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Research Article

Predicting the Effects of Medication and Nutrients on Orthodontic Tooth Movement via the Receptor Activator of Nuclear Factor kappa-B Ligand/Osteoprotegerin (RANKL/OPG) Ratio in Cultured Human Periodontal Ligament Fibroblasts

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Abstract

Aim: To develop an *in-vitro* system that predicts how specific medications and nutrients affect Orthodontic Tooth Movement (OTM), facilitating personalized treatment recommendations for orthodontic patients.

Methodology: Human Periodontal Ligament (PDL) fibroblasts were isolated from extracted teeth and cultured. The cells were exposed to Levocetirizine (5 μM and 10 μM), Risperidone (50 μM and 100 μM), Red Bull® energy drink (10% and 20% v/v), ATRA (2.0 and 4.0 μM) and Propylene Glycol (PG, 3% and 4% v/v). Cell viability was assessed using a Cell Counting Kit-8 (CCK-8) assay, which measures mitochondrial activity. A LUMINEX bead assay was used to quantify RANKL and OPG levels in the culture medium. Immunofluorescence staining and Fiji2 analysis were performed to quantify membrane-bound RANKL expression.

Results: Propylene glycol significantly increased both Receptor Activator of Nuclear Factor kappa-B Ligand (RANKL) and Osteoprotegerin (OPG) levels, indicating a potential enhancement of OTM. Risperidone (100 μM), the energy drink (10% v/v), ATRA (2.0 and 4.0 μM) and Propylene Glycol (3% and 4% v/v) significantly decreased OPG levels without affecting RANKL levels, suggesting increased OTM. Levocetirizine (5 and 10 μM), Risperidone (50 μM) and energy drink (20% v/v) had no significant effect on RANKL or OPG levels, implying a negligible impact on OTM.

Conclusion: This *in-vitro* system effectively predicted the effects of various substances on the RANKL/OPG ratio, providing valuable insights for orthodontists in advising patients on how medications and dietary habits may influence orthodontic outcomes.

Keywords: Orthodontic Tooth Movement; RANKL; Orthodontic Tooth Movement (OTM); Periodontal Ligament Fibroblasts; Bone Remodeling

Abbreviations

ATRA: All-Trans Retinoic Acid; CCK-8: Cell Counting Kit-8; DMSO: Dimethyl Sulfoxide; DMEM: Dulbecco's Modified Eagle Medium; MMPs: Matrix Metalloproteinases; OPG: Osteoprotegerin; OTM: Orthodontic Tooth Movement; PDL: Periodontal Ligament; PG: Propylene Glycol; RANKL: Receptor Activator of Nuclear Factor kappa-B Ligand; Risp: Risperidone

Introduction

Orthodontic Tooth Movement (OTM) occurs when orthodontic forces are applied to a tooth causing it to shift through the alveolar bone.1 While the precise biological mechanisms underlying this process are not fully understood, it is well established that Periodontal Ligament (PDL) fibroblasts, osteoblasts and osteoclasts are critical for OTM [1].

Significant inter-individual variability in OTM rates is observed under identical experimental conditions, with patients categorized as either "slow movers" or "fast movers" [2]. This variability reflects the multifactorial nature of OTM, which is influenced not only by the magnitude of the applied force but also by factors such as medication use, growth, environmental exposures and genetic background [3]. Differences in medication and nutritional intake are likely contributors to this variability, highlighting the importance of understanding factors that affect OTM in order to enable more personalized orthodontic care.

OTM can be divided into four distinct phases. In the initial phase, which occurs seconds after the application of orthodontic force, the tooth shifts slightly within its socket1 as the PDL matrix and alveolar bone are strained, initiating movement [4]. Compression of blood vessels during this phase reduces blood flow and deforms endothelial cells, resulting in local ischemia and necrosis of PDL cells [5]. Lysosomal enzymes are then released to degrade the extracellular matrix [5]. Before further tooth movement can occur, the necrotic, hyalinized tissue must be cleared by granulocytes and macrophages [5]. During the second or "hyalinization," phase, undermining bone resorption begins as osteoclasts form in the medullary cavities of the alveolar bone, likely in response to fluid shifts causedby bone deformation [5]. Osteocytes may transmit such signals, stimulating osteoclastogenesis, while factors from mechanically stimulated PDL cells further promote osteoclast precursor activation and differentiation [1]. Once the hyalinized tissue is removed by phagocytes, new blood vessels form, followed by the deposition of collagen [5]. The subsequent phases are characterized by tension-side PDL collagen fiber elongation, which activates osteoblasts and cementoblasts. As the process continues, osteoclasts and osteoblasts coordinate bone resorption on the compression side and bone deposition on the tension side of the tooth [6,7]. The third phase involvesslow tooth movement, whereas the fourth phase is marked by linear progression. Osteoclastogenesis is primarly regulated by Receptor Activator of Nuclear Factor Kappa B Ligand (RANKL), which is expressed on PDL fibroblasts and osteoblasts in response to orthodontic force [7,8].

