used risedronate (a bisphosphonate) topically by injecting the solution into the sub-periosteal region to enhance orthodontic retention.

In a search for non-invasive biological modality to retain teeth after treatment and building on promising previous reports (Dutra et al., 2017; Kaipatur et al., 2013; Adachi et al., 1994), the aim of this study was to evaluate the utility of bisphosphonates in reducing relapse of orthodontically moved teeth when injected intra-periodontally in goats.

2. Material and methods

The study was conducted in the Experimental Surgery and Animal Laboratory at King Saud University from January to July 2020. Ethical approval for the animal research was obtained from the institutional research ethics committee (No. KSU-SE-19–88). Veterinarians performed general examination of Somali goats to evaluate their general, dental, and periodontal health. Four adult goats were included in this study, with a mean age of 2 years and average live body weight of 35 kg. The goats were housed in a shaded yard (Wafi, Riyadh, Saudi Arabia) throughout the experiment and received a commercial complete pelleted diet containing 13% crude protein. Each goat had eight permanent incisors; four on each side. A split-mouth design was utilized to assign right and left sides to either a bisphosphonate group (BPG) or a control group (CG). First and third incisors on each side were included in the study, while the second incisor on each side was extracted. During the whole experimental period, the teeth were brushed with a tooth brush and toothpaste (Oral-B, Procter & Gamble Co., US) three times daily.

For each instance of anesthesia, a solution of 50% ketamine (0.2 ml) and 2% xylazine (0.2 ml) was administered intravenously via the jugular vein. Both right and left second lower incisors were removed from each goat and the site was allowed to heal (Fig. 1A). After two weeks, the goats were anesthetized and cleaning of the labial surface of both right and left lower first and third incisors was performed with pumice paste (Nada Co., USA) in a rubber prophy cup using low-speed handpiece (Layan Medical Co., Saudi Arabia). After cleaning, the labial surface of first and third incisors was roughened with a carbide round bur (size 2.1 mm) (Medical Vision Est., Saudi Arabia) using slow pace (5,000 rpm) of a low-speed handpiece. Then, the tooth surface was etched with 38% phosphoric acid (Pulpdent Co., USA) for 30 s, rinsed with water for 15 s, and dried by an air syringe. Each etched surface was then coated by a thin Transbond XT (3 M Unitek Corp., USA) and light cured with Ortholux Luminous curing light (3 M Unitek Corp., USA) for 20 s. Orthodontic bonding was performed with 0.018-inch slot victory series Roth prescription pre-coated orthodontic brackets (Victory Series; 3 M Unitek Corp., USA) to the labial surface of first and third incisors on each side. Each bracket was bonded midway mesiodistally along the long axis of the tooth and with the same vertical height so not to produce any vertical discrepancy between the bonded incisors. All brackets were light-cured for 20 s with Ortholux Luminous curing light. The first and third incisors on each side were paired with a sectional, 0.016×0.022-inch, stainless-steel orthodontic arch wire (3 M Unitek Corp., USA). To achieve an initial force of 150 gm, the first and third incisors on each side were engaged with an elastomeric chain (3 M Unitek Corp., USA) to approximate them towards each other (Fig. 1B). The elastomeric chain was replaced every week to maintain active force until the first and third incisors were in contact (Fig. 1C). After orthodontic movement, the orthodontic appliance was held in place for another two weeks.

The bisphosphonate alendronate sodium (Alendro™, Jamjoom Pharmaceuticals Co., Saudi Arabia) was prepared for injection by formulating it into a gel using the technique described by Reddy et al. (2005). One side on each animal was randomly selected to receive a bisphosphonate injection into the periodontal space of the mesial and distal aspects of the first and third incisors by using a 27-gauge-1 cc– ø-inch insulin syringe (Mckesson Corp., US). The injection site was standardized by marking a reference notch 2 mm above the gingival margin with a diamond bur on the mesial and distal sides of each first and third incisor. From that point, the needle was inserted 7 mm into the mesial and distal periodontal space and 1 ml of the material was injected. Normal saline (Al-Razi Pharma Ind., Saudi Arabia) was used for injection on the contralateral side using the same injection technique. Following the injection procedure, the orthodontic appliance was kept

Fig. 1 Photographs of the anterior region of animal's mandible showing: (A) After 2nd incisor extraction and healing. (B) Space closure between 1st and 3rd incisors on each side with an elastomeric chain. (c) Complete space closure btween 1st and 3rd incisors on each side.

in place and brackets of the first and third incisors were ligated together passively with a 0.012-inch steel ligature wire (Ormco Corp., USA) for 12 weeks. After this period, the fixed orthodontic appliance was de-bonded using bracket removing plier (Ormco Corp., USA) and an impression of the lower jaw was made (M1) with vinyl polysiloxane impression material (3 M Unitek Corp., USA) loaded into a customized resin tray. Six weeks after debonding, another impression of the lower jaw was made (M2) using the same material and technique. All impressions were poured with Prep-Stone™ Ivory (ETI Empire Direct Tech., USA) to prepare M1 and M2 orthodontic study models.

