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Effect of RANKL Inhibitor Osteoprotegerin - FC on Orthodontic Tooth Movement - A Systematic Review of Animal Studies

Research Article

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Abstract

Objective: The objective of this review was to systematically evaluate and appraise the quality of all the animal studies done to study the effect of OPG in inhibiting orthodontic tooth movement.
Methods: The protocol for the systematic review was registered in PROSPERO. Three electronic databases were searched for articles until February 2020. Hand searching of articles from the reference list of selected articles was also done. Animal studies which simulated orthodontic tooth movement after injecting osteoprotegerin and evaluated the rate of tooth movement were included for the review. Data collection, risk of bias and study quality was assessed by the two authors individually. SYRCLE's tool was used for risk of bias assessment whereas ARRIVE guidelines were used to check the study quality. The level of evidence and grade of recommendation was assessed using the OCEBM table.
Results: The search strategy identified a total 163 studies, of which 5 were included for the systematic review. Two studies had low risk of bias while the other three studies had unclear risk of bias. All the studies were of moderate quality according to the ARRIVE guidelines. The result of all the studies emphasized that osteoprotegerin was effective in inhibiting orthodontic tooth movement. Level of evidence 'V' and Grade of recommendation 'D' was identified.
Conclusion: From the available evidence it can be said that osteoprotegerin is effective in inhibiting orthodontic tooth movement. Before it can be directly incorporated into human trials, it is safe to conduct these studies on nude mice. This is to negotiate the alterations in immune-inflammatory response in rats produced by human OPG-Fc.

Keywords: RANKL Inhibitor; Osteoprotegerin; Tooth Movement; Systematic Review.

Introduction

Rationale

In orthodontics, anchorage is prevention of unwanted tooth movement. Anchorage has an important role in the orthodontic treatment of almost all types of malocclusions. Good anchorage control helps to achieve excellent treatment results. It means that there is a minimal or no movement of the anchorage unit during the orthodontic treatment. The movement of the anchorage unit can be related to the Newton's Third Law of Motion, where every action has an equal and opposite reaction. Here, the movement of the desired teeth is the "action", whereas movement of the anchorage unit is the "reaction" [1]. Various methods have been designed to enhance the anchorage control, namely, headgears, transpalatal arch, Nance palatal arch, lingual stabilizing arch, intermaxillary elastics, miniscrews, mini-plates, mini-implants, pharmacological agents etc.

The conventional methods of anchorage control such as headgears and intermaxillary elastics required high patient compliance and also the increasing esthetic demands among the patients made the use of these aids questionable. Studies have also shown that transpalatal arch, Nance palatal arch, lingual stabilizing arch were effective only when used with other adjunctive aids for anchorage

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control [2, 3]. Moreover patients find it difficult to talk with these appliances in place and also the chances of iatrogenic injuries and allergy are also high. Temporary anchorage devices such as mini-plates, mini-screws and mini-implants are known for providing absolute anchorage. Even though this is an advantage, there are some drawbacks with these intraoral skeletal anchorage devices, such as, damage to the surrounding structures such as tooth root, nerve or blood vessel damage, penetration into the nasal cavity or maxillary sinus, implant fracture during insertion or removal, soft tissue inflammation. The stability of the mini-implants or screws also depends on the quantity and quality of the bone [4, 5]. Given these disadvantages, we can search for pharmacological agents which can prevent the underlying biological events that take place during orthodontic tooth movement.

The basis for orthodontic tooth movement is the activity of osteoblasts and osteoclasts which are responsible for bone formation and bone resorption respectively. Osteoclast differentiation is regulated by receptor activator of nuclear factor kB (RANK)which is found on mature osteoclasts and their precursors. The RANK receptor is activated by RANK ligand (RANKL) seen on the surface of osteoblasts and periodontal ligament (PDL) cells. This is the critical step involved in the process of bone resorption [6, 7]. Conversely, osteoprotegerin (OPG) acts as a competitive inhibitor of RANK by binding to RANKL and preventing osteoclastogenesis and bone resorption [8]. OPG reverses osteoporosis [9] and also increases bone strength by improving cortical and trabecular bone architecture [10]. Considering these properties of OPG, it shows that it can be used for anchorage control in orthodontics by preventing bone resorption in relation to the anchor unit.