Osteoprotegerin (OPG) acts as a decoy receptor for RANKL, inhibiting osteoclast formation by preventing RANKL from binding to RANK [9]. The RANKL/OPG balance is crucial in controlling bone resorption and osteoclastogenesis. The importance of RANKL-RANK signaling in bone remodeling and OTM has been well established: RANKL promotes OTM, whereas RANKL depletion inhibits it [7]. Fibroblasts play a key role in PDL remodeling, while osteoclasts are responsible for bone resorption that drives tooth movement [1].

Both medication and nutrition intake influence OTM rates [4]. Certain drugs, such as NSAIDs, tend to slow OTM, whereas nutrients like vitamin D accelerate it [4,10]. However, the effects of many commonly used medications and nutrients on OTM remain unclear (Table 1). This study aims to develop an *in-vitro* system to elucidate these effects by examining the influence of selected foods and medications on the RANKL/OPG ratio, a known predictor of osteoclast formation and OTM. This knowledge may help orthodontists provide more tailored recommendations regarding treatment planning and duration, based on an individual patient's medication and dietary profile. The introduction should present the background of the study, the research question and the objectives. It should also briefly review relevant literature and explain the significance of the study.

Substance	Characteristics	Indication or Use	References
Levocetirizine Sigma)	Antihistaminic	Allergy symptoms	11
Risperidone (Sigma)	Atypical antipsychotic	Autism, spectrum disorder, schizophrenia, bipolar disorder	12
Energy drink (Red Bull ®)	A beverage containing caffeine and taurine	Used to provide an energy boost	13
All-Trans Retinoic Acid (ATRA)	Active metabolite of Vitamin A	Fish, eggs, dairy products, fruits, vegetables, etc.	14
Propylene glycol	Solvent	Electronic cigarette liquid, mouthwash, pharmaceuticals, edible items, etc.	15, 16

Table 1: Study's selection of frequently used foods and medications.

Methodology

Cell Culture

Human Periodontal Ligament (PDL) tissue was obtained from a healthy extracted third molar, as described by Henneman, et al. [17]. The tissue explants were placed in a 24-well culture plate and incubated at 37°C in a humidified atmosphere with 5% CO_2 in air. The culture medium was refreshed three times per week.

CCK-8 Cell Viability Assay

A Cell Counting Kit-8 (CCK-8) assey was used to detect mitochondrial activity and assess the effects of the various solutions on cell viability. After removing the medium, 200 µL of 10% CCK-8 reagent was added to each well of a 96-well plate containing adherent PDL cells, which were then incubated for 1.5 hours at 37°C. Absorbance at 450 nm was measured using a BioTek Gen microplate reader. Following the CCK-8 assay, cells were fixed with formalin for subsequent immunofluorescence staining [5].

Preparation of Conditioned Medium After PDL Cell Exposure to Test Compounds for Luminex Analysis

PDL cells (10^4 cells/well) were seeded into 96-well culture plates and cultured in DMEM for 24 hours. After washing with DMEM, cells were incubated for 48 hours with various concentrations of Levocetirizine (5 and 10 µmol/L in 0.1% DMSO), Risperidone (50 and 100 µM), energy drink (Red Bull ®) (10 and 20% v/v), ATRA (2.0 and 4.0 µM), Propylene Glycol (PG, 3 and 4% v/v) in DMEM [18-20]. Three wells were used per concentration, four as controls and six for DMSO (0.1%) controls. After 48 hours of incubation, the medium was collected, centrifuged (1500 rpm, 5 minutes) and the supernatant stored at -20°C for Luminex analysis of RANKL and OPG.

