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THE EFFECT OF CHLORHEXIDINE ON THE RECEPTOR ACTIVATOR OF NF-κB LIGAND (RANKL) AND OSTEOPROTEGERIN (OPG) EXPRESSION IN CHRONIC PERIODONTITIS IN HUMANS AND COMPANION ANIMALS

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Periodontal disease is a chronic, multi-factorial disease of the tissues supporting the teeth. Periodontitis in companion animals is an almost identical disease to that in humans in terms of disease course and clinical presentation.

Receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG) are bioactive molecules that control bone resorption. This study aims to evaluate the effect of Chlorhexidine (CXD) on the RANKL and OPG expressions in gingival crevicular fluid (GCF) collected from subjects with chronic periodontitis.

GCF was obtained from subjects with chronic periodontitis.10 subjects (CXD1) rinsed the mouth with 0.12% CXD, 10 subjects (CXD2) utilized 0.20% CXD and the last 10 (PL) used Placebo solution for 7 days. RANKL and OPG concentrations in GCF were measured by enzyme-linked immunosorbent assays ELISA at baseline and after 7 days. Periodontal clinical variables: clinical attachment loss (CAL), probing pocket depth (PPD), papilla-bleeding index (PBI) were evaluated in all groups.

After 7 days in CXD1 and CXD2 group RANKL/OPG ratio exhibited a significant decrease ($p<0.05$) in contrast to the PL group where results showed similar values of RANKL/OPG ratio at baseline and after the observation period. RANKL/OPG ratio was positively correlated with PPD, CAL and PBI before and after the observation period in both Chlorhexidine (CXD1, CXD2) groups.

In an existing inflammatory response, chlorhexidine reduced the level of periodontal inflammation, which leads to reduction of RANKL/OPG relative ratio. Decrease of RANKL/OPG ratio will apparently induce maintenance of alveolar bone and slow down periodontal tissue breakdown.

Key words: chlorhexidine, chronic periodontitis, ELISA, OPG, RANKL

INTRODUCTION

Periodontal disease is a destructive process that targets tooth-supporting structures through complex etiopathogenic processes. Alveolar bone destruction in periodontitis, one of the major causes of teeth loss in humans, is interceded by the host immune and inflammatory response to microbial challenge.

Periodontitis in companion animals is an almost identical disease to that in humans in terms of disease course and clinical presentation. It has been estimated that approximately 80% of dogs and cats demonstrate some degree of periodontal disease by 4 years of age (Harvey and Emily, 1993; Penman and Harvey, 1990). Companion animal periodontitis is a serious infection that can have medical consequences such as anorexia and weight loss, chronic pain, sore or loose teeth, swollen gums, tooth decay, breakage or loss of teeth, and breakage of the maxillary or mandibular bone. If left untreated, periodontal bacteria may spread to other sites in the body and lead to renal, coronary, or hepatic diseases (DeBowes *et al*., 1996; Scannapieco *et al*., 2003; Okuda *et al*., 2004). The accelerated disease progression observed in companion animals compared to humans may be due to a relative lack of routine dental care.

Periodontal disease is pathologic condition that affects normal bone remodeling. Recently, a novel approach of complex mechanisms underlying bone remodeling has been revealed. Receptor activator of nuclear factor-kappa B ligand (RANKL) and osteoprotegerin (OPG) have been shown to play a key role in regulating this balance.

RANKL binds its receptor RANK on the osteoclast precursor surface, determining their activation and differentiation to mature osteoclasts (Mogi *et al*., 2004; Vernal *et al*., 2004; Garlet *et al*., 2006). Osteoprotegerin (OPG) is a natural inhibitor of RANKL that plays an important role in the homeostatic control of osteoclast activity (Cochran, 2008). OPG is capable to bind to RANKL and neutralize its activity by inhibiting the cell-to-cell signaling between osteoblast/bone stromal cells and osteoclast precursor cells, resulting in the inhibition of osteoclast formation. RANKL and OPG are crucial molecules that act as positive and negative regulators, respectively, in osteoclastogenesis and bone resorption (Kirkwood *et al*., 2007). Under normal physiologic conditions, there is a balance between bone resorption and bone formation.

