Number of osteoclasts, receptor activator of nuclear factor kappa

by Rini Devijanti

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Original Research Article

Number of osteoclasts, receptor activator of nuclear factor kappa-b ligand and osteoprotegerin expression in electrolyzed reduced water-treated orthodontic tooth movement in Wistar rats

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Abstract

Purpose: *To analyse the potential use of electrolyzed reduced water effect (ERW) in the treatment of orthodontic tooth movement in Wistar rats by means of osteoclast number, receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) expressions.*
Methods: *ERW was produced by an electrolysis machine that rendered the water pH alkaline. A dose of ERW 2.5 ml/100 g body weight was used for treatment for 7 days. The orthodontic tooth movement animal study was done by means of a rubber separator, with 0.0284 N force applied to the maxillary incisive tooth for 7 days. The rats were euthanized on days 3, 5 and 7 with the maxilla bone subsequently removed for immunohistochemistry examination. RANKL and OPG expression were evaluated by immunohistochemical staining and the osteoclast number determined with the aid of haematoxylin-eosin stain.*
Results: *ERW decreased the osteoclast number in the treatment group on day 3 and OPG expression on day 7 and there was significant difference between the groups ($p < 0.05$). RANKL expression decreased on Day 7. There was a significant difference between treatment groups on Days 5 and 7.*
Conclusion: *ERW significantly inhibits the number of osteoclasts, RANKL and OPG expression during orthodontic tooth movement after 3 and 7 days. ERW is thus a potential therapy for enhancement of bone remodeling in patients with orthodontic tooth movement.*

Keywords: *Electrolyzed reduced water, RANKL, Orthodontic tooth movement, Osteoclast, Osteoprotegerin*

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INTRODUCTION

Malocclusion is the third most common dental health problem after caries and periodontal disease [1]. The prevalence of malocclusion has reached 80 % and continues to increase annually [2]. Orthodontic Tooth Movement (OTM) occurs as a result of orthodontic mechanical strength

applications occupying an area as large as 20 - 25 g/cm^2 around the tooth. [3] This represents one determinant of successful treatment.

Tooth movement resulting from orthodontic treatment places the periodontal ligament under pressure and tension causing bone apposition and resorption [4]. Several attempts to accelerate the process of orthodontic treatment, including the administration of prostaglandins (PGE1 and PGE2) that have been used to enhance the bone resorption. Application of low-intensity laser therapy (LILT) involving the application of 4 joules / cm^2 at a wavelength of 650 nm can decrease the expression of Heat Shock Protein-70, Matrix Metalloproteinase - 8, and bone alkaline phosphatase [5-7].

Electrolyzed reduced water (ERW) is one medical therapy using water from the electrolysis process which demonstrates potential as an anti-inflammatory and antioxidant [8]. In a previous study, hydrogen-rich water consumption was found capable of suppressing ligature - induced inflammation in mice [9]. The ERW anti - inflammatory effect is thought to decrease the expression of the receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG) and the number of osteoclasts during OTM [10].

The purpose of this study was to investigate the effect of ERW on the number of osteoclasts, RANKL and OPG expression during OTM.

EXPERIMENTAL

The sample consisted of 36 healthy male Wistar rats (Rattus novergicus) aged 10 - 12 weeks and weighing 200 - 250 gram. 36-samples were randomly divided into six groups each further sub - divided into three control groups and three treatment groups. The control group was provided with mineral water, while sample of the treatment group received the dose of ERW 2.5 mL / 100 g for seven days via a standart animal laboratory drinking bottle.

The application of a 0.0284 N (Newton) power rubber separator to the maxillary left incisor for seven days was intended to induce orthodontic tooth movement in the control and treatment groups. This represented the optimum force for promoting orthodontic tooth movement in Wistar rats (R. norvegicus). According to the method by Lerner et al. [11], the optimal force for OTM in rat is 10g/mm^2 [12]. All samples were sacrificed on day 3, day 5 and day 7, anesthetized by means of rodent's anesthesia. The maxillary bone tissue was subsequently removed and placed in a buffered formalin solution (10 % buffer formalin solution with pH 7.0). 3.3'-Diaminobenzidine (DAB) and monoclonal antibody were used to examine the expression of RANKL and OPG. Meanwhile, Hematoxylin Eosin (HE) staining used to determine osteoclast number. Immunohistochemical results (IHC) were then observed with a light microscope (Olympus, Japan) at 400x magnification.

