Effects of Adjunctive Local or Systemic Metronidazole with Non-Surgical Periodontal Therapy on Periodontal Clinical Parameters and Gingival Crevicular Fluid Biomarkers

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ABSTRACT

Objective: The aim of this study was to investigate the effects of local or systemic metronidazole as adjunctives to non-surgical periodontal treatment on the clinical parameters and gingival crevicular fluid (GCF) levels of matrix metalloproteinase-8 (MMP-8) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in chronic periodontitis patients.

Material and Method: Thirty patients were allocated into 3 groups: 10 were treated with scaling and root planing (SRP) only; 10 were treated with SRP and received adjunctive local metronidazole; 10 were treated with SRP and received adjunctive systemic metronidazole. Periodontal clinical parameters including plaque index, sulcus bleeding index, probing depth and relative attachment level were recorded before and after the treatments. The MMP-8 and TIMP-1 levels in GCF samples collected on days 0 and 49 were determined by using the enzyme-linked immunosorbent assay.

Results: All groups showed significant reductions in plaque index, sulcus bleeding index and probing depth (p<0.01). Although attachment gain was observed in all groups, only the gain in the SRP plus local metronidazole group was significant (p<0.05). The GCF MMP-8 levels decreased significantly (p<0.05), whereas the GCF TIMP-1 levels increased. No significant differences were found between the groups.

Conclusion: Within the limits of this study, our results suggest that local or systemic use of metronidazole combined with SRP did not provide an additional benefit in terms of the clinical parameters and GCF levels of MMP-8 and TIMP-1 over the SRP alone.

Key Words: Chronic periodontitis, metronidazole, scaling, root planing, matrix metalloproteinase-8, tissue inhibitor of metalloproteinase-1 **Nobel Med 2012; 8(1): 89-94**

MEKANİK PERİODONTAL TEDAVİYE DESTEK OLARAK KULLANILAN LOKAL VEYA SİSTEMİK METRONİDAZOLÜN KLİNİK PARAMETRELER VE DİŞETİ OLUĞU SIVISI BİYO-MARKERLERİ ÜZERİNE ETKİSİ

ÖZET

Amaç: Bu çalışmanın amacı, kronik periodontitis hastalarında cerrahi olmayan periodontal tedaviye ek olarak kullanılan lokal veya sistemik metronidazolün klinik parametreler ve dişeti oluğu sıvısı matris metalloproteinaz-8 (MMP-8) ve metalloproteinaz doku inhibitörü-1 (MDI-1) seviyelerine etkisini araştırmaktır.

Materyal ve Metod: Otuz hasta rastgele 3 gruba ayrıldı: 10 hasta sadece diş ve kök yüzeyi temizliği (DKYT); 10 hasta DKYT'ye ek olarak lokal metronidazol uygulaması; 10 hasta DKYT'ye ek olarak sistemik metronidazol uygulaması ile tedavi edildi. Periodontal klinik parametreler (plak indeks, dişeti oluğu kanama indeksi, sondalama derinliği, rölatif ataşman seviyesi) tedavi önce ve sonrasında ölçüldü. Sıfır ve 49 günlerde toplanan dişeti oluğu sıvısı örneklerindeki MMP-8 ve MDI-1 seviyeleri enzime bağlı immun deney ile belirlendi.

Bulgular: Bütün gruplarda plak indeks, dişeti oluğu kanama indeksi, sondalama derinliğinde anlamlı azalmalar görüldü (p<0.01). Bütün gruplarda ataşman kazancı sağlanmakla birlikte sadece DKYT artı lokal metronidazol grubundaki değişim anlamlıydı (p<0.05). Dişeti oluğu sıvısı MMP-8 seviyeleri anlamlı azalırken (p<0.05), MDI-1 seviyeleri arttı. Gruplar arasında anlamlı fark bulunmadı.

Sonuç: Bu çalışmanın sınırları içinde, sonuçlar kronik periodontitis hastalarında DKYT ile birlikte lokal veya sistemik metronidazol uygulamasının periodontal klinik parametreler, dişeti oluğu sıvısı MMP-8 ve MDI-1 seviyeleri açısından tek başına DKYT uygulamasına kıyasla ilave bir fayda sağlamadığını göstermektedir.

