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

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Abstract

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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 sialoprotein reduced movement of the anchoring tooth during orthodontic treatment and provided higher stability for the anchoring tooth.

Keywords: Sialoprotein, Mini-implant, Dental Anchorage.
mineralized tissue, polyglutamic acid sequences.\textsuperscript{10,11} Primarily BSP expressed in osteoblasts, osteoclasts and hypertrophic chondroblasts but it’s also expressed in breast and lung cancers.\textsuperscript{12,13} In a study done by Goldberg et al.,\textsuperscript{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 sialoprotein. 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.\textsuperscript{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.\textsuperscript{16} All previous studies concerning the effect of BSP on bone homeostasis were done on cell culture.\textsuperscript{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 3\textsuperscript{rd} 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 3\textsuperscript{rd} 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 sialoprotein 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 (7\textsuperscript{th}, 20\textsuperscript{th} and 33\textsuperscript{rd} 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 1\textsuperscript{st} 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 3\textsuperscript{rd} 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 3\textsuperscript{rd} incisor form an angle with the inter-palatal line, the value of this angle
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).

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

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

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 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 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 and Gonzales et al 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 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 and Siefi et al 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. In vitro studies have suggested that BSP may function at several steps in...
bone modeling and remodeling. In addition to its ability to nucleate hydroxyapatite crystal formation and promote mineralization,\textsuperscript{25,26} BSP was reported to increase osteoclastogenesis and bone resorption.\textsuperscript{27,28} Furthermore, BSP expression is coincident with de novo bone formation\textsuperscript{29} and ectopic calcification.\textsuperscript{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.\textsuperscript{31,32} The osteoinductive effect of BSP was also reported after coating femoral implants which inserted into bones subjected to mechanical loading,\textsuperscript{33} and the pre-coating of the rough implant surface with BSP enhanced the osteoinductive effect much more than did collagen precoating.\textsuperscript{34} Malaval et al\textsuperscript{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\textsuperscript{9,10,16,35} and the BSP expression is increased in osteoblasts subjected to mechanical stimulation.\textsuperscript{36}

Conclusion

The localized use of BSP could be a beneficial therapeutic adjunct for orthodontic treatment. The local injection of sialoprotein 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

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