Statistical analysis

The data were subjected to analysis by Shapiro-Wilk test and subsequently by analysis of variance (ANOVA, $p < 0.05$) using a Statistical Package for the Social Sciences 20.0 (SPSS inc, IBM Corporation, Illinois, Chicago, USA).

RESULTS

In this study, all data was distributed normally (Tables 1, 2 and 3). Osteoclast was expressed in the periodontal tissue (Figure 1). The highest number of osteoclast found in K5 group and the lowest found in T3 group (Figure 2). There were significant different in osteoclast number between groups ($p < 0.05$) (Table 4). The RANKL expression was positively shown in periodontal tissue (Figure 3). The highest RANKL expression found in K5 group and the lowest found in T3 group (Figure 4). There were significant different in RANKL expression between K7 and T7 groups ($p < 0.05$) (Table 5). The RANKL expression was positively shown in periodontal tissue (Figure 5). The highest OPG expression found in K5 group and the lowest found in T7 group (Figure 6). There were significant different in RANKL expression between groups ($p < 0.05$) (Table 6).

Table 1: Normality test result for osteoclast number

Time (day)	Group	Normality
3	K3	0.197
	P3	0.154
5	K5	0.642
	P5	0.999
7	K7	0.800
	P7	0.056

Table 2: Normality test result for RANKL expression

Time (Day)	Group	Normality
3	K3	0.200
	P3	0.200
5	K5	0.915
	P5	0.882
7	K7	0.200
	P7	0.056

Table 3: Normality test result for OPG expression

Time (Day)	Group	Normality
3	K3	0.915
	P3	0.996
5	K5	0.847
	P5	0.999
7	K7	0.999
	P7	0.594

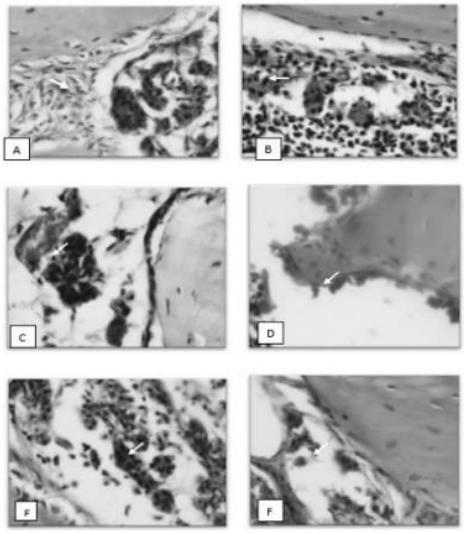

Figure 1: Number of osteoclasts in Wistar rat's periodontal tissue (white arrow) was observed by means of light Microscope (Olympus, US) at 400x magnification. A. Osteoclast number in the K3 group; B. Osteoclast number in the P3 group; C. Osteoclast number in the K5 group; D. Osteoclast number in the P5 group; E. Osteoclast number in the K7 group; F. Osteoclast number in the P7 group.

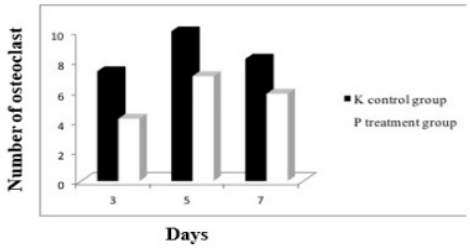

Figure 2: The number of osteoclast in each group

Table 4: ANOVA data for osteoclast number confirmed significant difference between groups