The expression of RANKL and OPG are tightly regulated by systemic and local factors, including hormones, inflammatory mediators, bacterial products, and immunosuppressive drugs (Lerner, 2004). Interleukin-1 and tumor necrosis factor-á were reported to induce osteoclast differentiation directly by modulating receptor activator of NF-κB ligand (RANKL) expression (Lerner, 2004; Taubman *et al*., 2005) When OPG concentrations are high relative to RANKL expression, OPG binds RANKL, inhibiting it from binding to RANK. Preventing the binding of RANKL to RANK leads to reduced formation of osteoclasts and apoptosis of preexisting osteoclasts and bone preservation (Mogi *et al*., 2004; Vernal *et al*., 2004; Garlet *et al*., 2006; Cochran, 2008).

Upregulation of RANKL has been seen also in inflamed periodontal tissues, indicating that RANKL strongly participates in the processes of periodontal tissue

destruction (Taubman *et al.*, 2005). On the other hand, the RANKL/OPG ratio is increased in periodontitis compared to non-diseased individuals, suggesting that this molecular interface may be important in modulating local bone loss. The RANKL/OPG ratio was found to be significantly increased in gingival crevicular fluid (GCF) from patients with periodontitis compared to healthy individuals (Mogi *et al*., 2004; Vernal *et al*., 2004; Kawasaki *et al*., 2006; Lu *et al*., 2006; Nishijima *et al*., 2006). Another line of research has emphasized the expression of these molecules in diseased periodontal tissues, rather than in GCF (Garlet *et al.,* 2006; Crotti *et al*., 2003; Kawai *et al*., 2006).

Chronic periodontitis was associated with RANKL mRNA upregulation and increased RANKL/OPG mRNA expression ratio (Garlet *et al.*, 2006). Few studies presented a positive correlation between concentration of *P. gingivalis* and upregulation of RANKL in clinically obtained human gingival tissues (Okahashi *et al*., 2004; Wara-Aswapati *et al*., 2007).

Chemotherapeutic agents have the potential to inhibit plaque growth, reduce gingivitis and improve oral health beyond tooth brushing alone. As an effective antibacterial agent, chlorhexidine (CHX) still remains the gold standard, unsurpassed by other agents (Gjermo *et al*., 1970; Paraskevas, 2005).

The efficacy of chlorhexidine digluconate (CXD) as a bacteriostatic and bactericidal agent has long been demonstrated (Loe and Schiott, 1970; Lang *et al*., 1982). Several studies comparing CXD to other chemical agents have shown CXD to be most effective in plaque control (Ernst *et al*., 1998; Renton-Harper *et al*., 1996). This antiplaque activity of CXD seems to be related to high levels of adsorption in multiple sites in the oral cavity, and to its substantivity (Mandel, 1988). Another line of studies showed that the impact of Chlorhexidine rinsing reduced bacterial load, especially subgingival levels of *P. gingivalis* (Sekino *et al.*, 2004; Persson *et al*., 2007). CXD had a strong anti-inflammatory effect by reducing the basal concentration of leucocytes migrating into the chamber. It also significantly reduced the levels of the pro-inflammatory cytokines (Houri-Haddad *et al.*, 2008).

The present study was focused on evaluation of relationship and the influence of Chlorhexidine solution on the expression of RANKL and OPG in periodontitis.

MATERIALS AND METHODS

Subjects recruitment

Thirty subjects with chronic periodontitis were enrolled in the study during a 2-month period (September to October 2008) at the Perio Dpt. School of Dentistry in Belgrade. The definition of chronic periodontitis was based on the classification system of the 1999 International Workshop for a Classification of Periodontal Diseases and Conditions (Armitage, 1999).

The University Ethical committee approved the protocol for human subjects. All participating subjects signed a consent form approved by an institutional review board. Every participant completed a comprehensive medical questionnaire confirmed by their medical care providers. All patients were

systemically healthy and without any significant history of systemic diseases. Similarly, all patients underwent a full-mouth dental and periodontal examination, performed by the same examiner.