Orthodontic relapse was assessed by measuring the distance between the adjacent proximal surfaces of the lower first and third incisors at mid-crown level on M1 and M2 study models using a digital caliper (Neiko 01407A Stainless Steel Electronic Digital Caliper; Ridgerock Tools Inc., USA). The relapse was calculated by subtracting each M2 measurement from the corresponding M1 measurement. All measurements were performed twice at an interval of two weeks and the mean of each measurement was used as the representative value. All animals were sacrificed for the purpose of microcomputed tomography and histological analysis. A size 21sharp blade (Swann Morton Co., England) was placed in the attached soft tissue to separate the mandible of individual animals, and a thick diamond saw (Buehler Co., USA) was used to section the anterior part of each mandible 5 mm distal to the fourth incisor.

Microcomputed tomography (micro-CT) was performed using a SkyScan 1172 high-resolution micro-CT (Bruker SkyScan, Belgium) to scan the entire specimen. The scanning parameters were as follows: voltage of 93 kV, anode current of 106 μA, exposure time of 158 ms, pixel size of 26 μm, rotation time (360° rotation) of 0.3 s, frames averaged for an improved signal-to-noise ratio of 4, and random movement to reduce ring artifacts of 8. To correct for variations in camera pixel sensitivity, flat-field correction was performed before the scanning process. Following scanning, digital reconstruction was performed with NRecon© Version 1.6.9.4 software (Bruker Skyscan, Belgium) to create longitudinal images. The PDL width was measured at the mesial and distal aspect of each first and third incisors at mid-point level between the crest of the alveolar bone and the root apex, along a line perpendicular to the long axis of the root canal (Fig. 2).

For histological analysis the specimens were fixed for 2 weeks in 10% formaldehyde, buffered with phosphate-buffered saline at pH 7.2. The fixed blocks were rinsed in running water and decalcified for 5–6 weeks in a solution of 8%

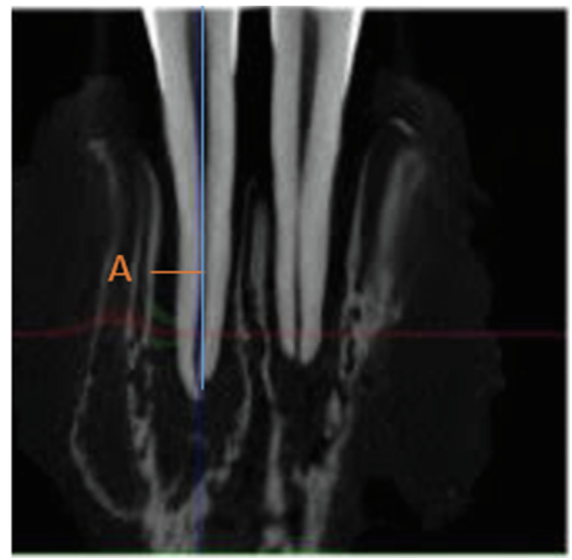

Fig. 2 Micro-CT image showing the midpoint reference for measurement of PDL width between the alveolar crest and root apex (A).

hydrochloric and 8% formic acid. Then, each incisor was sectioned into a separate block for paraffin processing using a size 20 surgical blade (Swann Morton, England). Five-micrometer-thick serial longitudinal sections were made and stained with hematoxylin and eosin using standard laboratory protocol. Histological sections were viewed using Aperio Scanscope scanner (Aperio Technologies Inc., US). The periodontal space of each first and third incisors was examined longitudinally within an area of 2 mm above and 2 mm beneath the injection site. Bone-like formations and changes within PDL were compared between the experimental and control sides.

2.1. Statistical analysis

Statistical analysis was performed using SPSS version 23 (Statistical Package for Social Science, IBM Inc., USA). The normal distribution of continuous data was tested with Kolmogorov-Smirnov Z test. Comparison between the two groups was done with Mann-Whitney *U* test for relapse measurements and Unpaired *t*-test for PDL width measurements depending upon the data distribution. For all analyses, a P-value (two-tailed) of ≤ 0.05 was considered to indicate statistical significance.

3. Results

Based on millimetric measurements obtained from orthodontic study models, significantly less relapse ($p \leq 0.05$) was noticed in the BPG compared to CG (Table 1).

Micro-CT measurements demonstrated significant difference at the mean PDL width ($p \leq 0.05$) between BPG and CG, with narrower PDL in the BPG (Table 2).