Objective

The objective of this review was to systematically evaluate and appraise the quality of all the animal studies done regarding the effect of OPG in inhibiting orthodontic tooth movement.

Materials And Methods

Protocol and registration

The protocol for the systematic review was registered in PROSPERO (ID- CRD42019150387). PRISMA statement was followed for developing the protocol as well as during conduct and reporting [11].

Eligibility criteria

Inclusion criteria

- Studies involving healthy animals with orthodontic appliance exerting force on the molars
- Administration of osteoprotegerin injection during the start of force application
- Rate of anchorage loss compared between the osteoprotegerin group and control group which is injected with phosphate buffered saline solution or no injection
- Qualitative data on the rate of movement of molar

Exclusion criteria

- No control group or alternative drug used as control
- Review articles, systematic reviews and meta-analysis

Information sources and search strategy

Three electronic databases PubMed, Cochrane Library and Google Scholar were searched for articles until February 2020. The search strategy was designed by the two authors. There were no date restrictions used in the search strategy. Reference lists of the selected articles were also searched.

Study selection

The study articles were selected by two authors independently and then combined together. The preliminary selection of articles was based on the title and abstract. The selected articles were completely assessed according to all the inclusion and exclusion criteria. Disagreements among the authors were resolved by discussions. Authors of the respective articles were contacted in case of any unreported data.

Data collection and data items

Data extraction was done by the same two authors independently and then combined. Any disagreements were resolved by discussion. Data collection forms were used to record the following details:

- Name of the first author and year of publication
- Characteristics of the animals
- Mode of tooth movement
- Particulars of intervention
- Outcomes measured
- Results

Risk of bias in individual studies

The risk of bias was assessed using SYRCLE's risk of bias tool by the same authors as mentioned above [12]. Disagreements were resolved by discussion.

Summary measures and synthesis of results

Meta-analysis was to be done if it was possible to combine the results of the included studies.

Risk of bias across studies and additional analyses

'Small study effects' and other additional subgroup analyses were planned if sufficient information could be extracted from the included studies. The level of evidence and grade of recommendation was assessed using Oxford Centre for Evidence-Based Medicine (OCEBM) [13].

Results

Study selection

The PRISMA flow chart for study selection is shown in Figure 1. A total of 163 articles were obtained. 162 articles were identified through electronic database searching and 1 article was identified from the reference list of the selected articles. 2 duplicate articles were removed from the total list 163 articles. 161 articles were

screened and out of these 155 articles were excluded based on the title and abstract. 6 full text articles were assessed completely for eligibility and 1 was excluded since it was a hypothesis article. Therefore, finally 5 articles were included for qualitative synthesis in this systematic review [14-18].

Study Characteristics

The characteristics of the included studies are listed in Table I. All the studies were experimented on male rats. The methodology of the study was almost similar for all the studies except for the study by Keles et al [18]. Orthodontic force was applied using closed NiTi coil spring for a period of 3 to 4 weeks producing mesial movement of the molars. The intervention was given as a local injection adjacent to the molars. Tooth movement was measured from scanned images of study models which were obtained from polyvinyl siloxane impressions of the teeth. In study by Keles et al., [18] there was no mention about the Animal Welfare Committee approval; the orthodontic force was applied through a Y shaped stainless steel spring that exerted a buccal/palatal force to the molars; the intervention was given as a subcutaneous injection and the tooth movement was measured through radiographs. In study by Fernandez et al., [16, 17] no treatment was performed on the contra lateral side because of the possible systemic effects of the drug. Fernandez et al. [16] and Keles et al. [18] also compared the effect of OPG to zoledronate and pamidronate respectively. Other than the tooth movement measurements, histomorphometric analysis and micro-computed tomography were done by Dunn et al. [14], Sydorak et al. [15] and Fernandez et al. [15-17]. Sydorak et al. [15] also did serum analysis to evaluate the circulating levels of OPG. Osteoclast recruitment rate and apoptosis was assessed by Keles et al. [18]. Rate of incisor retraction and the subsequent anchorage loss ratio was evaluated in the studies by Dunn et al. [14] and Sydorak et al. [15]. The study quality was moderate for all the studies according to the Animal Research: Reporting in Vivo Experiments (ARRIVE) guidelines [19].

