Dental Research Journal

Original Article

Effects of platelet-rich plasma local injection on dentin sialo protein and dentin matrix acidic phosphoprotein 1 levels of secretion in gingival sulcular fluid during orthodontic movement in animal samples

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Received: 22-Sep-2021
Revised: 23-Jan-2022
Accepted: 03-Mar-2022
Published: 16-Aug-2022

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ABSTRACT

Background: Prolonged orthodontic treatment causes complications such as root resorption, gingivitis, and caries. Platelet-rich plasma (PRP) can accelerate the dental movements of orthodontic treatments. The aim of the present study was to evaluate the effect of local PRP injection on the secretion of dentin sialoprotein (DSP) and dentin matrix acidic phosphoprotein (DMP1) biomarkers in gingival crevicular fluid during orthodontic movements in animal samples.

Materials and Methods: In this experimental study, one maxillary quadrant of six beagle dogs was randomly selected as the experimental group, and the other side was considered as the control group. Afterward, maxillary of the first premolars was extracted, and then, a titanium nickel coil spring (150 g) was then used between the second premolar and the canine. PRP that was previously activated with CaCl 2-thrombin was injected intraligaman to the experimental side at days 0, 21, and 42. Moreover, the mixture of the CaCl 2-thrombin with placebo was injected in to the control side. The study period was 63 days. Sampling was performed on days 0, 1, 2, 7, 21, 42, and 63. Then, in all the samples, the DSP and DMP1 level was measured using special kits by ELISA method. Data were analyzed by the analysis of variance and *t*-test.

Results: Upon the application of orthodontic forces, the mean DMP1 and DSP levels in the experimental group at different times were significantly increased compared to the control group ($P < 0.001$).

Conclusion: PRP injection enhanced DMP1 and DSP level with probable rise in the rate of root resorption.

Key Words: Dentin matrix protein, dentin sialo protein, orthodontic treatment, platelet-rich plasma

INTRODUCTION

Prolonged orthodontic treatment can cause complications such as root resorption, gingivitis, and caries. When orthodontic force is applied, the periodontal fibers change significantly on the side where orthodontic force is applied.[1] In addition,

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www.ncbi.nlm.nih.gov/pmc/journals/1480

How to cite this article: Hosseini SM, Farhad SZ, Esfahani NE, Shirani N. Effects of platelet-rich plasma local injection on dentin sialo protein and dentin matrix acidic phosphoprotein 1 levels of secretion in gingival sulcular fluid during orthodontic movement in animal samples. Dent Res J 2022;19:68.

since dental movements are key to orthodontic treatment, efforts have been made to accelerate dental movements by affecting bone remodeling.[2] Dental movements are a biological response to controlled mechanical and long-term orthodontic forces, which causes areas of pressure and tension in the periodontium and boosts the production of inflammatory mediators such as prostaglandins and leukotrienes. Eventually, the dental socket will be displaced due to bone resorption and formation by osteoclasts and osteoblasts.[3] Just as osteoclasts cause bone resorption and tooth movement, root cement is also affected. Root resorption is a complication of orthodontic treatment that occurs in most orthodontic patients and may involve up to one-third of the root length.[4]

Root resorption is known as a multifactorial phenomenon and it seems that the factors related to external root resorption can be divided into two general categories of biological and mechanical factors. Mechanical factors include the amount of dental movement, the type of movement, the amount of orthodontic force, and the duration of the force. Biological factors also include genetic predisposition and systemic factors such as hormonal imbalance, root morphology, tooth agenesis, and prescribed medications.[4]

Analysis of dentin proteins in gingival crevicular fluid (GCF) is a safe method for estimating the rate of root resorption compared to conventional radiographic methods.[5]

As a mineral tissue of teeth, dentin contains a combination of minerals, organic matter and water. The organic matrix contains type 1, 3 and 5 collagens. Dentin matrix acidic phosphoprotein (DMP1), dentin sialoprotein (DSP) and DPP originate from dentin and are found in adult cartilage and bone. They are associated with the cellular protein matrix family, which includes osteopontin and bone sialoprotein.[6] DSP is the second abundant compound in dentin structure after dentin phosphoprotein (DPP). Large amounts of dental proteins are released during orthodontic damage due to orthodontic movements and can be used as a biomarker to detect and observe root damage. Therefore, DSP can be released from these damaged teeth.[7] DMP1 plays an important role in tooth formation and interstitial and cartilaginous ossification as well as cellular connections and mineralization.[8]

PRP is part of the blood plasma that contains high levels of platelets. It is also obtained by the centrifugation of the patient's own blood, and includes growth factors that play a role in creating and maintaining wound healing, accelerating bone formation, and increasing fibroblast proliferation; it plays an important role in tissue healing.[9] The relationship between PRP and orthodontic tooth movement has studied by some researchers in the recent years. For instance, based on an animal study, Rashid *et al*.[10] and Güleç *et al*.[11] investigated the effects of local injection of PRP on the rate of tooth movement. These studies endorse the positive correlation between these two determinants. In this regard, a recent study by El-Timamy *et al* in 2020[12] showed that PRP may accelerate the rate of tooth movement in the 1st months after the injection.