The study was a double-blind study with 3 parallel groups using three different solutions from the same manufacturer: 0.12% chlorhexidine digluconate (Curasept® 212 Curaden Swiss), 0.2% chlorhexidine digluconate (Curasept® 220 Curaden Swiss) and a placebo (PL group).

After enrollment and examination, subjects received their first 1 week supply of the assigned rinse. The active rinse consisted of a 0.12% (CXD1) and 0.2% chlorhexidine digluconate (CXD2) mouthrinse. The placebo rinse was prepared with quinidine and a color additive similar to the color of the CXD rinse.

10 subjects (CXD1) rinsed the mouth with 0.12% CXD mouth rinse twice a day for 1 week. The other 10 participants (CXD2) rinsed the mouth with 0.2% CXD mouth rinse for 1 week. The last 10 subjects rinsed the mouth with the placebo solution. Patients were instructed to rinse for 30 seconds with 10 mL of their undiluted mouthrinse twice a day for 7 days and to avoid eating or drinking for 1 hour afterward.

GCF sampling

After enrollment into the study, subjects were recalled for GCF sampling. GCF samples were collected from the mesiobuccal aspect of non-adjacent single-rooted teeth exhibiting PPD of 6–8 mm. The selected sites were cleared of supragingival plaque usind sterile currete, isolated with cotton rolls and dried with a gentle stream of air to prevent saliva contamination. One paper strip was used for each collection site. Two sites were selected in each participant. A sterile Periopaper strip (ProFlow Inc., Amityville, NY, USA) was gently inserted into the periodontal pocket until 1 mm and left in place for 30 s. Mechanical irritation was avoided and strips contaminated with blood were discarded. Strips were placed in a sterile polypropylene tube and kept at -40ºC until being analysed. Paper strips for each participant were pooled, and the GCF was extracted and assayed for the content of RANKL and OPG. GCF was extracted from the paper strips with buffer (50 mM phosphate buffer, pH 7.2, containing protease inhibitors: 0.1 mM phenylmethylsulphonylfluoride and 5 μg/mL each of leupeptin, pepstatin, amastatin, chemostatin, and antipain), and collected following centrifugation.

GCF sampling was repeated at 1 week.

Clinical assesments

The periodontal clinical variables clinical attachment level (CAL), probing pocket depth (PPD) and papilla bleeding index (PBI) were evaluated in all groups. Clinical parameters were recorded at baseline and after 1 week follow-up by the same examiner using a periodontal probe (PCP-UNC 15; Hu Friedy, Leimen, Germany), at six sites per tooth.

Detection of RANKL and OPG in GCF

The amount of RANKL and OPG in the GCF samples was evaluated using commercially available human-specific ELISA in accordance with the manufacturer's instructions (total sRANKL ELISA kit: Immundiagnostik AG, Bensheim, Germany, and Osteoprotegerin ELISA kit: Biomedica, Vienna, Austria).

These assays measure the total levels of RANKL or OPG present in the GCF, including both their unbound-free forms, and their RANKL-OPG complex form. Estimation of the RANKL and OPG concentration in each GCF sample was performed by dividing the total amount of RANKL or OPG by the volume of the sample [RANKL or OPG concentration (pg/μL)=total RANKL or OPG (pg)/volume (μL)].

Statistical analysis

Statistical comparisons were assessed with Mann–Whitney *U*-tests, and $p<0.05$ was considered to be statistically significant. In order to evaluate the correlations between GCF RANKL/ OPG ratio levels and clinical parameters, Spearman's rank correlation analysis was used and $p<0.01$ was considered as significant.

RESULTS

The study participants consisted of 16 females and 14 males ranging in age from 38 to 47 years with a mean age of 44.14 ± 14.81 years. The mean baseline PPD, PBI and CAL scores of sampling sites in CXD1, CXD2 and PL groups were similar. No significant differences were detected between results obtained at baseline and after 1 week of of treatment in CXD1, CXD2 and PL groups regarding the PPD and CAL of sampling sites ($p>0.05$). CXD1 and CXD2 groups showed significantly decreased mean PBI scores of sampling sites after inspection period 2.03 ± 1.24, 1.67 ± 1.35 and 2.15 ± 1.34, 1.51 ± 1.47 ($p<0.05$) (Table 1).

