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Efficacy of Local Minocycline HCl 2% Gel as Adjuvant for Scaling and Root Planing in Chronic Periodontitis: A Prospective Randomized Open Blinded Endpoint Study

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Introduction

Chronic periodontitis is an inflammation of periodontal tissue caused by bacterial infection. The clinical symptoms are loss of attachment, bone destruction and tooth mobility. In Indonesia, a survey by the Indonesian Ministry of Health in 2010 showed that 157,485 people suffered from chronic periodontal disease (Kassebaum *et al* 2014). Since 1970, there has been an increasing awareness of bacterial involvement in the etiology of periodontal disease (Socransky 1970). Chronic periodontitis is caused by pathogens called the red complex, which includes *Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia*. These pathogens dominate the subgingival layers and are recognized as the most important pathogens in adult periodontitis (Slots *et al* 1979, Socransky *et al* 1998). The number of pathogens are usually higher in inflamed gingiva than in healthy (Socransky *et al* 1998). Mechanical debridement may not always be able to be carried out to an optimal level due to the variety and complexity of tooth anatomy, which may make it difficult to properly insert instruments into the periodontal pocket. Thus, a direct approach using antibacterial agents in systemic or topical administration has become an important part of periodontal disease management (Eickholz *et al* 2005, Petersilka *et al* 2002, Radvar 1996).

Routine use of systemic antibiotics is contraindicated due to the risk of antimicrobacterial resistance and possible systemic side-effects (Eickholz *et al* 2005). Therefore, local application of antibiotics directly at the subgingival area (into periodontal pockets) has become an alternative. Scaling and root planing, combined with local antibiotics, has been shown to have better results compared to scaling and root planing alone (Radvar *et al* 1996, van Steenberghe *et al* 1993, Timmerman *et al* 1996). A critical period of seven to ten days of routine antibiotic application is needed to provide a long term effect (Eickholz *et al* 2005).

One local antibacterial agent which is stable and has sustained action for at least seven days in periodontal pockets is the topical application of 2% minocycline (Radvar *et al* 1996, van Steenberghe *et al* 1993, Timmerman *et al* 1996). Minocycline is a member of the tetracycline class of antibiotics, and is effective in eradicating the

periodontal pathogens implicated in chronic periodontitis (Sunstar 2011). Subgingival application of minocycline can be used as an adjuvant therapy for chronic periodontitis after scaling and root planing (Ciancio *et al* 1980, Ciancio *et al* 1982, Nakagawa *et al* 1991, Perno *et al* 2001). Minocycline can reduce osteoid degradation by inhibiting osteoblast collagenase. Minocycline may also enhance bone formation by increasing the alkaline phosphatase and collagen synthesis produced by osteoblasts (Gomes *et al* 2007). Unfortunately, minocycline gel is not yet available in many countries. In developing countries such as Indonesia, the scattered distribution of dental devices and experts requires development of practical, durable and effective treatment options for chronic periodontitis. Thus, in this study, we aimed to analyze the efficacy of minocycline HCl 2% gel administered subgingivally as an adjuvant treatment to scaling and root planing in a patient population.

Type of study

A prospective randomized open blinded endpoint study was conducted in a single center (Periodontic Clinic, Faculty of Dentistry, Universitas Indonesia) from November 2013 to November 2014. Ethics approval was given by the Research Ethics Committee of the Faculty of Dentistry, Universitas Indonesia. Patients were consecutively randomized into two parallel groups; minocycline group and control group.

Methods

Subjects

Patients were aged 30 to 55 years with localized chronic periodontitis, who had 4 to 6 mm proximal pocket depth (PD), clinical attachment loss (CAL) equal to or greater than 4 mm and gingival bleeding on probing. Inclusive criteria were patients that had not take any antibiotics in the last three months and had no periodontal treatment in the last six months. Patients were excluded if they were suffering from a systemic disease, were allergic to doxycyclin hyclate, had proximal tooth restorations, had proximal or cervical caries, pregnant or breastfeeding women, smokers, poor oral hygiene, malocclusion and patients on continuous medication.

Study drug

Minocycline HCl 2% gel (Periocline, Sunstar Japan) is a pale yellow coloured gel used for periodontal treatment. It contains 20 mg minocycline HCl (2% potent) per gram as microcapsules. It packed in a syringe (0.5 g) for every patient. The drugs were locally applied into the gingival pocket by inserting the gel at the periodontal pocket base, and then slowly pulling the ends of the syringe while continuing the injection.