LUMINEX Beads Assay for RANKL and OPG Quantification

RANKL and OPG in the conditioned medium were measured using a Luminex Human ProcartaPlex Simplex Kit on a Luminex Bio-Rad Bio-Plex™ 200 System, per manufacturer's (BIO-RAD) instructions. Samples, standards and controls, were tested in triplicate with antibody-coated fluorescent beads to detect Osteoprotegerin (OPG) or RANKL. After incubation and washing, detection was completed using biotinylated detection antibodies and streptavidin-phycoerythrin.

Immunofluorescence Staining of RANKL and Quantification

To visualize and quantify RANKL on PDL cells, immunofluorescence staining was performed. Cells were first preincubated in a blocking buffer (1% Normal Donkey Serum (NDS), 10% w/v Bovine Serum Albumin (BSA), 0.5% v/v Triton X-100 and 0.5% v/v Tween-20 in PBS) for 30 minutes. Next, the cells were incubated overnight at 4°C with the primary rabbit anti-RANKL antibody (1:200; Korain Biotech, Shanghai, China). Two wells served as negative controls for the primary antibody. After washing, Alexa Fluor 647 goat anti-rabbit antibody (1:200; Invitrogen, Waltham, USA) was applied for 1 hour. Nuclei were stained with DAPI (4′,6-diamidino-2-phenylindole, 0.4 µg/mL in PBS) for 10 minutes. Finally, 20 µL of mounting medium (0.5% DABCO (1,4-diazabicyclo-(2.2.2)octane) in PBS) was added to each well.

Imaging and Quantification

Multi-channel 8-bit images of RANKL immunofluorescence staining were acquired at 100x magnification on an Axio Observer 7 fluorescence microscope (Zeiss, Jena, Germany), with constant saturation, brightness and exposure settings. RANKL expression levels in widefield epifluorescence images were quantified using a custom FIJI macro (https://github.com/jelupo/FIJI-macros/tree/main/WagenerHasanzadah%20expression%20quantification). Briefly, nuclei (channel 1) and cell-populated areas (channel 1) were separately identified using user-specified Labkit classifiers.

Various intensity distribution measurements in channel 2 (signal of interest) were reported. These included total cell area, average fluorescence in cell regions, user-specified background value-corrected cumulative signals and fluorescence normalized per detected nucleus. GPU acceleration via CLIJ2 is supported but optional. ImageJ/FIJI software version 2.3.0 was used for the image analysis. A Fiji macro sequence was designed to quantify the average fluorescence in cell regions, corrected for background levels and was automatically applied to all images. Macro validity was confirmed by high/low value comparisons (Fig. 1).

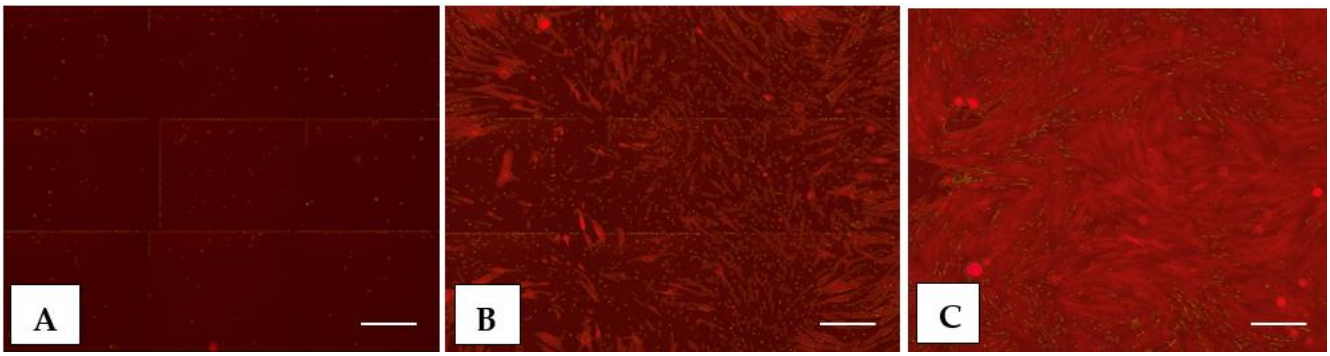

Figure 1: Expression levels in widefield epifluorescence images were quantified using custom FIJI macro (github.com/jelupo/FIJI-macros/blob/main/Wagener-Hasanzadah expression quantification). Briefly, RANKL fluorescence in cell regions was measured in a negative control without the first antibody (A), low-expressing (B) and high-expressing RANKL PDL fibroblasts (C). Scale bar for all images: 500 μm.