Table 1. Clinical parameters of the sampling areas in the study groups (mean ± SD)

	CXD 1		CXD2		PL	
	baseline	1 week	baseline	1 week	baseline	1 week
PPD	6.05±1.45	5.81±1.70	6.21±1.28	5.90±1.42	6.11±1.05	6.15±1.15
CAL	7.15±1.22	7.10±1.32	7.22±1.37	7.18±1.20	7.25±1.19	7.28±1.20
PBI	2.03±1.24	1.67±1.35†	2.15±1.34	1.51±1.47†	2.25±1.28	2.30±1.18

†Significant difference from baseline results, Mann–Whitney *U*-test, $p<0.05$)
PPD, probing pocket depth; CAL, clinical attachment loss; PBI, papilla bleeding index, CXD1; group treated with 0.12% chlorhexidine; CXD2, group treated with 0.20% chlorhexidine; PL, group treated with placebo solution

RANKL and OPG were detected in all GCF samples. All groups demonstrated similar levels of RANKL, which were 325.9 ± 18.14, 327.4 ± 17.43 and 323.9 ± 13.87 pg/μL, respectively before treatment. CXD1 and CXD2 groups exhibited significant decrease of RANKL after observation period (291.40 ± 8.28, 292.3 ± 7.17 pg/μL). Placebo group showed similar amount of RANKL 322.10 ± 13.41 pg/μL after 7 days. Baseline recordings of OPG levels in all three groups showed similar results 119.70 ± 3.09, 120.2 ± 5.14, 120.6 ± 5.02 pg/μL. After surveillance CXD1 and CXD2 groups showed significant enhancement 198

± 5.31, 196.70 ± 4.40 pg/μL. In contrast to CXD1 and CXD2 groups Placebo group exhibited a similar concentration of OPG 120.50 ± 5.44.

The relative RANKL/OPG ratio was further investigated. Baseline results showed similar values in all 3 groups (CXD1, CXD2, PL) 2.72 ± 0.17, 2.73 ± 0.23, 2.69 ± 0.18 and there were no significant differences among these groups (Figure 1). After 7 days in CXD1 and CXD2 group RANKL/OPG ratio exhibited significant decrease 1.47±0.06, 1.49±0.05. In contrast to CXD1 and CXD2 groups, PL group showed similar value of RANKL/OPG ratio after observation period 2.67 ± 0.17 pg/μL (Table 2, Figure 2).

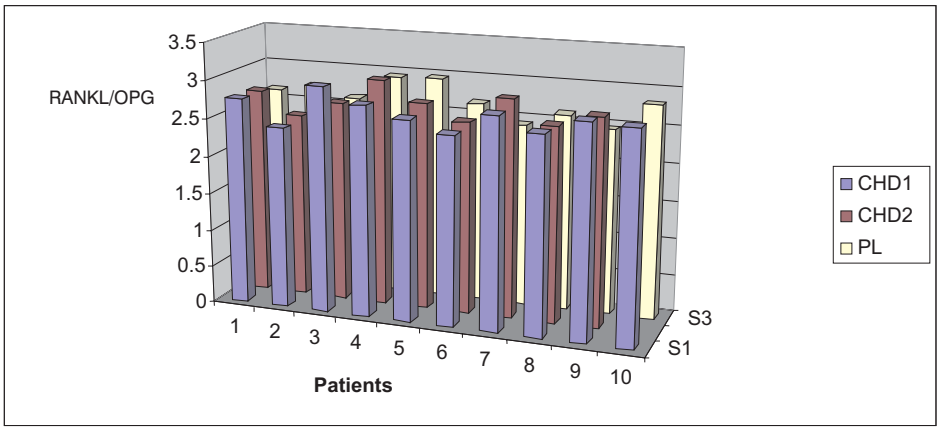

Figure 1. Distribution of relative receptor activator of NF-κB ligand (RANKL)/osteoprotegerin (OPG) ratio levels in gingival crevicular fluid (GCF) from CXD1 n=10 , CXD2 n=10 and Placebo group n=10 before treatment
Series 1 = CXD1, Series 2 = CXD2, Series 3 = PL