However, the need for further investigation in this field of research has suggested by some studies.[13]

In a study of biomarkers and the rate of root resorption, Balducci *et al*.[14] concluded that DSP and DPP can be suitable biological markers to evaluate the process of root resorption during orthodontic treatment. However, Tarallo *et al*.[15] showed that DSP and DMP1 in GCF cannot diagnose root resorption due to orthodontic movements.

Considering the increasing need for orthodontic treatments with minimal time and complications and the fact that PRP is a new orthodontic adjuvant treatment, the aim of the present study was to investigate the effect of the local injection of PRP on the DSP and DMP1 level in the periodontal ligament. The aim of the present study is to investigate the role of injectable PRP in orthodontic treatment, specifically by focusing on its effect on root resorption.

MATERIALS AND METHODS

This was an experimental *in vitro* study approved by the Animal Research Ethics Committee at the School of Dentistry of Isfahan Azad University. A total of 6 adult male dogs aged 10–12 months weighing 15–20 kg were included in the study. All surgical procedures were performed under deep anesthesia using xylazine hydrochloride and ketamine hydrochloride.

Blood samples were first prepared. To prepare PRP from canine cephalic veins, blood samples were centrifuged in 5 cc test tubes containing sodium

citrate in a centrifuge plasma rich in growth factors (PRGF, BTI, Spanish) at 460 g for 8 min. Plasma was isolated from erythrocytes after the completion of centrifugation. In the centrifuged test tube, red blood cells were placed at the bottom with a thin layer of white blood cells spotted on it. At the top, the plasma was divided into three layers from bottom to top: PRGF, plasma with growth factors (0.5 cc) and plasma poor in growth factors (0.5 cc). Each layer was poured into separate tubes. After separating the PRGF layer, it was kept at the same temperature of 36°C until use.

In each dog, the first left and right maxillary premolars were extracted, and then the NiTi close coil (9 mm nickel-titanium wire; Dentaline GmbH) was closed to the second premolars with an average force of 150 g from the canine tooth. Each dog underwent random intraligamentary injection of thrombin-calcium chloride mixture (0.5 cc) and PRP (0.5 cc) on one side around the second premolar at 8 points (mid-buccal, midpalatal, distobuccal, distopalatal, a point in the middle of the distal surface, mesiopalatal, mesiobuccal and a point in the middle of the mesial surface), which was considered as the experimental group. In the control group, only calcium chloride (0.5 cc) and thrombin (0.5 cc) were injected. Injection was performed on days 1, 21, and 42. The study period was 63 days and sampling was performed on days 0, 1, 2, 7, 21, 42, and 63. Each time sampling was carried out by point papers, and the samples were immediately placed in containers of the intermediate agent. They were then transported to the laboratory under cold temperature in a dry ice box (2–5°C). They were frozen at a temperature of −70°C, then in all samples, the DSP and DMP1 levels were measured by special kits for these mediators by ELISA method.

Data were analyzed by independent *t*-test, ANOVA and SPSS 25 software.[16]

RESULTS

ANOVA was used to investigated the mean DSP level in GCF during orthodontic forces at different times. Results showed that mean DSP level in the experimental group increased significantly at different times ($P = 0.02$) but there was no significant difference at different times in the control group ($P = 0.46$). Based on independent *t*-test, there was no significant difference between the two groups in terms of the mean DSP level at baseline ($P = 0.09$) but this level was significantly higher in the experimental group than the control group at other times ($P < 0.05$) [Figure 1].

In the evaluation of the mean DMP1 mediator in the gingival crevice fluid of the teeth during the application of orthodontic forces at different times based on analysis of variance test, the mean DMP1 mediator of the experimental group at different times had a significant increase ($P = 0.03$). However, there was no significant difference in the mean DMP1 levels in the control group at different time intervals times ($P = 0.27$). Independent *t*-test also showed no significant difference between the two groups at baseline in terms of the mean DMP1 level ($P = 0.12$), but it was significantly higher in the experimental group than the control group at other time intervals ($P < 0.001$) [Figure 2].

DISCUSSION

According to the results of the present study, PRP increases the DSP and DMP1 levels, which can indicate a rise in root resorption.

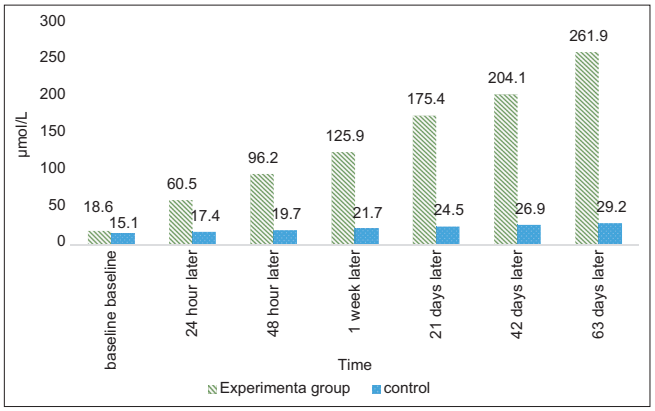

Figure 1: The mean dentin sialoprotein level (µmol/L) in gingival crevicular fluid during orthodontic forces at different times.