Anahtar Kelimeler: Kronik periodontitis, metronidazol, diş ve kök yüzeyi temizliği, matriks metalloproteinaz-8, metalloproteinaz doku inhibitörü-1 **Nobel Med 2012; 8(1): 89-94**

INTRODUCTION

Periodontitis is a chronic inflammatory disease affecting the gingiva, cementum, periodontal ligament and alveolar bone, namely the periodontal tissues. While mild to moderate forms of periodontal disease affect a majority of adults, a total of 5 to 20% of the population has severe, generalized periodontitis.[1] The pathogenesis involves predominantly gram-negative bacteria in microbial dental plaque that initiates a localized inflammatory host reaction, leading to release of inflammatory mediators, destruction of connective tissue, periodontal pocket formation and alveolar bone resorption and, ultimately, tooth loss.[2]

Matrix metalloproteinases (MMPs) are produced by both infiltrating and resident cells of the periodontium that play a role both in physiological events and pathological processes including periodontitis.[3] An imbalance between activated MMPs and host-derived tissue inhibitors of metalloproteinases (TIMPs) leads to pathological breakdown of the extracellular matrix during periodontal disease[3]. One prominent member of this family, MMP-8 is produced and released mainly by polymorphonuclear neutrophils, and is the main type of collagenase in gingival crevicular fluid (GCF) from chronic periodontitis patients.[4]

Periodontal therapy involves elimination of periodontal pathogens by non-surgical mechanical procedures such as scaling and root planing (SRP) and, further periodontal surgery when required. Due to infectious nature of periodontal disease, adjunctive pharmacological agents have been suggested in the non-surgical treatment of periodontitis.[5] Metronidazole with a selective anaerobic spectrum is effective against various gram-negative anaerobic rods and spirochetes, and is found in GCF at levels high enough to inhibit specific anaerobic periodontal microorganisms.[6] A number of studies evaluating the effect of systemic metronidazole alone or in combination with non-surgical periodontal treatment have reported clinical and microbiological improvements and reduced surgical treatment needs.[7-10] A recent development has resulted in a metronidazole 25% gel for local application into periodontal pockets to provide higher drug concentration at the target site.[11] Previous studies have shown that treatment with local metronidazole resulted in significant improvements in clinical parameters and reduction in the number of anaerobic periodontopathogens.[9,10,12-14]

The purpose of the present study was to investigate the effects of local or systemic metronidazole in combination with non-surgical periodontal treatment in terms of GCF levels of MMP-8 and TIMP-1 as well as periodontal parameters. Besides, the efficacy of three treatment modalities on periodontal tissue breakdown was evaluted by determining the changes in the GCF MMP-8 and TIMP-1 profile. →

MATERIAL and METHOD

Subject Population

In this prospective randomized clinical trial, 30 non-smoking patients (24 males and 6 females), 35 to 58 years old (mean age 43.1±5.6 years) with chronic periodontitis[15] were included. Inclusion criteria were healthy subjects having a minimum of 15 teeth and at least 3 single rooted teeth with a probing depth (PD)≥5 mm. Exclusion criteria included any systemic disease, treatment with antibiotics or periodontal therapy in the last 6 months and any drug known to affect periodontal tissues.

Thirty patients were randomly divided into three groups: 10 were treated with SRP only; 10 were treated with SRP and received adjunctive local metronidazole (SRP+LM group); 10 were treated with SRP and received adjunctive systemic metronidazole for 10 days (SRP+SM group). The protocol of the study was approved by the Ethics Commitee of the Ministery of Health, Turkey. The nature of the study was described thoroughly to all patients and each of them provided signed informed consent before entry into the study.

Clinical Examination and Treatment Procedures

The study protocol is shown in Figure 1. One week prior to non-surgical periodontal therapy, oral hygiene instructions were given to each subject and the 2 GCF sampling site of single rooted teeth with a probing depth (PD) ≥5 were selected. Periodontal assessment was performed at baseline (day 0) and on day 49. The following measurements were taken: plaque index (PI), sulcus bleeding index (SBI), PD and relative attachment level (RAL). PI was assessed at four locations for each tooth (mesio-buccal, mid-buccal, distobuccal and mid-lingual/palatal aspects) as described by Silness and Löe.[16] The appearance of gingiva and existence of gingival bleeding upon gentle probing were evaluated according to Mühlemann and Son and assigning a score of 0 to 5 for SBI, with 0 indicating a healthy appearance and no bleeding.[17] PD, the distance between the gingival margin and the most apical extent of probe penetration within the pocket, and RAL, the distance between a reference point and the base of the pocket, were measured with a periodontal probe (PCPUNC15, Hu-Friedy Ins. Co, USA) using an individual occlusal stent as a reference point for probe placement.