Study procedures

At day zero of this study, patients who matched inclusion and exclusion criteria were examined for their oral hygiene. Subjects were examined for papilla bleeding index (PBI), PD and CAL. Subjects that fulfilled inclusion and exclusion criteria were consecutively randomized using a prepared randomization list. Patients with traumatic occlusion were given occlusal adjustments and evaluated for one week before receiving further treatment. We performed supragingival scaling and root planing in all subjects at baseline (day 1).

In the minocycline group, subgingival minocycline HCl gel 2% was applied. The same procedures were repeated at day 7 and day 14. Oral hygiene instruction was given to the patients after each procedure.

At day 14, the amount of plaque was scored

using Löe and Sillness Index (Löe 1967). Papilla bleeding index was scored using Muhleman Modification Index (Muhlemann and Son 1971). At day 21, plaque index, PBI, PD and CAL were scored. PD and CAL were examined using bite registration for probing. At month two, we measured the bleeding scores, PD and CAL in both groups.

The subjects were followed up for six months. At month 3 and month 6, subgingival plaque samples were taken for microbiologic test prior to clinical examinations. We scored the plaque index, PBI, PD and CAL during the follow up period. Radiographic images were taken to analyze the bone density and bone height using bite registration for radiographic film.

Outcome evaluation

In this study, we determined the efficacy of the applications of minocycline based on the rate of clinical and microbiological parameters. For clinical parameters, we measured PBI, PD and CAL at baseline, day 21, month 2, month 3 and month 6. In addition, we measured the increase in bone density at month 3 and 6 with radiographic imaging (Ellis *et al* 2002).

PBI were measured using a Hu-Freidy periodontal probe by carefully inserting the probe in the marginal gingival sulcus (Muhlemann and Son 1971). The mesial surface was measured from the labial/buccal site, while the bleeding on the distal surface was measured from the palatal/lingual site. The intensity of bleeding was measured after 20 to 30 seconds. The PBI was scored as follows: 0 for no bleeding; 1 for bleeding in form of point; 2 for bleeding in form of line; 3 for bleeding in form of triangle; and 4 for wide spread bleeding.

Periodontal pocket depth (PPD) was measured from the base of the gingival pocket with millimeter scale probe. Pocket depth was determined to the nearest millimeter. Pocket examination was measured from labial to buccal and from palatal to lingual (distal). Based on pocket depth, periodontitis can be described as: mild (pocket depths 1 to 3 mm), moderate (pocket depths 4 to 6 mm), and severe periodontitis (pocket depths more than 6 mm) (Cobb 1996, Novak 2006, Ranwey 1993).

CAL was measured from the cemento-enamel junction to pocket base. Measurement was done with periodontal probe in a millimeter scale. Increased or decreased attachment loss was defined as the difference in distance before and after treatment. We categorized the CAL as mild being 1 to 3 mm, as moderate when 4 to 6 mm and as severe being more than 6 mm attachment loss.

Plaque index was measured using an index of Loe and Silness (Löe 1967). We selected teeth 12, 16, 24, 32, 36 and 44. We examined the labial or buccal surface divided into facial, mesio facial, facial and disto facial, while the palatal or lingual were considered as single surfaces. The teeth were dried before plaque index scoring. Score 0 was noted if no plaque adheres to the tip of the explorer when a probe is passed along the cervical portion of the tooth surface. Score 1 was noted when a film of plaque adheres to the tip of explorer when a probe is passed along the cervical portion of the tooth surface. Score 2 was noted for a thin to moderate accumulation of plaque seen on the tooth surface at the cervical portion of the crown. Score 3 was noted when an abundance of plaque is seen on the tooth surface at the cervical portion of the crown. Individual plaque index was calculated as the average plaque score of mesiofacial, facial, distofacial, palatal surfaces. The individual scores of all teeth were then added and divided by the total number of teeth examined.

We performed radiographic evaluation from intraoral digital radiographs of alveolar bone loss in order to measure bone density

(International Association of Maxillofacial Radiology 1995, White *et al* 2004). Radiographic bone density were measured on teeth with 4 to 6 mm pocket depth using periapical projection on the mesial and distal. Radiographic bone density was measured with mean gray levels in an 8 bit computer ranging from 0 to 256 pixels (International Association of Maxillofacial Radiology 1995). We categorized the bone density with a cut-off point of pixel intensity value of 100, as low or high bone density (Hedstr *et al* 2010).

Statistical procedure

In this study, the sample size was calculated based on a power of 80% and alpha level of 5% using standard deviation of 0.8 mm and precision of 0,3736 (Piantadosi 2005). The minimal sample size was determined as 36 subjects for each group.

