The effect of IL-1 receptor antagonist on orthodontic tooth movement in mice

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A B S T R A C T

Objective: Orthodontic tooth movement (OTM) is achieved by alveolar bone remodelling induced by mechanical loading. Whilst interleukin-1 (IL-1) is directly involved in OTM, the role of interleukin-1 receptor antagonist (IL-1Ra), a naturally occurring IL-1 antagonist, is not completely defined. Therefore, the aim of this study was to investigate the effects of IL-1Ra on OTM.

Methods: An orthodontic appliance was placed in C57BL6 mice treated with vehicle or IL-1Ra (10 mg/kg/day). OTM and TRAP-positive osteoclasts were evaluated after 12 days of mechanical loading and the levels of cytokines on periodontal tissues were analysed by ELISA after 12 and 72 h.

Results: Mice treated with IL-1Ra showed diminished OTM and decreased numbers of TRAP-positive osteoclasts. In line with this, lower levels of IL-18 and TNF-α, and higher levels of IL-10, were observed on periodontal tissues of IL-1Ra-treated mice in relation to the vehicle-treated group.

Conclusion: The present study suggests that IL-1Ra downregulates OTM, probably by its anti-inflammatory actions.

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1. Introduction

Orthodontic tooth movement (OTM) occurs through remodelling of alveolar bone after mechanical stimuli. The orthodontic forces generate and propagate signalling cascades through all paradental tissue cells, triggering important changes in the homeostatic periodontal environment.¹ ²

The orthodontic loading leads to a focal tissue injury and, consequently, an aseptic inflammatory response characterised by the release of several important inflammatory mediators on periodontal tissues,² ³ such as the cytokine interleukin-1 (IL-1).⁴ IL-1 is directly involved in bone resorption by taking part in the survival, fusion and activation of osteoclasts and it exerts its activities by binding to two types of receptors, IL-1-R1 and IL-1-RII.⁵ Whilst the latter has no
described signalling properties and acts as a “decoy” target for IL-1, the former develops pro-inflammatory functions, such as cell recruitment and release of other cytokines, which also are involved in bone resorption. However, IL-1 functions are physiologically controlled by the naturally occurring interleukin-1 receptor antagonist (IL-1Ra), which competitively blocks the interactions of IL-1 with its receptors and inhibits its activity.\(^7\)\(^8\)

IL-1Ra has long been studied in clinical and experimental surveys as a physiological and therapeutic target in inflammatory conditions related to bone resorption, such as rheumatoid arthritis\(^9\)\(^,\)\(^10\) and periodontal disease.\(^11\)\(^,\)\(^12\) These studies reported that administration of exogenous IL-1Ra may be a useful strategy to control bone resorption, mainly for its anti-inflammatory properties related to the antagonism of IL-1\(^1\)\(^,\)\(^9\)\(^–\)\(^11\)\(^,\)\(^13\) However, only a few studies have investigated the effect of IL-1Ra on OTM, showing a positive correlation between decreased IL-1Ra gingival expression and faster OTM in humans.\(^14\)\(^–\)\(^17\)

Despite these findings, there is a lack of evidence describing the effects of IL-1Ra therapy on bone remodelling after mechanical loading. Therefore, the aim of this study was to investigate the effects of IL-1Ra administration on OTM in a mouse model.

### 2. Materials and methods

#### 2.1. Experimental animals

Thirty five ten-week-old wild-type mice (WT) (C57BL/6J) were used in this study. For histomorphometric analysis, 10 mice with orthodontic appliance were used. In this set of experiments, the left side of maxillae (without orthodontic appliance) was used as control. For biochemical analysis, 20 mice received the orthodontic appliance and treatments described below, and 5 mice did not receive appliance, being used as the control group. All animals were treated under ethical regulations for animal experiments, defined by the Institutional Ethics Committee. Each animal’s weight was recorded throughout the experimental period and there was no significant loss of weight.

#### 2.2. Experimental protocol

The experimental protocol was based on a previous study.\(^18\) Briefly, mice were anaesthetized and a Ni-Ti 0.25 mm × 0.76 mm coil spring (Lancer Orthodontics, San Marcos, CA, USA) was bonded by a light-cured resin (Transbond, Unitek/3M, Monrovia, CA, USA) between the maxillary right first molar and the incisors. The force magnitude was calibrated by a tension gauge (Shimpo Instruments, Itasca, IL, USA) to exert a force of 0.35 N applied in the mesial direction. There was no reactivation during the experimental period. Thereafter, mice were randomly divided in two groups for histomorphometric analysis: mice treated with vehicle (PBS) (vehicle group) or with IL-1Ra (daily administration [s.c.] of 10 mg/kg/day IL-1Ra [Biogen INC, Geneva, Switzerland]) (IL-1Ra group). For biochemical assays, three groups were created: mice without appliance (control group) and mice with activated coil spring (experimental group) treated with PBS (vehicle group) or with IL-1Ra (IL-1Ra group). At the end of the experiments, mice were euthanized with an overdose of anaesthetic at the following times: 12 days after orthodontic appliance placement for histological measurements, and 12 h and 72 h for biochemical analysis. For every set of experiments, 5 mice/group were used for each time-point.