Histologically, the PDL space of BPG showed multiple spicules of bony-like formation with osteoblastic and osteoclastic activity, indicating active bone remodeling (Fig. 3). In the apical region and along the root surface of the BPG, layers of newly-formed scalloped cementum were observed coating a layer of irregular cementum (Fig. 4A), which indicates a repair process of previously resorbed cementum. However, in the CG an apical root resorption was observed (Fig. 4B).

Table 1 Comparison of orthodontic relapse between study groups.

Group	N	Mean	SD	P-value
BPG	4	0.575	0.1258	0.029*
CG	4	3.275	0.6801	

Man-Whitney *U* test, *Statistical significance at $p \leq 0.05$.

Table 2 Comparison of PDL width between study groups.

Group	N	Mean	SD	P-value
BPG	16	0.5301	0.19430	0.001*
CG	16	0.9019	0.35365	

Unpaired *t*-test, *Statistical significance at $p \leq 0.05$.

4. Discussion

The goat model is considered suitable for studies involving the dentoalveolar structure, as an adult goat has eight lower incisors that anatomically and periodontally resemble human teeth (Pearce, et al., 2007; Hassan et al., 2010). In the present study, alendronate sodium gel was used as it is reportedly retained in the PDL, allowing the slow release of bisphosphonate (Reddy at al., 1994). The gel was delivered intra-periodontally in our study, which is considered a safe route and clinically-applicable method of drug delivery (Moore et al., 2011). This is in contrast to the *trans*-mucosal delivery of DynaGraft reported by Hassan et al. (2010), following the creation of small windows using mini-screws. Nevertheless, our histological findings were similar to that reported by Hassan et al. (2010) in showing signs of newly-formed bone in the PDL of experimental groups, which is most likely responsible for the observed less relapse in these groups.

In addition, the results of the present study are in accordance with those of Adachi et al. (1994) in terms of the ability of bisphosphonates to enhance post-orthodontic stability. However, in contrast to the technique described by Adachi et al. (1994) where the bisphosphonate liquid was injected subperiosteally on multiple doses over 21 days in rats, a single dose of bisphosphonate in a gel format was injected intra-periodontally in our study to allow for its slow release over a longer period of time with a simple non-invasive modality. A split-mouth design was used in this study to control for potential internal confounders, such as body weight, oral hygiene, occlusal interferences, and rate of tooth movement.

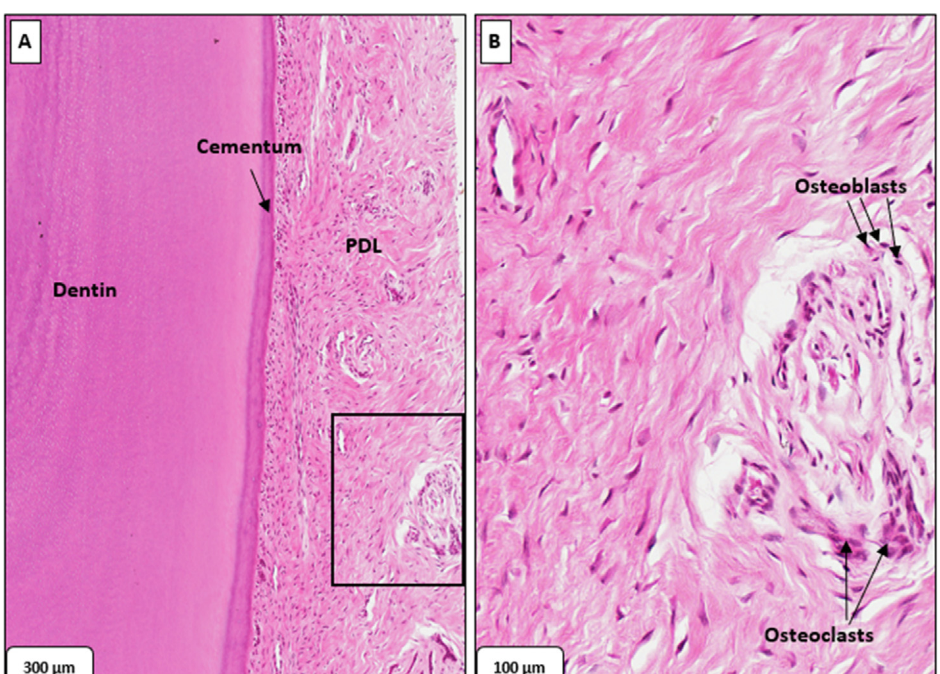

Fig. 3 (A) A photomicrograph showing islands of bone formation in the PDL of BPG, (B) Higher magnification of framed area in (A) showing clusters of osteoblasts and osteoclasts in a bony-like formation.