Statisticians were blinded on the treatment arms. Difference between means of both groups at each time point were tested using student t-test for numerical data (PD, CAL, bone density) and Wilcoxon rank sum test for non-parametric, categorical data (PBI, bleeding index, calculus index). Changes between different timeframes (baseline vs month 3 vs month 6) were tested using Wilcoxon sign tests. Differences between means of both groups were tested using ANOVA and Friedmann Two Way Anova. Data were analyzed using SPSS 20.

Results

84 patients in the study were randomized into 42 patients in each group. Two patients from the treatment group dropped out from the study before receiving any treatment. This resulted in 40 patients in the treatment group

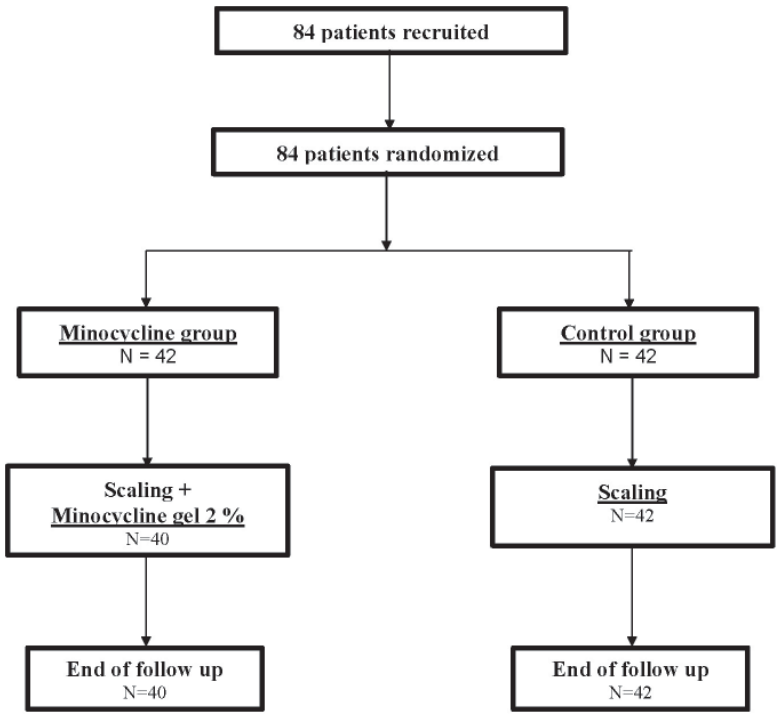

Figure 1. Study participant flow chart

Characteristics	Mean ± SD (unless stated otherwise)		P
	Minocycline group (N = 40)	Control group (N = 42)	
Female, n (%)	33 (78.6%)	31 (77.5%)	0.91
Age (years)	43.67 ± 6.88	44.24 ± 6.99	0.74
BMI (kg/m2)	25.83 ± 2.89	25.24 ± 3.89	0.44
Plaque index score	1.04 ± 0.54	1.08 ± 0.49	0.69
Calculus index score	1.26 ± 0.64	1.44 ± 0.84	0.42
Oral hygiene index score	2.31 ± 1.10	2.53 ± 1.13	0.35
Low bone density	78.67 ± 9.91	67.85 ± 13.09	0.00
High bone density	112.60 ± 9.86	105.01 ± 3.86	0.23

Table 1. Baseline characteristics of subjects.

(minocycline group) and 42 patients in the control group with mean age of 44 years that were followed for 6 months (Figure 1). From Table 1, we can see that the characteristics of test and control groups were similar except for patients with low bone density.

Clinical efficacy

Table 2 describes the CAL, pocket depth and papilla bleeding index based on baseline CAL. CAL, PD and PBI were observed in both groups for 6 months. CAL decreased rapidly in the minocycline group and was significantly different compared to the control group. PBI in the minocycline group were decreased and significantly different to control group with moderate and severe baseline CAL (>4 mm) at day 21 and month 2 follow-up ($p < 0.05$). This loss remained stable during the 6 months of follow-up. Pocket depths decreased in both groups and no significant difference ($p > 0.05$) was found.

In Table 3 we classified subjects based on their bone density and CAL at baseline. All patients with low bone density and moderate to severe CAL at baseline had increased bone density at month 3 and 6, although the increase was not significant. Similar findings were found in patients with low bone density and mild CAL (<4 mm) at baseline. The increase was significant for patients in the control group after month 3, and the increase was only significant for patients with minocycline at month 6.

Minocycline treated patients with high bone density and moderate to severe CAL at baseline had a significant reduction of bone density at month 3. A similar pattern was shown in minocycline patients with high bone density and mild CAL at baseline.