At baseline (day 0) and day 7, the patients in all groups received full-mouth mechanical instrumentation of tooth and root surfaces under local anesthesia using an ultrasonic scaler (Cavitron SPS, 30K TFI-10, Dentsply,
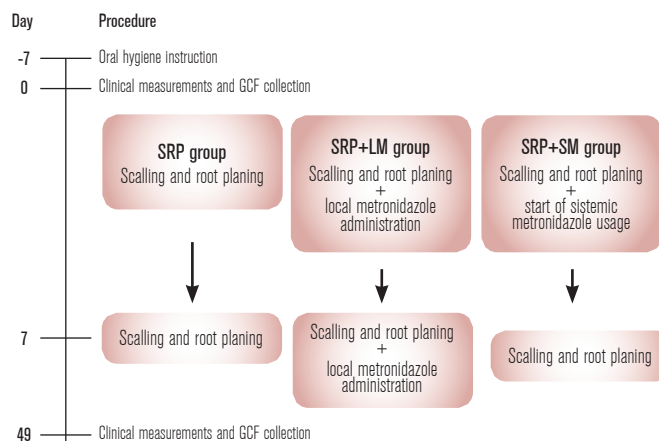

Figure 1. Flow chart of the study.

Table 1: Comparison of clinical measurements between baseline and day 49 in each group.

	SRP Group			SRP+LM Group			SRP+SM Group		
	Baseline	Day 49	p	Baseline	Day 49	p	Baseline	Day 49	p
PI	2.5±0.8	0.3±0.4	0.006	2.3±0.6	0.7±0.6	0.004	2.9±0.3	0.5±0.7	0.006
SBI	4.3±0.6	1.5±0.8	0.005	4.2±0.7	1.6±0.6	0.005	3.1±0.9	0.4±0.6	0.004
PD (mm)	6.2±2.2	4.7±1.3	0.016	6.1±1.2	3.9±1.1	0.005	5.3±0.7	4.0±1.3	0.011
RAL (mm)	10.8±2.8	9.7±1.9	0.089	10.0±2.1	8.6±1.5	0.016	9.3±0.9	8.6±1.1	0.072

Table 2: Multiple comparison of changes in clinical parameters using the Kruskall Wallis test.

	SRP Group	SRP+LM	SRP+SM	Chi-Square	p
PI	-2.20±1.03	-1.60±0.51	-2.40±0.84	5.82	0.054
SBI	-2.80±1.22	-2.60±0.96	-2.70±1.05	0.08	0.961
PD (mm)	-1.45±2.06	-2.15±1.37	-1.35±1.17	3.15	0.206
RAL (mm)	-1.10±2.27	-1.40±1.57	-0.70±0.94	0.80	0.669

USA) and curettes (Gracey, SG 3/4, 7/8, 11/12, 13/14, Hu-Friedy, USA). The SRP+LM group received additional full-mouth local administration of a gel containing 25% metronidazole placed directly into periodontal pockets following SRP procedure on days 0 and 7. This group of patients was instructed not to drink and eat within 2 hours following application of the gel. In addition, the SRP+SM group patients started to use systemic metronidazole at baseline (day 0) and continued for 10 days.

GCF Collection and Measurements of MMP-8 and TIMP-1

Two GCF samples were obtained from selected periodontal sites in each subject after the PI were scored and prior to the measurement of other clinical parameters. The selected sites were isolated with cotton rolls followed by removal of saliva and supragingival plaque.[18] Pre-cut strips of Whatman 3 MM chromatography paper (Whatman Lab. Sales Ltd.;→

Table 3: Comparison of GCF MMP-8 and TIMP-1 levels between baseline and day 49 in each group.

	SRP Group			SRP+LM Group			SRP+SM Group		
	Baseline	Day 49	p	Baseline	Day 49	p	Baseline	Day 49	p
GCF (µl)	0.58±0.17	0.44±0.09	0.023	0.72±0.12	0.53±0.21	0.026	0.61±0.17	0.38±0.12	0.027
MMP-8 (ng/µl)	160.3±98.1	66.94±60.84	0.013	152.50±128.10	51.73±69.80	0.037	166.97±115.42	63.01±105.04	0.022
TIMP-1 (ng/µl)	0.10±0.07	0.14±0.06	0.285	0.08±0.05	0.18±0.14	0.028	0.11±0.08	0.14±0.14	0.541

Table 4: Multiple comparison of changes in laboratory parameters using the Kruskall Wallis test.