Time (day)	Group	Sig.
3	K3 P3	0.000*
5	K5 P5	0.002*
7	K7 P7	0.040*

Note: *significant at $p < 0.05$

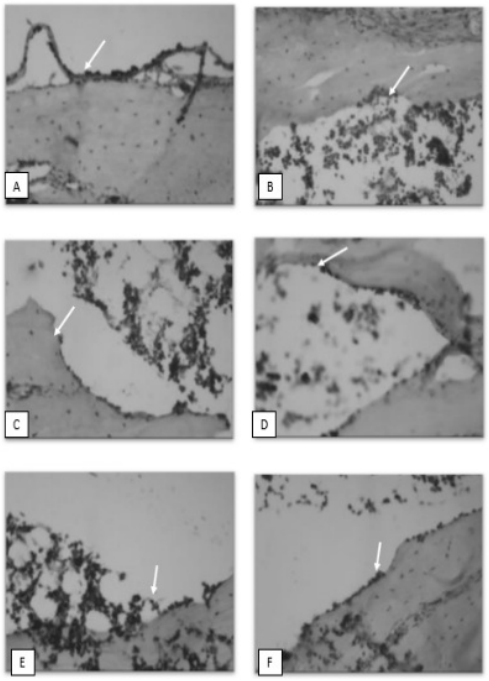

Figure 3: The positive RANKL expression in Wistar rat's periodontal tissue (white arrow) was observed by means of light Microscope (Olympus, US) at 400x magnification. A. RANKL expression in the K3 group; B. RANKL expression number in the P3 group; C. RANKL expression in the K5 group; D. RANKL expression in the P5 group; E. RANKL expression in the K7 group; F. RANKL expression in the P7 group

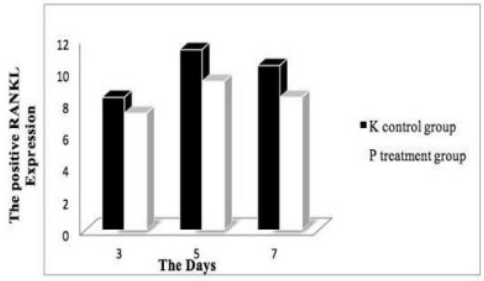

Figure 4: RANKL expression in each group

Table 5: ANOVA data for RANKL expression between groups

Time (day)	Group	Sig.
3	K3 P3	0.0599
5	K5 P5	0.0367*
7	K7 P7	0.0079*

*Significant at $p < 0.05$

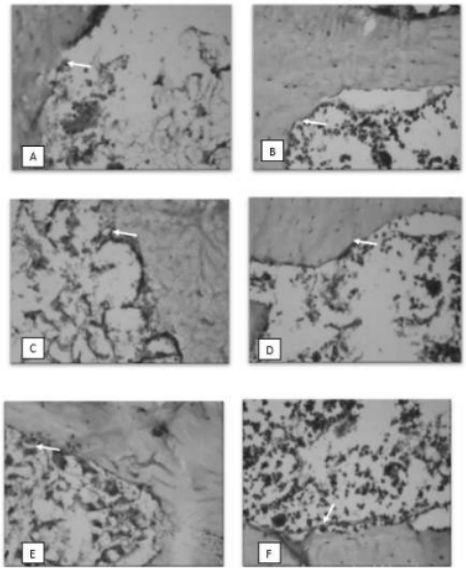

Figure 5: Positive OPG expression in Wistar rat's periodontal tissue (white arrow) was observed by means of light Microscope (Olympus, US) at 400x magnification. A. OPG expression in the K3 group; B. OPG expression number in the P3 group; C. OPG expression in the K5 group; D. OPG expression in the P5 group; E. OPG expression in the K7 group; F. OPG expression in the P7 group

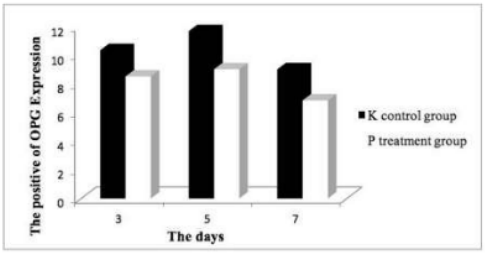

Figure 6: OPG expression in each group

Table 6: The result of ANOVA of OPG expression showed significant difference between groups