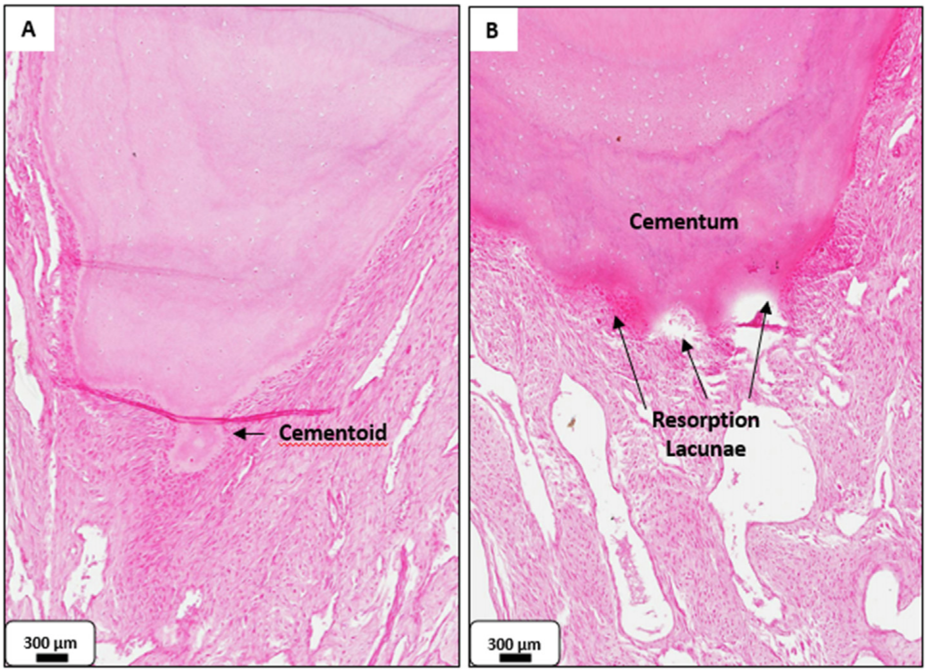

Fig. 4 Photomicrographs of the apical region showing: (A) cementoid formation in BPG, (B) active cementum resorption in CG.

A related study conducted by Kim et al. (1999) assessed the effect of systemic administration of bisphosphonate on orthodontic relapse after 10 days in rats. Their findings showed similar results to our study and support the fact that bisphosphonate can reduce initial relapse. Histologically, consistent findings were observed as they reported degeneration of osteoclasts, which reflects increased activity of osteoblasts and enhanced bone formation as observed in our study. The early stage at which Kim et al. (1999) observed the changes, i.e., 10 days, could explain the lack of bone formation as was observed in our study when we examined the tissue histologically after 18 weeks of drug administration. However, findings of both our study and Kim et al. (1999) study confirms the mechanism of action of bisphosphonate as it starts initially with inhibiting osteoclasts, and then directly inducing osteoblasts to start bone formation (Giuliani et al., 1998).

The measurements of PDL width on micro-CT images in this study revealed statistically significant difference between BPG and CG, which comes along with the histological finding of abundant newly-formed bone spicules observed in the PDL space. These observations support the reported ability of bisphosphonates to enhance bone formation (Giuliani et al., 1998) and therefore reducing the tendency for relapse.

Interestingly, in the histological sections of the BPG, newly formed cementum was observed on top of the orthodontically induced root resorption (OIRR) lacunae. This finding agrees with the conclusion of previous reports that the surface treatment of avulsed teeth with alendronate before replantation reduced root resorption (Najeeb, 2016). This could open new doors for investigation of the possible effect of bisphosphonates in prevention of OIRR.

Future studies are required to elaborate on the effect of bisphosphonates on post-orthodontic stability and to optimize the required dose of bisphosphonate for proper retention period. In addition, studies in humans will be needed to confirm these findings. Moreover, studies are required to evaluate the effects of bisphosphonates on cementum repair.

5. Conclusion

Within the limitations of this study (sample size, study duration, and limited data on anabolic activity), our preliminary results suggest that bisphosphonate injection enhances post-orthodontic stability and the repair of orthodontic-induced root resorption.

Ethical satement

This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. The study design was approved by the appropriate ethics review board. We have read and understood your journal's policies, and we believe that neither the manuscript nor the study violates any of these. There are no conflicts of interest to declare.

CRediT authorship contribution statement

Hana A. Tokhtah: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Data curation, Project administration. **Adel M. Alhadlaq:** Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

We thank King Abdulaziz City for Science and Technology for funding this project.

Also, special thanks to Dr. Razan Tokhtah and Dr. Yasser A. Al-Shawkir for their help during various stages of the experiment.

Funding

This study was supported by King Abdulaziz City for Science and Technology

(KACST) by the Research and Development Grants Program for National Research

Institutions and Centers (GRANTS) for graduate students' research program in Riyadh- Kingdom of Saudi Arabia, reference no. (1-1-03-001-038).

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