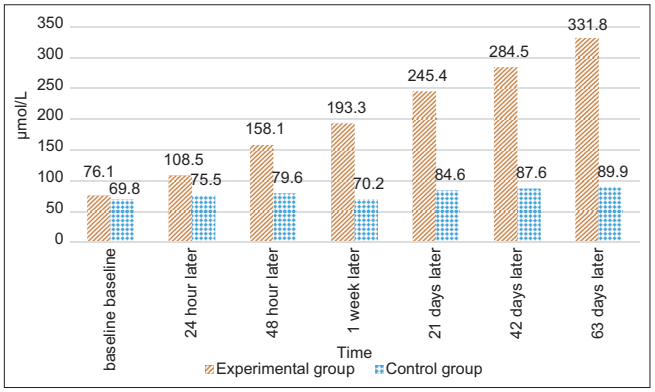

Figure 2: The mean dentin matrix acidic phosphoprotein mediator (µmol/L) in gingival crevicular fluid during orthodontic forces at different times.

Root resorption during orthodontic treatment is a major challenge with irreversible defects on the tooth.[17] So far, no conclusive evidence has been provided to determine the cause of root resorption.[18] The incidence of further resorption is significantly associated with the use of heavier and uncontrolled forces.[19] However, it has been reported that hormonal changes and genetic factors can cause root resorption.[20]

The role of biomarkers such as DMP1 and DSP can be mentioned while studying root resorption. In their study, Kumar *et al*.[21] found DSP in luxated teeth, the amount of which depended on the degree of activity and the pathological process. DSP, therefore, has a noninvasive potential for root detection. In their study of DPP in GCF during root resorption, Mah and Prasad.[6] concluded that the experimental group had a much higher DPP in GCF compared to the control group. In a systematic review study of the GCF mediators to detect root resorption in patients undergoing orthodontic treatment, Tarallo *et al*.[15] concluded that DSP and DMP1 in GCF could not effectively diagnose root resorption due to orthodontic movements.

Tooth movement occurs following the application of mechanical force. The main cause of this movement is inflammation in the periodontal ligament tissue and alveolar bone.[4] Dominant cells in this inflammatory process accelerate the remodeling of periodontal ligament and alveolar bone by affecting the activity of osteoblasts and osteoclasts. Natural bone remodeling depends on the balance between bone formation and resorption.

DSP and DMP1 are similar in many respects in terms of gene structure and protein, and DSP is derived from DMP1 with a similar approach to gene replication.[6] PRP is also composed of platelet-derived growth factor, epithelial-vascular growth factor, transfer growth factor (β1-β2-β3), and insulin-like growth factor (IGF), which is considered as an accelerator for orthodontic movements.[22-24]

In their study of the effect of PRP on orthodontic tooth movement in mice, Güleç *et al*.[11] concluded that PRP increases dental movement and osteoclast activity in the experimental group and expressed the greatest effect of PRP injection after 60 days. In the present study, the DSP level in the experimental group was augmented over time after PRP injection, which indicates that PRP is effective in increasing osteoclast activity.

In their study, Yeom *et al*.[25] found significant and positive increase in the incidence of mRNA and odontoblastic protein markers such as DMP1 and DSP following PRP injection during orthodontic movements as compared to the control group. PRP also increased alkaline phosphatase (ALP) activity. It stimulates the formation of mineral nodules over time, in the presence of stimulants such as ascorbic acid and β-Glycerophosphate *in vitro*.

In their study of DSP and DMP1 as two phosphorylated proteins in mineral tissues, Suzuki *et al*.[17] concluded that PRP injection can increase the DMP1 and DSP levels, which is consistent with the results of the present study. Creeper *et al*.[26] also achieved similar results in the study of the effect of PRP on differentiation, proliferation, cell function and migration of osteoblasts, and periodontal ligament cells. They stated that platelet-rich plasma (PRP) can have a positive effect on osteoblasts and periodontal cells and DMP1 and DSP levels increase after PRP injection.

In the study of transgenic expression of DSPP in rats, Gibson *et al*.[27] found that the DMP1 and DSP levels remained unchanged after PRP injection, which is inconsistent with the results of the present study, which could be due to genetic differences in experimental samples and different PRP concentrations. In the present study, dogs were used as a sample, but rats were used as experimental samples in the Gibson's study.

Since DMP1 and DSP are inflammatory factors related to root resorption, PRP injection can raise the level of these mediators, which indicates higher root resorption during orthodontic movements. The limitation of this study was the lack of the assessment of bone remodeling parallel with root resorption. Another limitation was the lack of histopathological measurement of root resorption with root resorption biomarkers in GCF. Therefore, for future studies, we suggest to use bone formation markers such as osteoprotogerin, osteonectin, osteocalsin, and bone morphogenic protein immunohistochemically for better understanding of the new bone formation mechanism with PRP injection on tooth movement together with root resorption biomarkers.

CONCLUSION

PRP injection raises DMP1 and DSP levels. Furthermore, as an accelerator of dental movements, it could accelerate root resorption.

Financial support and sponsorship
Nil.

Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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