#### 2.3. Histopathological analysis

The right and left halves of maxillae, including first, second, and third molars, were dissected, fixed in 10% buffered formalin (pH 7.4) and rinsed in distilled water. Thereafter, each semi-maxilla was decalcified in 14% EDTA (pH 7.4) for 14 days and embedded in paraffin. Samples were cut into sagittal sections of 5 μm thickness. Sections were stained for tartrate-resistant acid phosphatase (TRAP; Sigma–Aldrich, St. Louis, MO, USA), counterstained with haematoxylin, and used for histological examination. The first molar distobuccal root, on the coronal two-thirds of the mesial periodontal site, was used for osteoclast counting on 5 non-consecutive sections (40 μm apart one from the other) per mouse. Osteoclasts were identified as TRAP-positive, multinucleated cells on the bone resorption lacunae.

#### 2.4. Measurement of tooth movement

Image J software (National Institutes of Health) was used to quantify the amount of tooth movement, as previously described.\(^18\) Tooth movement was obtained through the difference between the distance of the cementum-enamel-junction’s (CEJ’s) of the first molar and the second molar (1st and 2nd molar distance) of the experimental side (right hemi-maxilla) in relation to the control side (left hemi-maxilla) of the same animal. Five sections per mouse were evaluated under a microscope Axioskop 40 (Carl Zeiss, Göttingen, Niedersachsen, Germany) adapted to a digital camera (PowerShot A620, Canon, Tokyo, Honshu, Japan). Three measurements were conducted for each evaluation and the variability was below 5%.

#### 2.5. Measurement of cytokine levels

Periodontal ligament and surrounding alveolar bone samples from the areas adjacent to the upper first molars were obtained using a stereomicroscope. Samples were weighed and homogenized in PBS (0.4 mM NaCl and 10 mM NaPO\(_4\)) containing protease inhibitors (0.1 mM PMSF, 0.1 mM benzethonium chloride, 10 mM EDTA, and 0.01 mg/mL aprotonin A) and 0.05% Tween-20 at 1 mg/mL. The homogenate was centrifuged (8946 × g) at 4 °C for 10 min. The supernatant was then collected and stored at −70 °C until further analysis. The levels of IL-1β, TNF-α and IL-10 were evaluated by double-ligand enzyme-linked immunosorbent assay (ELISA), according to the manufacturer’s protocol (R&D Systems, Minneapolis, MN, USA). The results were expressed as picograms of cytokine/100 mg of tissue.

#### 2.6. Statistical analysis

The results were expressed as the mean ± standard error of the mean (SEM). Comparison amongst the groups was
statistically analysed by one-way analysis of variance (ANOVA), followed by the Newman–Keuls multiple comparison test. \( P < 0.05 \) was considered statistically significant.

3. Results

3.1. Tooth movement and TRAP-positive cells

The amount of OTM was significantly less in mice treated with IL-1Ra (Fig. 1A), as well as the number of TRAP-positive osteoclasts (Fig. 1B), when compared to the vehicle group after 12 days of mechanical loading.

Histological characterisation of periodontal tissues also revealed that IL-1Ra treated mice demonstrated a decreased TRAP activity and a smaller number of osteoclasts in the pressure side of the periodontium (Fig. 2E and F), when compared to the experimental tooth of vehicle treated mice (Fig. 2C and D).

3.2. Levels of cytokines in periodontal tissues

The smaller amount of OTM observed in IL-1Ra treated mice led us to investigate the effects of such therapy on the expression of cytokines involved in bone remodelling. Mechanical loading applied to tooth triggered a significant release of pro-inflammatory and bone resorptive cytokines in periodontal tissues just after 12 h of stimulation. Whilst the levels of IL-1\( \beta \) (Fig. 3A) and TNF-\( \alpha \) (Fig. 3B) increased approximately 6 and 5.5 fold, respectively, IL-10 levels (Fig. 3C) were not altered when compared to control mice. After 72 h of mechanical loading, IL-1\( \beta \) levels were almost 10 times higher than control (Fig. 3A), and the levels of TNF-\( \alpha \) (Fig. 3B) and IL-10 (Fig. 3C) were similar to the basal condition. In contrast, treatment of mice with IL-1Ra reduced the inflammatory milieu observed in periodontal tissues after stimuli. IL-1Ra therapy induced a decrease of 66% and 76% in the levels of IL-1\( \beta \) (Fig. 3A) and TNF-\( \alpha \) (Fig. 3B), respectively, when compared to vehicle-treated mice, whilst the levels of IL-10 (Fig. 3C) enhanced approximately 2 fold either at 12 or at 72 h after mechanical loading.