Statistical Analysis

Data are presented as means. GraphPad Prism version 9.00 (GraphPad Software, San Diego, CA, USA) was used to analyze the data. Statistical significance was assessed using one-way ANOVA with a Tukey's post-hoc test after Kolmogorov-Smirnov normality testing. P-values < 0.05 were considered statistically significant.

Results

Effects of Putative OTM Modulators on Cell Survival

PDL fibroblast cell viability (cell survival) was assessed using the CCK-8 assay, which measures mitochondrial activity, after exposure to Levocetirizine (5 and 10 μM in 0.1% DMSO), Risperidone (50 and 100 μM), Energy drink (10 and 20% v/v), All-Trans Retinoic Acid (ATRA)(2 and 4 μM) and Propylene Glycol (3 and 4% v/v). Fig. 2 presents absorbance values for Risperidone, energy drink, ATRA and Propylene Glycol relative to the control (normalized to 1). Levocetirizine results were compared to a DMSO control (also set at 1).

All treatment groups exhibited comparable mitochondrial activity. No statistically significant differences in mitochondrial activity were found between any treated group and their respective controls (Fig. 2). These findings indicate that the tested concentrations of Levocetirizine, Risperidone, Energy drink, ATRA and Propylene Glycol do not impair the mitochondrial activity or overall survival of PDL fibroblasts.

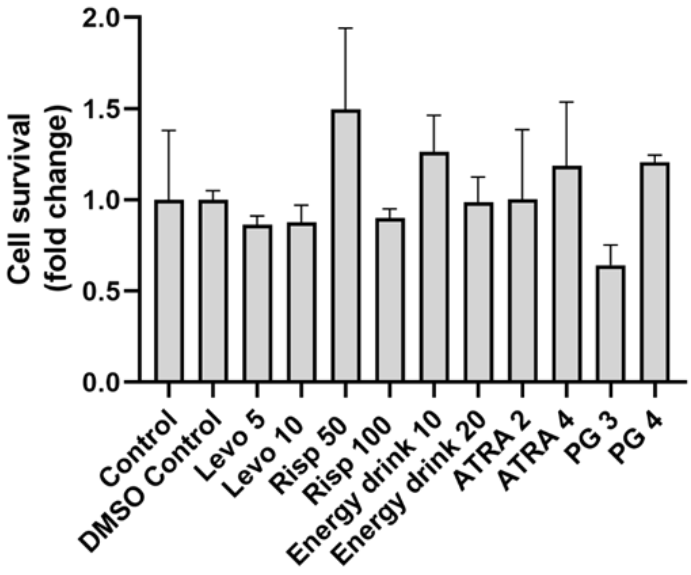

Figure 2: CCK-8 assay evaluating the potential cytotoxicity of selected treatments on PDL fibroblasts.

Cells were incubated for 48 hours with Levocetirizine (Levo, 5-10 μM), Risperidone (Risp, 50-100 μM), Energy drink (Red Bull, 10-20% (v/v)), ATRA (2-4 μM) or Propylene Glycol (PG, 3-4% (v/v)). Mitochondrial activity was measured 1.5 hours after reagent addition. Absorbance readings for Risperidone, Energy drink, ATRA and Propylene Glycol are shown relative to the untreated control (set at 1); Levocetirizine values are compared to the DMSO control (also set at 1). No significant differences in mitochondrial activity were observed across groups (P>0.05), indicating no detectable cytotoxicity at the tested concentrations.

Effects of Putative OTM Modulators on RANKL and OPG in Conditioned Medium of Treated PDL Cells

A Luminex bead assay was used to quantify OPG and RANKL levels in the conditioned medium following a 48-hour exposure of PDL fibroblasts to Levocetirizine (5 and 10 μM), Risperidone (50 and 100 μM), Energy drink (10% and 20% v/v), ATRA (2 and 4 μM) and Propylene Glycol (3 and 4% v/v). RANKL concentrations in the conditioned medium were below the assay's detection limit (data not shown), while OPG was readily detectable.