Risk of bias within studies

Summary of risk of bias within the studies is presented in Table II. Studies by Dunn et al. [14] and Fernandez et al. [17] had low risk of bias while the other three studies [15, 16, 18] had unclear risk of bias. The studies presented an unclear risk of bias in terms of randomization, allocation concealment, randomised housing of the animals, blinding of caregivers/investigators and information on confounding factors. Regarding the outcome assessor blinding, two studies [14, 17] were rated low and three were rated high [15, 16, 18]. All the studies except for the study by Keles et al. [18] had low risk of bias in the domain of selective outcome reporting.

Results of individual studies

Osteoprotegerin injection was effective in inhibiting orthodontic tooth movement [14-18]. OPG inhibited tooth movement more effectively compared to zoledronate and pamidronate [15, 17]. OPG also increased the bone density and bone volume fraction in the site of injection [16-18]. Sydorak et al., [15] showed that microsphere encapsulated OPG had more localised effect compared to non encapsulated OPG of the same dosage whereas non-encapsulated OPG of higher dosage was the most effective in inhibiting tooth movement but entered the systemic circulation. RANK, Runx, vimentin, MMP-9 and tissue inhibitor metalloproteinase 1 immunoreactivity were reduced significantly in OPG treated animals [16, 17].

Risk of bias across studies and additional analyses

With the available data from the included studies, it was not possible to do analyses for small study effects or other additional analyses. Level of evidence 'V' and Grade of recommendation 'D' was identified using OCEBM table [13].

Discussion

Summary of evidence

The effect of pharmacological agents in inhibiting orthodontic tooth movement was studied by various authors. Most of the studies focused on the effect of bisphosphonates in preventing tooth movement by inhibiting bone resorption [20-23]. Even though bisphosphonates proved to be effective in inhibiting orthodontic tooth movement, these are not indicated for orthodontic use currently due to its potential side effect of osteonecrosis of the jaw [24, 25]. It resides in the bone for a long time and the effects are irreversible [22, 23]. Therefore, researchers started analysing the biology of the tooth movement and the cellular mediators involved in it. Thus came the RANKL inhibitors, which could inhibit the activity of RANKL by binding to RANK and prevent osteoclastogenesis [8].

The RANKL inhibitor, osteoprotegerin, proves to be effective in inhibiting orthodontic tooth movement by preventing bone resorption [14-18]. Local injection of 5mg/kg OPG, twice weekly

Figure 1. PRISMA flow chart.

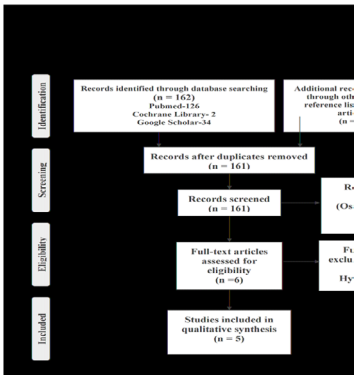

Table 1: Study characteristics.