4. Discussion

Interleukin-1 (IL-1) has been one of the most studied cytokines and it is one of the major soluble proteins related to osteoclast activation and bone resorption.\(^5\,^6\) Evidence for the existence of IL-1 controlling pathways indicates that the IL-1 receptor antagonist (IL-1Ra) can be important for the \textit{in vivo} regulation of IL-1\( \beta \) activity in bone resorptive conditions.\(^7\,^8\) However, the effect of IL-1Ra on bone remodelling after mechanical loading is not well described. In the present study, administration of IL-1Ra diminished OTM by reducing the expression of the pro-inflammatory cytokines IL-1\( \beta \) and TNF-\( \alpha \), and by increasing the levels of IL-10, a negative regulator of bone resorption.

When an orthodontic force is applied on teeth, it leads to a transient aseptic inflammation of the periodontium that culminates in bone remodelling.\(^1\) In this context, bone resorption is a fundamental step and several cytokines associated to osteoclast differentiation and activation, such as TNF-\( \alpha \) and IL-1\( \beta \), are early released in the periodontium after mechanical loading.\(^3\,^4\,^18\,^20\) Accordingly, the levels of these cytokines were increased in our experimental conditions, whilst the levels of IL-10, a cytokine known to control bone resorption and osteoclast activation,\(^2\) were not affected.

In view of the importance of this inflammatory milieu to bone resorption, it has been suggested that the control of such inflammation could affect OTM. A previous study showed that an interference with TNF-\( \alpha \) activity might decrease osteoclast migration and, consequently, diminish OTM.\(^18\) In this regard, administration of IL-1Ra to interfere with IL-1\( \beta \) activity could also alter mechanically induced bone remodelling. IL-1Ra, first called IL-1 inhibitor, was cloned and identified as an IL-1 receptor antagonist after being noticed to bind to IL-1 receptors but not to transduce the same signals that IL-1\( \beta \) did.\(^22\,^23\) Thus, IL-1Ra acts by competitively blocking the interactions of IL-1 to their receptors, inhibiting its activities.\(^7\,^8\) Indeed, the administration of exogenous IL-1 receptor antagonist has been shown to be effective in reducing signs of IL-1-related bone resorptive conditions, such as rheumatoid arthritis\(^10\) and periodontal disease,\(^11\) concomitantly with a
reduction of pro-inflammatory cytokines. In this regard, a decreased physiological IL-1Ra expression in gingival crevicular fluid has been shown to correlate with faster OTM in humans. 

In the present study, mice treated with IL-1Ra showed significantly diminished OTM and osteoclast numbers than vehicle-treated animals. This phenotype was associated with reduced early release of TNF-α and IL-1β, concomitantly to increased expression of IL-10 on periodontal tissues. The present results give support to previous findings showing that administration of soluble IL-1 receptors reduces the amount of OTM in rats and go further when showing that this effect occurs by controlling the expression of cytokines. The data strongly indicates that interfering with the IL-1β pathway may impair the pro-inflammatory and pro-osteoclastic milieu on periodontal tissues, and, consequently, decrease OTM. In accordance, in vitro studies have shown that IL-1 receptor antagonists can inhibit the compressive force-induced expression of RANKL (receptor activator of nuclear factor kappa B ligand), a positive regulator of osteoclast differentiation and activation, by periodontal ligament cells. Similarly, the expression of TNF-α after application of compressive forces in vitro was decreased with the addition of IL-1Ra to cell cultures.

In conclusion, the present study suggests that IL-1Ra might affect bone remodelling after mechanical loading probably by its anti-inflammatory actions, such as the reduction of pro-inflammatory and bone resorptive cytokines and the increase in IL-10 expression.
of anti-inflammatory cytokine. Furthermore, analysis of our data provides new insights into the development of future therapeutic interventions with IL-1Ra, which could modulate the amount of OTM and restrain the relapse of the final orthodontic result.

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**Competing interests:** The authors state no conflict of interest.

**Ethical approval:** Mice were treated under ethical regulations for animal experiments, defined by the Institutional Ethics Committee (Universidade Federal de Minas Gerais), which approved the experimental procedures adopted in the study (protocol number 135/08).

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