Fig. 3 displays OPG levels in the medium for Risperidone, Energy drink, ATRA and Propylene Glycol relative to the control (set at 1); Levocetirizine values are shown relative to the DMSO control (also set at 1). Significant reductions in OPG were observed following exposure to Risperidone (100 μM), Energy drink (10% (v/v)), ATRA (2.0 and 4.0 μM) and Propylene Glycol (3% and 4% (v/v)) compared to non-treated control cells (769 pg/ml). Among these, the medium from PDL cells exposed to Risperidone (100 μM) yielded the highest OPG value (482 pg/ml), while exposure to 4% PG solution resulted in the lowest OPG level (111 pg/ml) (Fig. 3). For Levocetirizine (5 and 10 μM in 0.1% DMSO;) OPG levels did not differ significantly from the DMSO control.

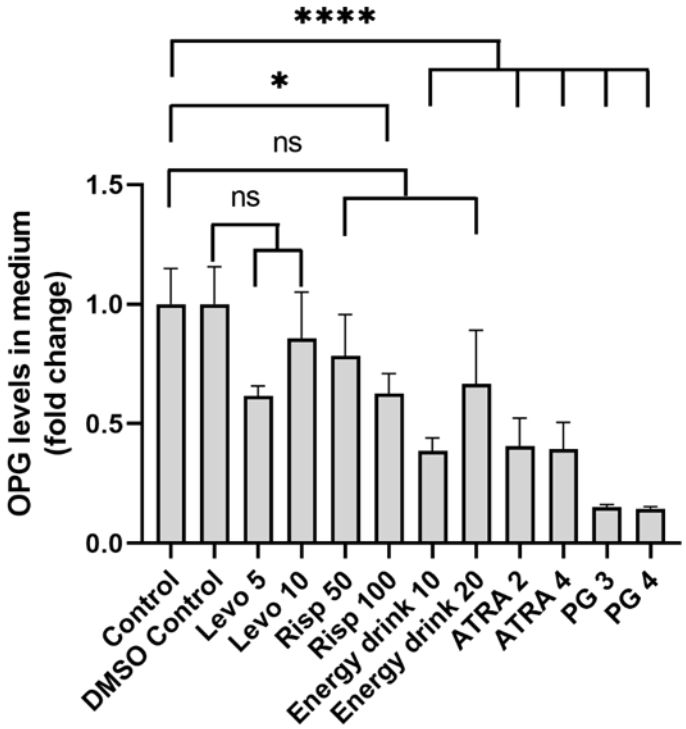

Figure 3: OPG levels in the culture medium of PDL fibroblasts after 48 hour incubation with Levocetirizine (Levo, 5-10 μM), Risperidone (Risp, 50-100 μM), Energy drink (Red Bull, 10-20% (v/v)), ATRA (2-4 μM) and Propylene Glycol (PG, 3-4% (v/v)), quantified by Luminex bead assay.

OPG values for Risperidone, Energy drink, ATRA and Propylene Glycol are shown relative to untreated controls (set at 1); Levocetirizine is compared to DMSO control (set at 1). * $P \leq 0.05$, **** $P \leq 0.0001$.

Effects of Putative OTM Modulators on Cellular RANKL Expression

Since RANKL was not secreted into the medium, membrane-bound RANKL expression on PDL cells was analyzed using immunofluorescence staining and quantified via FIJI (Fig. 4). Representative images are shown in Fig. 4, where RANKL is

visualized in red and the cell nuclei are blue. Notably, PDL fibroblasts exposed to 4% PG (Fig. 4) displayed a more rounded morphology compared to controls (Fig. 4). To quantify the amount of membrane-bound RANKL, the average immunofluorescence intensity in cell regions was measured using an image processing program, allowing for comparisons (see Materials and Methods). Fig. 5 depicts normalized RANKL immunofluorescence intensity for each treatment. Only PDL fibroblasts treated with 4% PG exhibited significantly higher RANKL signal compared to control cells. No significant changes were observed for Levocetirizine (5 and 10 μM), Risperidone (50 and 100 μM), Energy drink (10% and 20%) or Propylene Glycol at 3% relative to their controls. The lowest fluorescence intensities were observed with 20% Energy drink and 10 μM Levocetirizine (both circa 30), while 4% PG produced the highest signal (circa 89) (Fig. 5).