Table 2. Concentration of RANKL, OPG pg/μL, and relative (RANKL)/ (OPG) ratio levels in gingival crevicular fluid

	RANKL		OPG		RANKL/OPG	
	baseline	1week	baseline	1week	baseline	1week
CXD1	325.9±18.14	291.4±8.28	119.7±3.09	198±5.31	2.72±0.17	1.47±0.06
P	0.0024969*		0.0001570522*		0.0001570522*	
CXD2	327.4±17.43	292.3±7.17	120.2±5.14	196.7±4.40	2.73±0.23	1.49±0.05
p	0.00058284*		0.0001570522*		0.0001570522*	
PL	323.9±13.87	322.1±13.41	120.6±5.02	120.5±5.44	2.69±0.18	2.67±0.17
p	0.677584		0.96985		0.96985	

*Significant difference from baseline results, Mann–Whitney *U*-test, $p<0.05$).
CXD1, group treated with 0.12% chlorhexidine; CXD2, group treated with 0.20% chlorhexidine; PL, group treated with placebo solution

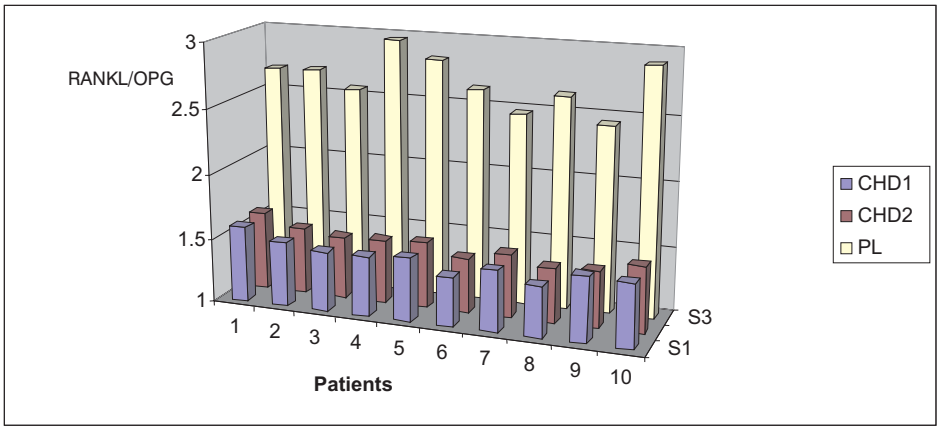

Figure 2. Distribution of relative receptor activator of NF-κB ligand (RANKL)/osteoprotegerin (OPG) ratio levels in gingival crevicular fluid (GCF) from CXD1 n=10 , CXD2 n=10 and Placebo group n=10 after observation period
Series 1 = CXD1, Series 2 = CXD2, Series 3 = PL

Comparation of RANKL/OPG relative ratio recorded in CXD1 and CXD2 group showed high level of nominal and statistical equivalence before and after chlorhexidine utilization, 2.72 ± 0.17, 2.73 ± 0.23 and 1.47 ± 0.06, 1.49 ± 0.05 ($p>0.05$). RANKL/OPG relative ratio recorded in CXD1 and CXD2 referring to RANKL/OPG relative ratio obtained from PL group (2.69±0.18) at baseline exhibited similar results. After observation period RANKL/OPG values recorded in PL group (2.67±0.17) were significantly higher ($p<0.05$) comparing to CXD1 (1.47±0.06) and CXD2 (1.49±0.05) group.

The correlation of RANKL/ OPG relative ratio with clinical parameters was investigated by Spearman's rank correlation analysis. RANKL/OPG ratio was positively correlated with PPD, CAL and PBI ($p.01$) before treatment with CXD and after observation period in both Chlorhexidine groups (Tables 3, 4).