	SRP Group	SRP+LM	SRP+SM	Chi-Square	p
GCF (µl)	-0.16±0.17	-0.19±0.19	-0.23±0.24	0.30	0.860
MMP-8 (ng/µl)	-93.43±101.98	-100.77±118.33	-103.96±118.88	0.01	0.995
TIMP-1 (ng/µl)	0.04±0.13	0.09±0.10	0.04±0.13	1.26	0.532

Maidstone, UK) (2 x 10 mm) were placed at the entrance of the crevice and left in position for 2 min.[19,20] The volumes of GCF collected were measured by weighing the strips using a microbalance accurate to within 0.01 mg.[21] Samples were stored at -40^{0}C prior to use.

The frozen GCF paper strips were thawed at room temperature for 30 min. For elution, 50 µl of phosphate-buffered saline was added to each tube containing the strip and centrifuged at 11 000 rpm for 15 min.[22] This step was repeated, the eluates combined and the total volume of 100 µl stored at 4∞C for up to 24 h prior to use. The levels of MMP-8 and TIMP-1 in the GCF samples were determined using commercially available Colorimetric Sandwich ELISA Kits (Quantikine DMP800 and DTM100, R&D Systems; Minneapolis, MN, USA) according to the manufacturer's instructions.

Statistical analysis

For the clinical data, the mean of periodontal sites with PD ≥5 mm in each patient was chosen as the unit of measurement. For the laboratory data, the mean of biomarker level of 2 GCF samples in each patient was calculated. Non-parametric techniques were used for statistical analysis since it was found that the distribution of data was not normal. The Wilcoxon Signed Ranks test was used to determine whether there was a significant difference in clinical and laboratory parameters within each group. The Kruskal Wallis test was used to determine any significant difference in changes of parameters between the groups. Values of p<0.05 were set as statisticaly significant.

RESULTS

Clinical characteristics

The results in Table 1 show the clinical measurements of the three groups at the selected sites on days 0 and 49. Oral hygiene measures in the SRP, SRP+LM and SRP+SM group patients improved as evidenced by significant reductions in the PI values (p<0.01). Similarly, clinical signs of gingival inflammation measured as SBI, decreased significantly in all groups (p<0.01). The baseline PD was found to be significantly reduced by an average of 2.15 mm in the SRP+LM group (p<0.01), while marked reductions were also observed in both the SRP and SRP+SM groups (p<0.05). Although RAL decreased in all groups suggesting gain in attachment level, only the change in the SRP+LM group was found significant (p<0.05). However, comparison of the changes in the PI, SBI, PD and RAL values revealed that there were no significant differences between the three groups after therapy (Table 2).

GCF volume and MMP-8 and TIMP-1 levels

Based on the total of 120 GCF samples collected from periodontal sites of the 10 SRP, 10 SRP+LM and 10 SRP+SM subjects, the results in Table 3 show that the mean GCF volumes of all three groups decreased significantly on day 49 (p<0.05). All samples of GCF were found to have detectable levels of biomarkers investigated in this study. The mean concentration of MMP-8 in GCF samples of the SRP, SRP+LM and SRP+SM groups decreased almost to the two thirds of their respective baseline values (p<0.05), as shown in Table 3. On the contrary, the mean concentration of TIMP-1 in GCF samples of all groups increased. However, the changes in the GCF level of both MMP-8 and TIMP-1 were not significant between the three groups (Table 4).

DISCUSSION

The present randomized, parallel-group study was designed to evaluate the effects of local or systemic metronidazole as an adjunct to SRP in patients with chronic periodontitis. The efficacy of adjunctive metronidazole on periodontal clinical parameters as well as GCF MMP-8 and TIMP-1 levels were tested.
Non-surgical mechanical periodontal treatment including thorough removal of supra- and subgingival bacterial deposits by scaling and root planing is the cornerstone of periodontal therapy and the first recommended approach to the control of periodontal infections.[23] This treatment is still considered to be the gold standard to which other treatment methods are compared. In addition to obtaining a high level of personal oral hygiene, SRP clinically induces the resolution of inflammation and halting the progression of periodontal disease, which leads to the reduction in PD, decrease in the number of gingival sites with bleeding on probing, attachment gain, and a shift →

from a predominantly gram-negative obligate anaerobe to a gram-positive aerobe subgingival microbiota.[24] Increased knowledge about the infectious nature of periodontal diseases has prompted the use of adjunctive antibiotics in the treatment of periodontitis patients.[5] However, systemic versus local administration of antibiotics in periodontal therapy is still a matter for further study. While the systemic route allows the antibiotic to enter the periodontal pocket via GCF, local crevicular administration has the potential advantage of minimizing adverse effects seen with systemic therapy and producing local concentrations exceeding those achievable by systemic administration. Hence, both routes of antibiotic administration were used in this study in order to test their efficacy on clinical measures and GCF biomarkers.