Study(Name of the first author and year of study)	Subject characteristics (Species, sex, age, weight, total number)	Tooth movement model	Group characteristics (number, injecting agent, dosage, frequency, route, administration)	Assessment of tooth movement	Results
Dunn et al, 2007 [14]	Sprague-Dawley rats; male; 250-300g; 39	Closed coil Ni-Ti spring from Mx M1 to Mx CI; Force- 54+/-2g 21 days	Group 1: 10; with appliance, OPG-Fc 5mg/kg; twice weekly Group 2: 10; with appliance; OPG-Fc 0.5mg/kg; twice weekly Group 3: 10; with appliance; PBS; twice weekly 3 animals: no appliance; no injection 3 animals: no appliance; vehicle injected 3 animals: no appliance; high dose OPG Site of injection- Palatal mucosa adjacent to mesial surface of Mx M1 Needle- 33 gauge microneedle Sample size calculation- No	Time interval- 0,3,7,10,14,17 and 21 days after appliance placement PVS impression-stone models-scanned-magnified 100X using Adobe Photoshop Mesial movement of Mx M1- distobuccal grove of Mx M1 to the most distal surface of Mx M3. Distal movement of Mx CI - Mesiodistal center of CI at the facial gingival margin to distal surface of Mx M3	After 21 days, Mesial movement of Mx M1- Group 1:0.20+/-0.03mm Group 2:0.75+/-0.10mm Group 3:0.93+/-0.07mm Distal movement of Mx CI- Group 1:1.05+/-0.03mm Group 2:1.53+/-0.03mm Group3: 2.17+/-0.05mm Ratio- Incisor retraction: molar movement Group1- 5.2:1 Group 2- 2:1 Group 3- 2.3:1
Keles et al, 2007 [18]	C57Bl/6 mice; male; 8 week	0.2mm diameter coiled stainless steel wire fashioned into a Y-shaped spring from Mx M1 to Mx CI Experiment group-constricted Control group- passive	Group 1: Sterile saline with Pamidronate 5mg/kg; daily up to 8 days Group 2: Sterile saline with OPG 10mg/kg; daily up to 8 days Route of administration- subcutaneous injection Sample size calculation- No	Time interval- 0,1,4,8 and 12 days Low speed dental X-ray film exposed using HP Faxitron; exposure time 30s at 30W- developed films scanned as tif file-digitized using COREL DRAW 11 software- magnified X50- tooth movement calculated as one-half of the intermolar distance between the bonded wire tips	After 8 days, Molar tooth movement- 1.Control- 0.06+/-0.02mm Pamidronate- 0.04+/- 0.01mm 2. Control- 0.07+/-0.02mm OPG- 0.02+/- 0.06mm
Fernandez et al, 2016 [17]	Sprague-Dawley rats; male; 6 months; 420-450g; 42	Super elastic Ni-Ti closed coil spring from right Mx M1 to anterior mini-screw (6mm length) placed behind and between the roots of the Mx incisors Force-50g 21 days	Group 1: 21; with appliance; 50µl PBS with human OPG-Fc 5mg/kg; twice weekly Group 2: 21, with appliance; 50µl PBS vehicle Site of injection- palatal mucosa; mesial and distal surface of right Mx M1 and vestibule above the M1; 3/10ml syringe Sample size calculation- Yes	Time interval- 7,14 and 21 days PVS impressions-die stone-scanned at 1400 dpi- magnified 300X using Adobe Photoshop	After 21 days, Mesial movement of molar- Group 1: 0.22+/-0.01mm Group 2: 0.98+/-0.05mm
Fernandez et al, 2016 [16]	Sprague Dawley rats; male; 420-460g; 36	Super elastic Ni-Ti closed coil spring from right Mx M1 to anterior mini-screw (6mm length) placed behind and between the roots of the Mx incisors Force-50g 21 days	Group 1: 12; with appliance; 50µl PBS with 16µg zoledronate; single dose Group 2: 12; with appliance; 50µl PBS with human OPG-Fc 5mg/kg; twice weekly Group 3: 12; with appliance; 50µl PBS Site of injection- palatal mucosa; mesial and distal surface of right Mx M1; 3/10ml syringe Sample size calculation- Yes	Time interval- 7,14 and 21 days PVS impressions-die stone-scanned- magnified 100X using Adobe Photoshop	After 21 days, Mesial movement of molar- Group 1: 0.30+/-0.01mm Group 2: 0.21+/-0.01mm Group 3: 0.99+/-0.03mm
Sydorak et al, 2019 [15]	Sprague Dawley rats; male; 360g; 42	Ni-Ti coil spring from Mx M1 to Mx CI Force-25g 28 days	Group 1: 6; with appliance; empty microspheres; single dose Group 2: 6; with appliance; 1mg/kg microsphere encapsulated OPG; single dose Group 3: 6; with appliance; 1mg/kg non-encapsulated OPG; single dose Group 4: 6; with appliance; 5mg/kg non-encapsulated OPG; multiple dose-every 3 days once up to 28 days Group 5: 6; no appliance; empty microspheres; single dose Group 6: 6; no appliance; 1mg/kg microsphere encapsulated OPG; single dose Group 7: 6; no appliance; 1mg/kg non-encapsulated OPG; single dose Site of injection- palatal mucosa adjacent to the mesial surface of Mx M1 Sample size calculation- No	PVS impressions- stone models-scanned at 1200 dpi- magnified 300X using Adobe Photoshop Mesial movement of Mx M1- distal grove of Mx M1 to the distal surface of Mx M3. Distal movement of Mx CI - Facial surface of Mx CI at the gingival margin to distal surface of Mx M3	After 28 days, Mesial movement of Mx M1- Group 1: 0.8+/-0.1mm Group 2: 0.6+/-0.1mm Group 3: 0.8+/-0.1mm Group 4: 0.2+/-0.1mm Distal movement of Mx CI- Group 2: no reduction compared to Group 1 Group 3: no reduction compared to Group 1 Group 4: Significant reduction compared to Group 1,2 and 3 Ratio- Incisor retraction : mesial molar movement Group 2: 1.4times greater compared to Group 1 Group 3: No significant difference compared to Group 1 Group 4: 2.3 times greater when compared to Group 1 1.7 times greater when compared to Group 3