Table 3. Correlations between RANKL/OPG ratio with clinical parameters (CXD1 group)

CXD1	RANKL/OPG Baseline	RANKL/OPG 7days
PPD	**0.809	**0.783
CAL	**0.777	**0.798
PBI	**0.881	**0.594

**$p<0.01$
Spearman's rank correlation analysis was used
PPD, probing pocket depth; CAL, clinical attachment level; PBI, papilla bleeding index

Table 4. Correlations between RANKL/OPG ratio with clinical parameters (CXD2 group)

CXD2	RANKL/OPG Baseline	RANKL/OPG 7days
PPD	**0.823	**0.765
CAL	**0.751	**0.807
PBI	**0.872	**0.581

**$p<0.01$
Spearman's rank correlation analysis was used
PPD, probing pocket depth; CAL, clinical attachment level; PBI, papilla bleeding index

DISCUSSION

Disorder of the RANKL-OPG interaction may lead to alveolar bone resorption as has been shown in experimental periodontitis models (Teng *et al.*, 2000; Taubman *et al*., 2005). In the present study, the main objective was to assess the effects of chlorhexidine mouth rinse on the levels of RANKL, OPG, as well as their relative ratio, in gingival crevicular fluid from periodontally diseased subjects. Baseline results of the RANKL, OPG levels in all three groups (CXD1, CXD2 and PL) were in agreement with previous studies (Mogi *et al.*, 2004; Vernal *et al*., 2004; Kawasaki *et al*., 2006; Lu *et al*., 2006; Bostanci *et al.*, 2007) demonstrating a high level of similarity of RANKL and OPG in the GCF from patient with chronic periodontitis. RANKL showed to be significantly decreased after one week of CXD consumption in both experimental groups treated with chlorhexidine. Instantly the amount of OPG exhibited a significant increase. That can be explained as a result of chlorhexidine influence on periodontopathogen load and positive correlation between concentration of *P. gingivalis* and upregulation of RANKL (Sekino *et al.*, 2004; Persson *et al*., 2007).

Chlorhexidine had a strong anti-inflammatory effect by reducing the basal concentration of leucocytes migrating into the chamber compared with the control group. It also reduced the levels of the pro-inflammatory cytokines, resulting in a strong anti-inflammatory effect (Houri-Haddad *et al*., 2008). Placebo group showed high level of similarity for RANKL and OPG concentration before and after 1 week of placebo solution utilization as expected result. RANKL OPG relative ratio confirmed significant decrease in both experimental groups (CXD1, CXD2) after the observation period as a result of impact of CXD on bacterial load and reduction of inflammation (Sekino *et al*., 2004; Persson *et al*., 2007; Houri-Haddad *et al*., 2008).

Change of RANKL and OPG levels reflected changes in their relative RANKL/OPG concentration ratio in gingival crevicular fluid. No significant differences in RANKL/OPG ratio were detected among the patients with chronic periodontitis before utilization of chlorhexidine solution or Placebo mouthrinse which is in agreement with previous reports (Mogi *et al*., 2004; Vernal *et al*., 2004; Lu *et al.*, 2006; Bostanci *et al.*, 2007). RANKL/OPG relative ratio confirmed

significant decrease in both experimental groups (CXD1, CXD2) after an observation period as result of strong anti-inflammatory effect of CXD on periodontal tissues. In the same time RANKL/OPG relative ratio in the placebo group showed high level of similarity with values of RANKL/OPG ratio detected before utilization of Placebo solution and that was the expected result.

Very interesting data is obtained from the relationship between RANKL/OPG ratio in the CXD groups after the surveillance period. No significant differences in RANKL/OPG ratio were detected among groups treated with 0.12 and 0.2% CXD after 7 days. It implies that there are no differences in efficacy of those two tested chlorhexidine concentrations on reduction of periodontal inflammation and influence on periodontopathogen load.