Time (day)	Group	Sig.
3	K3 K3	0.007*
5	K5 K5	0.012*
7	K7 K7	0.004*

Note: *Significant at $p < 0.05$

DISCUSSION

Orthodontic Tooth Movement (OTM) depends on the balance of alveolar bone remodeling. Consumption of Electrolyzed Reduced Water (ERW) / alkalin water can improve this process. In this study, a significant difference was found in the osteoclast number between groups. Orthodontic pressure produced cellular reactions characterized by increased pro - inflammatory cytokines and stimulated RANKL expression. RANKL binds to Receptor Activator Nuclear Kappa-B (RANK) on osteoclast precursors that trigger differentiation and osteoclast proliferation resulting in osteoclasts becoming active. Osteoclast activation result in bone resorption [11]. The decreased RANKL expression and osteoclast number on day 7 indicated a decrease in osteoclast activity. Previous study used rats as OTM experimental model showed that RANKL expression decreased on Day 7. There was a difference in RANKL expression between each study group on Day 5 and Day 7. This decrease in RANKL expression was due to increased production of pro-inflammatory cytokines that play an important role in osteoclast proliferation and differentiation [12].

The formation and activation of osteoclasts is controlled by cytokines composed of RANKL, RANK and OPG which constitute a family group of TNF [13]. RANKL, RANK and OPG are key regulators of bone remodeling, being directly involved in osteoclast differentiation and activation [14]. The formation of RANKL is controlled in response to pro-inflammatory cytokines, such as TNF - α and IL - 1. When RANKL interacts with RANK, intracellular signals may result in the formation of mature multinuclear osteoclasts from osteoclast precursors [13-15]. The role of RANKL in orthodontic tooth movement is that of binding to RANK to activate osteoclasts resulting in resorption. On the other hand, OPG (osteoprotegerin) acts as an antagonist receptor. Consequently, OPG binds to RANKL, it will activate osteoblasts resulting in bone apposition on the tensile area side [16].

In this study, OPG expression was significantly different between groups. Pro -inflammatory cytokines induced by orthodontic forces inhibit the expression of OPG [12]. OPG inhibits RANKL binding of RANK to osteoclast precursors and stimulates osteoclast apoptosis to inhibit osteoclastogenesis. The low RANKL/OPG ratio indicates regeneration and tissue repair. The analytical results for OPG expression showed an increase on day 5 (P5) compared to day 3 and a decrease on day 7 in the treatment group. This decrease in OPG expression is due to increased production of pro-inflammatory cytokines, thus inhibiting OPG expression [17].

In a previous study, it was found that hydrogen-rich water/alkaline water consumption can suppress periodontal inflammation triggered by the use of ligature wire in mice [18]. The

hydrogen molecule is known to have antioxidative and anti-inflammatory properties [19]. Hydrogen-rich water consumption causes down-regulation of NF-kB gene expression in periodontal tissue where IL-1β production is involved [20]. Consumption of hydrogen - rich water may decrease the expression of IL - 1β protein, but this study did not show any change in the expression of IL-1β protein in experimental models. The consumption of alkaline water can protect against the oxidative damage associated with tissue aging.

Alkaline water has the potential to decrease the effects of systemic oxidative damage on periodontal tissues [21]. Oxidative damage contributes to osteoclast differentiation [22]. Hydrogen - rich water consumption can suppress the osteoclast differentiation that accompanies periodontal inflammation. Consumption of hydrogen water can decrease the number of TRAP-positive osteoclasts on the surface of alveolar bone along with aging. This suggests that hydrogen-rich water has an aging effect on osteoclast differentiation by reducing oxidative damage. Hydrogen - rich water consumption is aimed at reducing alveolar bone loss effectively as a result of aging and/or periodontal inflammation [20-22].

CONCLUSION

ERW significantly inhibits the number of osteoclasts, RANKL and OPG expression during orthodontic tooth movement after three and seven days. Thus, ERW is a potential therapy for enhancing bone remodeling in patients with orthodontic tooth movement.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was undertaken by the author(s) named in this article and all liabilities pertaining to claims relating to its contents will be borne by the authors. All the authors made substantial contributions to this study and/or manuscript and approved the final draft of the paper prior to its submission.

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