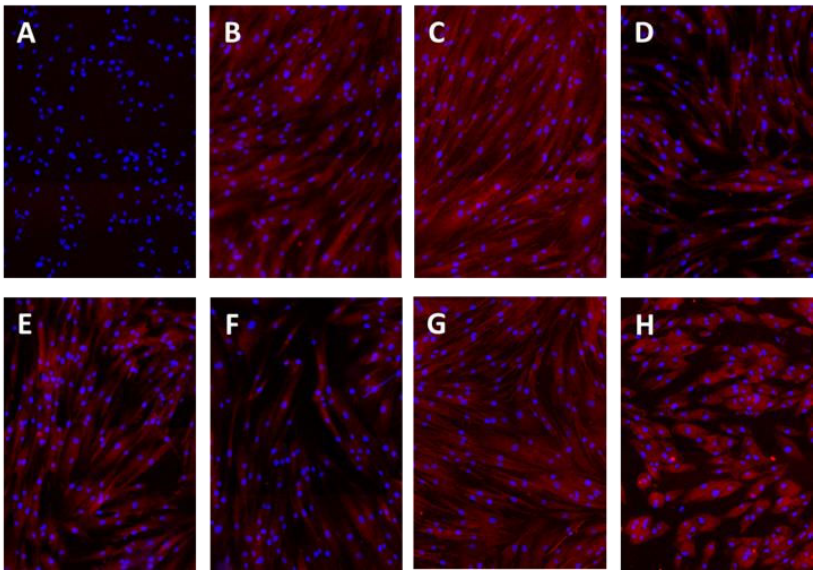

Figure 4: Effects of putative OTM modulators on RANKL immunofluorescence in PDL fibroblasts. RANKL is depicted in red; cell nuclei are stained blue with DAPI. Panels: (a) Negative control: PDL fibroblasts exposed only to the secondary antibody, displaying blue nuclei. (b) Untreated PDL fibroblasts. (c) cells incubated with 0.1% DMSO. (d) treatment with 10 μM Levocetirizine in 0.1% DMSO for 48 hours. (e) treatment with 100 μM Risperidone for 48 hours. (f) treatment with 20% Energy drink (Red Bull) for 48 hours. (g) treatment with 4.0 μM ATRA for 48 hours. (h) treatment with 4% Propylene Glycol for 48 hours. Scale bar: 200 μm for all panels.

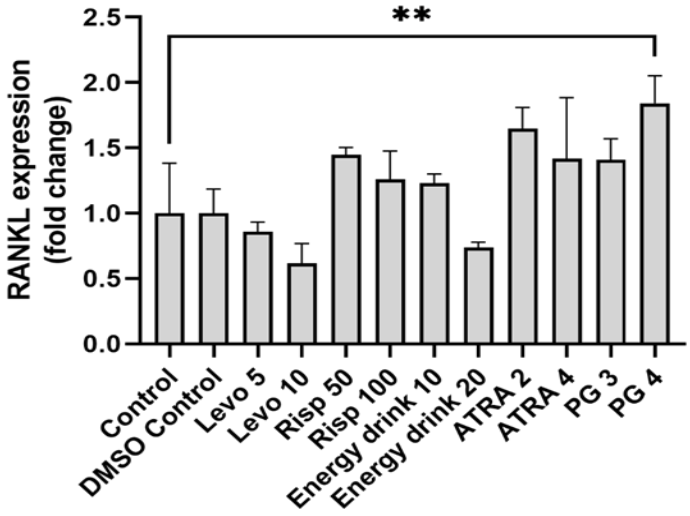

Figure 5: Quantification of RANKL expression in PDL cells as average immunofluorescence per cell region. The average immunofluorescence intensity was normalized to the control (set at 1). Data for Risperidone (Risp) (50-100 μM), Energy drink (Red Bull, 10-20% (v/v)), ATRA (2-4 μM) and Propylene Glycol (PG, 3-4% (v/v)) are compared to untreated control. Levocetirizine (Levo, 5-10 μM) is shown relative to DMSO control (set at 1). *P ≤ 0.05.

Effects of Putative OTM Modulators on the RANKL/OPG Ratio

Table 2 summarizes the predicted effects of Levocetirizine, Risperidone, Energy drink, ATRA and PG on the RANKL/OPG ratio and, hence, on OTM relative to controls (all values normalized to 1). Based on Fig. 3-5, PG at 4% was the only substance that both significantly increased RANKL levels (>1) and decreased OPG levels (<1), resulting in a pronounced increase in the RANKL/OPG ratio. Risperidone (100 μM), Energy drink (10%), ATRA (2.0 and 4.0 μM) and PG (2%) significantly decreased OPG levels (<1), without changing RANKL levels (=1), which may also elevate the RANKL/OPG ratio and enhance OTM. No significant effects were detected for Levocetirizine (5 and 10 μM), Risperidone (50 μM) or Energy drink (20%) on either RANKL (=1) or OPG levels (=1), indicating no expected change in OTM under these conditions.