Ni-Ti- Nickel Titanium; OPG-Fc-human recombinant Osteoprotegerin; PBS- phosphate buffered saline; Mx- maxilla; M1-first molar; M3-third molar; CI- central incisor; PVS- polyvinyl siloxane

was effective in significantly reducing the mesial movement of molar without inhibiting the distal movement of the anteriors [14-17]. Sydorak et al., in his study showed that 5mg/kg of non-encapsulated OPG was more effective in molar inhibition than a local injection of microsphere encapsulated 1mg/kg OPG. But the systemic circulation of OPG was high in animals injected with 5mg/kg of non-encapsulated OPG [15]. Therefore, localising the action of OPG by microsphere encapsulation could be a safer option in order to prevent unwanted systemic effects. OPG is not only known to inhibit orthodontic tooth movement, but it is

Table 2. Summary of risk of bias assessment.

Study	Signaling questions										
	1	2	3	4	5	6	7	8	9	10	Summary
Dunn et al, 2007 [14]	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	Low
Keles et al, 2007 [18]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	High	Unclear	Unclear	Unclear	Unclear
Fernandez et al, 2016 [17]	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	Low
Fernandez et al, 2016 [16]	Unclear	Low	Unclear	Unclear	Unclear	Unclear	High	Low	Low	Unclear	Unclear
Sydorak et al, 2019 [15]	Unclear	Low	Unclear	Unclear	Unclear	Unclear	High	Low	Low	Unclear	Unclear

1-Was the allocation sequence adequately generated and applied? ; 2- Were the groups similar at baseline or were they adjusted for confounders in the analysis? ; 3- was the allocation to different to groups adequately concealed during the study? ; 4-Were the animals randomly housed during the assessment? ; 5- Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment? ; 6- Were animals selected at random for outcome assessment? ; 7- Was the outcome assessor blinded? ; 8. Were incomplete outcome data adequately addressed? ; 9- Are reports of the study free of selective outcome reporting? ; 10. Was the study apparently free of other problems that could result in high risk of bias?

also a potential inhibitor post-orthodontic tooth relapse [26, 27]. Unlike bisphosphonates, RANKL inhibitors do not reside in the bone and their effects are reversible [28].

Strengths and limitations

This systematic review was based on the PRISMA guidelines. Electronic databases were searched using various combinations of search terms. All potentially eligible studies up to October 2019 were included in this review. Article screening, data extraction, assessment of study characteristics, risk of bias as well as assessment of level of evidence were performed independently by two authors and were combined together. All quality assessments were done based on the respective universal guidelines. Any disagreements aroused were resolved by discussion. All efforts were made to reduce the level of bias in the review.

The major limitation of the review would be the methodology of the included studies which used human recombinant OPG in animals. The dosage and the frequency of the OPG-Fc used in these animal studies might be higher than the effective human dosage as human OPG-Fc could alter the immune-inflammatory response in the rats [29]. Other limitations were that the database search was restricted to English language and hand search for the articles were not done. Also meta-analyses and other additional analysis could not be done with the available data.

Recommendations for future research

Before employing these results directly into human trials, it is better to study the dosage and frequency of OPG administration in nude mice that better resembles a human subject [30]. It is also important to carry out animal studies to find the long term local and systemic effects of OPG-Fc injection.

Conclusion

According to the results of this systematic review, osteoprotegerin is effective in inhibiting orthodontic tooth movement and it can be used to enhance anchorage control during canine-retraction, en-mass anterior retraction, and various other orthodontic tooth movements, taking into consideration the possible systemic effects it can cause.

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