Among the antibiotics found to be useful as adjuncts to mechanical periodontal therapy, tetracyclines and metronidazol are the most studied drugs and attracted the most attention . Tetracyclines and their chemically modified relatives have been shown to inhibit the activity of various MMPs by a mechanism independent of the antimicrobial efficacy which in turn may alter the progression of periodontal disease.[5,30] On the other hand, very little is known about the potential effects of metronidazole, which offers much promise in periodontology due to its safe and selective anaerobic spectrum, on MMPs and their inhibitor TIMPs.

In the present study, significant improvements in clinical measurements were observed in chronic periodontitis patients after SRP, SRP+LM and SRP+SM therapies. This was not surprising, since mechanical SRP procedure as the main treatment modality was included in all three groups. Nevertheless, the highest reduction in PD and the highest gain in attachment level were observed in the SRP plus local metronidazole group. This finding confirms previous studies of Yilmaz et al, Noyan et al., Griffiths et al. and Perinetti et al. who demonstrated favourable treatment outcomes in the group of SRP and local metronidazole combination.[9,10,25,26] In this study, the mean reduction in PD in SRP, SRP+LM and SRP+SM groups were 1.45±2.06 mm, 2.15±1.37 mm and 1.35±1.17, respectively. Furthermore, the mean gain in attachment level in these three groups were 1.10±2.27 mm, 1.40±1.57 mm and 0.70±0.94, respectively. However, intergroup comparisons revealed no statistically significant differences in the changes in PD and attachment gain between the groups. Similarly, Stelzel and Flores-de-Jacoby and, Hanes and Purvis reported that metronidazole gel administration combined with SRP was as effective as the mechanical instrumentation alone.[27,28]

The lowest concentration of metronidazole required to inhibit 50% of strains was reported to be below 1 µg/ml for the anaerobic bacteria associated with periodontal disease.[29] Stoltze found that following 1 application of a 25% gel into the pockets, metronidazole concentration was above 1 µg/ml for all chronic periodontitis patients at 4 and 8 hours; in 92% of patients at 12 hours; in 50% at 24 hours, and in 8% at 36 hours.[30]

To the best of our knowledge, this is the first study to investigate the effects of local and systemic metronidazole on the GCF levels of MMP-8 and its endogenous inhibitor of TIMP-1 in chronic periodontitis patients. MMP-8 plays a central role in the turnover and degradation of periodontal tissues, especially in the degradation of type I collagen. Since higher levels of MMP-8 have been found in GCF of periodontitis patients, this enzyme has been suggested to be suitable for monitoring periodontal conditions.[4,31] The pre-treatment GCF level of MMP-8 has been shown to decrease to levels found in periodontally healthy GCF, after non-surgical periodontal treatment.[31-33] Our data have shown that local or systemic usage of metronidazole as an adjunct to SRP resulted in GCF MMP-8 levels comparable to that of the SRP alone. Since mechanical therapy results in the resolution of inflammation, as evidenced by marked reduction in SBI values, a significant decrease in GCF levels of MMP-8 associated mainly with neutrophils is not surprising. MMP-8 is specifically inhibited by TIMP-1, a sialoglycoprotein with a molecular weight of about 28kDa. Our study confirms the findings of a number of studies which have reported that GCF TIMP-1 levels were increased significantly not only after SRP but also after local administration of metronidazole gel.[34-36]

Although metronidazole is effective in inhibiting gram negative anaerobe periodontopathogens, as evidenced by our previous studies and related literature, metronidazole applied either local or systemic appeared to have no direct effect on MMP-8 and TIMP-1 profile in the gingival crevice within the limits of selected experimental period and parameters.[5,9,10,12,26,28,29]

CONCLUSIONS

The findings of the present study indicate that systemic or local administration of metronidazol in combination with SRP can improve clinical periodontal parameters, reduce the GCF MMP-8 levels and increase the GCF TIMP-1 levels in patients with chronic periodontitis. Furthermore, our results suggest that local or systemic use of metronidazole combined with SRP did not provide an additional benefit both in terms of the clinical parameters and GCF levels of MMP-8 and TIMP-1 over the SRP alone.

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