The present study demonstrates a positive correlation of the RANKL/OPG ratio in gingival crevicular fluid with PBI, PPD and CAL in the groups treated with Chlorhexidine (CXD1, CXD2). Achieved results support the diagnostic value of the RANKL/OPG ratio, as they indicate that it may correlate with the condition of periodontal inflammation as the progression of periodontal destruction. Positive correlation of the RANKL/OPG ratio with PBI, in the groups treated with chlorhexidine indicates that it may strongly correlate with the state of periodontal inflammation and this finding may be in disagreement with previous studies (Vernal *et al*., 2004; Lu *et al.*, 2006; Bostanci *et al*., 2007). These studies exhibited a positive correlation of the RANKL/OPG ratio from GCF with PPD and CAL values, but did not identify a positive correlation between RANKL/OPG ratio and PBI values.

In conclusion, the study provides information on the influence of chlorhexidine on RANKL and OPG expression and downregulation in chronic periodontal diseases. In an existing inflammatory response, chlorhexidine reduced the levels of inflammation induced by a periodontopathogen challenge which obviously leads to reduction of RANKL/OPG relative ratio. Decrease of RANKL/ OPG ratio will apparently induce maintenance of alveolar bone and slow down periodontal tissue breakdown. Targeting RANKL and OPG regulation by host response modulation therapies may be a gainful approach to periodontal disease management (Salvi and Lang, 2005).

Oral disease (periodontitis) has been identified as one of the most prevalent diseases of companion animals (Harvey, 1998). Companion animals accumulate plaque and tartar much more rapidly than humans. The progression from a healthy state to gingival inflammation to periodontitis occurs more rapidly. The clinical features of periodontal disease in companion animals are very similar to that of humans, where destruction of the attachment tissue and alveolar bone are observed (Harvey, 1998; Hennet and Harvey, 1992). Owners often neglect dental hygiene in pets and treatment for periodontal diseases in dogs is a common problem in veterinary dentistry (Hoffmann and Gaengler, 1996). It is, therefore, important to establish alternative and simple treatments to prevent periodontal disease on the animals. Use chlorhexidine mouthrinse could be method of choice.

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Janković S *et al*.: The effect of chlorhexidine on the receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG) expression in chronic periodontitis in humans and companion animals

EFEKAT HLORHEKSIDINA NA EKSPRESIJU RECEPTOR AKTIVATORA NF-κB LIGANDA (RANKL) I OSTEOPROTEŽERINA (OPG) KOD LJUDI I KUĆNIH LJUBIMACA

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SADRŽAJ

Parodontopatije su hronična, multikauzalna oboljenja potpornog aparata zuba. Parodontalna oboljenja koja srećemo kod kućnih ljubimaca su prema toku i kliničkoj slici skoro identična onima koje se javljaju kod ljudi.

RANKL i osteoprotegerin (OPG) su bioaktivni molekuli koji kontrolišu koštanu resorpciju. Cilj ove studije je evaluacija efekata hlorheksidina na ekspresiju RANKL-a i OPG-a u gingivalnoj tečnosti (GT) uzetoj od pacijenata sa hroničnom parodontopatijom.

10 pacijenata (CXD1) su ispirali usta sa 0.12% CXD, 10 pacijenata (CXD2) su koristili 0.20% CXD i poslednjih 10 pacijenata (PL) su koristili placebo rastvor 7 dana. RANKL i OPG koncentracije u GT su merene ELISA testom na početku i posle sedam dana. Parodontalni klinički parametri CAL, PPD i PBI su evaluirani u svim grupama.

Posle 7 dana u CXD1 i CXD2 grupi RANKL/OPG odnos je pokazao signifikantno smanjenje ($p<0.05$) u poređenju sa PL grupom gde su zabaleženi slični rezultati na početku i nakon opservacionog perioda. RANKL/OPG odnos je pokazao pozitivnu korelaciju sa vrednostima PPD-a, CAL-a i PBI-a pre i nakon observacionog perioda u obe eksperimentalne grupe (CXD1, CXD2).

U prisutnom inflamatornom odgovoru hlorheksidin je redukovao nivo inflamacije, što je uslovilo redukciju RANKL/OPG odnosa. Rezultati istraživanja dokazuju da koncentracija hlorheksidina ne utiče statistički značajno na smanjenje RANKL/OPG odnosa.