Substance	RANKL	OPG	RANKL/OPG Ratio	Predicted OTM
Control	1	1	1	Unchanged
DMSO Control	1	1	1	Unchanged
Levo (5 μM)	1	1	1	Unchanged
Levo (10 μM)	1	1	1	Unchanged
Risp (50 μM)	1	1	1	Unchanged
Risp (100 μM)	1	<1	>1	Increased
Energy drink (10%)	1	<1	>1	Increased
Energy drink (20%)	1	1	1	Unchanged
ATRA (2 μM)	1	<1	>1	Increased
ATRA (4 μM)	1	<1	>1	Increased
PG (3%)	1	<1	>1	Increased
PG (4%)	>1	<1	>1	Increased

Table 2: Predicted effects of frequently used foods and medications on Orthodontic Tooth Movement (OTM).

Discussion

In this study, we aimed to create an *in-vitro* system for predicting the effects of common foods and medications on Orthodontic Tooth Movement (OTM) by assessing the RANKL/OPG ratio. Although the exact mechanisms affecting the RANKL/OPG ratio are not fully understood, this ratio remains a critical predictor of osteoclast formation, crucial for bone remodeling and orthodontic treatment outcomes. We tested our system by exposing PDL fibroblasts to various concentrations of frequently used substances, including Levocetirizine (the active R-enantiomer of the antihistamine cetirizine), Risperidone (an antipsychotic), Energy drink (Red Bull®), ATRA (an active metabolite of Vitamin A) and Propylene Glycol (PG, a solvent found in e-cigarettes and mouthwashes).

We measured and quantified RANKL and OPG concentrations in the medium of treated cells. All tested samples had RANKL levels in the medium below the detection limit, suggesting that membrane-bound RANKL on PDL fibroblast cells is normally not cleaved by Matrix Metalloproteinases (MMPs) to form soluble RANKL [21]. Significantly lower OPG concentrations were observed in the conditioned medium following exposure to Risperidone (100 μM), energy drink (10%), ATRA (2.0 and 4.0 μM) and PG (2% and 4%) compared to controls. This indicates that these substances generally inhibit OPG secretion by PDL cells, potentially increasing the RANKL/OPG ratio and enhancing OTM if RANKL expression remains constant or rises. Previous studies have shown that membrane-bound RANKL is more effective in stimulating osteoclastogenesis than its soluble form [22,23].

Membrane-bound RANKL was successfully detected and quantified via immunofluorescence staining. Among the tested solutions, only Propylene Glycol (PG, 4%) - a widely used humectant - significantly increased RANKL expression and reduced OPG levels relative to the controls. This suggests a potential rise in the RANKL/OPG ratio and may augment OTM. However, the specific impact of Propylene Glycol on the RANKL/OPG ratio or OTM is not well documented in the literature. For instance, a clinical study by Ibraheem, et al., reported elevated levels of both RANKL and OPG in crevicular fluid of e-cigarette users compared to non-smoking controls [24]. This contrasts with our findings of increased RANKL and decreased secreted OPG. Considering Propylene Glycol is just one component of e-cigarettes other ingredients may also influence these outcomes.

Levocetirizine, the biologically active enantiomer of cetirizine, did not significantly change RANKL or OPG levels compared to controls, suggesting it does not significantly affect OTM [25]. Although the highest tested concentration of Levocetirizine (10 μM) yielded the lowest RANKL expression, this reduction was not statistically significant. Previous studies on the parent drug of levocetirizine, cetirizine, by Meh, et al. and Križnar, et al., have demonstrated significantly decreased OTM [26,27]. However, recent research suggests this effect may depend on experimental context, warranting further investigation [28]. Risperidone, an atypical antipsychotic primarily administered for conditions such as autism and bipolar disorder, showed a dose-dependent effect on the RANKL/OPG ratio. At the highest concentration tested (100 μM), Risperidone significantly decreased OPG levels in the medium of PDL fibroblasts, while RANKL expression remained unchanged. This change could increase the RANKL/OPG ratio, consequently enhancing OTM. Although no studies have directly examined Risperidone's impact on OTM, *in-vitro* research by Zheng, et al., demonstrated significant downregulation of both OPG and RANKL expression in pre-osteoblast cells [18]. However, they did not examine these markers in human PDL fibroblasts. An animal study by Motyl, et al., found that Risperidone induced trabecular bone loss in mice, likely due to elevated RANKL expression, although no changes in OPG expression were observed [29].

Energy drinks, containing sugar, caffeine and taurine, also demonstrated a dose-dependent effect on the RANKL/OPG ratio. In our study, the lowest concentration tested (10% v/v) significantly lowered OPG levels in the medium of PDL fibroblasts, while RANKL expression on PDL fibroblasts remained unaffected. This could elevate the RANKL/OPG ratio and increase OTM. While no research to date has specifically assessed the effects of energy drinks on OTM, prior studies have explored the individual components. Zhang et al. showed that glucose upregulates RANKL and downregulates OPG in human PDL fibroblasts [30]. Yi, et al., found that caffeine significantly accelerated OTM and increased RANKL expression in PDL cells under compression, with no effect on OPG levels [31]. Moreno, et al., demonstrated that caffeine induces alveolar bone loss in rats during OTM, correlating with elevated RANKL and no change in OPG [32]. A recent systematic review and meta-analysis confirmed that caffeine speeds up OTM after three weeks of force application, although high doses may diminish this effect. These findings align with our observations regarding exposure to energy drinks [33]. For taurine, Yuan, et al., found that it decreased the number of osteoclasts but did not affect RANKL or OPG expression in osteoblasts, suggesting taurine may directly inhibit osteoclastogenesis through taurine transporter-mediated uptake [34]. Collectively, current evidence indicates energy drinks, particularly their caffeine component, may influence bone remodeling during OTM by modulating the RANKL/OPG ratio. Given the popularity of energy drinks, further studies are warranted to better understand their impact on orthodontics and bone metabolism.

All-Trans-Retinoic Acid (ATRA), the active metabolite of Vitamin A, led to a significant decrease in OPG levels in the medium without notably altering membrane-bound RANKL expression. This could potentially increase the RANKL/OPG ratio and consequently, promote OTM. The inhibitory effect of ATRA on OPG levels was also reported by Jacobson, et al., who found that ATRA downregulates OPG secretion in osteoblasts. Although the soluble RANKL in their initial osteoblast cultures was undetectable, they observed that ATRA upregulated RANKL transcriptional levels in mRNA, thereby increasing the RANKL/OPG ratio [19]. By contrast, Nishio, et al., found no effect of retinoic acid on OTM in rats, while Ferreira, et al., observed reduced OTM with retinoic acid administration, reflecting inconsistency in the literature [35,36]. This study has several limitations. It is important to recognize that RANKL and OPG are produced by various cell types, such as osteoblasts, which can also affect the RANKL/OPG ratio [7]. Furthermore, excessive RANKL expression in periodontal tissues may lead to orthodontically induced External Root Resorption (ERR). Therefore, an elevated RANKL/OPG ratio is not always beneficial for OTM [21].

Future research should aim to validate our findings through *in-vivo* studies and explore the broader implications of these commonly consumed substances on orthodontic treatments. Gaining a deeper understanding of these effects could help clinicians classify patients as slow or fast movers and provide more informed advice on how their dietary and medical choices may impact orthodontic outcomes.

Conclusion

In conclusion, we have successfully developed an *in-vitro* system for evaluating the impact of commonly used substances on the RANKL/OPG ratio in PDL fibroblasts, a key determinant of OTM. Our results show that Risperidone, Energy drinks, ATRA and Propylene Glycol, significantly decreased OPG levels, which could increase the RANKL/OPG ratio and potentially enhance OTM. Notably, Propylene Glycol was unique in also increasing RANKL expression, suggesting a potential enhancement of OTM.

Conflict of Interest Statement
All authors declare that there are no conflicts of interest.

Informed Consent Statement
Informed consent was obtained from the participant involved in this study.

Authors' Contributions
All authors have contributed equally to this work and have reviewed and approved the final manuscript for publication.

Financial Disclosure
The authors received no external financial support for this study.

Ethical Statement
Not applicable.

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