Discussion

In this study, we describe the efficacy of subgingival application of minocycline HCl 2% gel in an Indonesian population. Addition of minocycline HCl 2% to scaling and root planning in chronic periodontitis improved clinical outcomes in longer periods compared to scaling and root planning alone. The test drug also decreased the population of microbiological pathogens significantly.

Regeneration, repair, and a new attachment of periodontal tissues are the goals of treatment of periodontal disease. In our study, clinical attachment loss decreased rapidly in the minocycline group and was significantly

	Base-line	**P**	**21 days**	**P**	**2 mths**	**P**	**3 mths**	**P**	**6 mths**	**P**
Mild attachment loss <4.0 mm at baseline										
Clinical attachment loss										
Test	4.00 ± 0.00	1	2.67 ± 0.48	0.01*	2.58 ± 0.49	0.00*	2.72 ± 0.57	0.00*	2.72 ± 0.67	0.00*
Control	4.00 ± 0.00		2.87 ± 0.60		2.87 ± 0.63		3.25 ± 0.75		3.35 ± 0.78	
Pocket depth										
Test	4.00 ± 0.00	1.00	2.85 ± 0.47	0.34	2.73 ± 0.52	0.04*	2.72 ± 0.57	0.91	2.72 ± 0.67	0.76
Control	4.00 ± 0.00		2.73 ± 0.48		3.00 ± 0.47		2.75 ± 0.68		2.80 ± 0.63	
Papilla bleeding index										
Test	0.28 ± 0.13	0.24	0.07 ± 0.08	0.19	0.07 ± 0.11	0.49	0.05 ± 0.05	0.26	0.10 ± 0.10	0.88
Moderate to severe attachment loss >4.0 mm at baseline										
Clinical attachment loss										
Test	5.12 ± 0.34	0.97	3.50 ± 0.72	0.01*	3.41 ± 0.70	0.00*	3.29 ± 0.75	0.01*	3.33 ± 0.64	0.00*
Control	5.18 ± 0.52		4.50 + 1.71		4.65 + 1.66		4.31 + 1.63		4.45 ± 1.85	
Pocket depth										
Test	5.12 ± 0.34	0.68	3.50 ± 0.72	0.62	3.61 ± 0.94	0.64	3.29 ± 0.75	0.45	3.33 ± 0.64	0.49
Control	5.07 ± 0.61		3.41 ± 0.70		3.53 ± .03		3.13 ± 0.76		3.20 ± 0.75	
Papilla bleeding index										
Test	0.42 ± 0.42	0.10	0.03 ± 0.05	0.01*	0.07 ± 0.05	0.02*	0.06 ± 0.63	0.53	0.08 ± 0.05	0.10
Control	0.61 ± 0.42		0.10 ± 0.12		0.16 ± 0.17		0.07 ± 0.11		0.13 ± 0.14	

Table 2. Comparison of Mean ± SD of clinical data based mild attachment loss (<4 mm) and moderate to severe attachment loss (>4 mm) at baseline. Note: * difference between this time of follow up with the previous phase of follow up was significant with p <0.05.

	Base-line	P	3 mths	P	6 mths	P
Low bone density and mild CAL <4.0 mm at baseline						
Test	78.78 ± 12.23	0.01	79.47 ± 13.89	0.07	81.21 ± 13.87$	0.04*
Control	64.41 ± 11.94		69.05 ± 11.77		68.65 ± 13.01#	
High bone density and mild CAL <4.0 mm at baseline						
Test	115.36 ± 12.47	NA	111.69 ± 5.00*	NA	117.71 ± 11.44^	NA
Low bone density and moderate to severe CAL >4.0 mm at baseline						
Test	78.60 ± 8.43	0.01	82.37 ± 10.47	0.01*	83.05 ± 11.07	0.55
Control	69.13 ± 13.48		71.48 ± 13.35		80.20 ± 18.79	
High bone density and moderate to severe CAL >4.0 mm at baseline						
Test	105.01 ± 3.86	0.28	100.87 ± 6.82	0.18	106.69 ± 4.08	0.49
Control	109.15 ± 4.84		109.35 ± 7.39		111.44 ± 10.26	

Table 3. Comparison of Mean ± SD of radiographic data based on bone density and CAL status at baseline. Note: There were no patients in the control group in category of High Bone Density (>100 PI) with baseline CAL <4.0 mm. * Differences between baseline and month 3 were significant ($p < 0.01$); Differences between month 3 and month 6 were significant ($p < 0.05$); ^ Differences between baseline and month 6 were significant ($p < 0.01$); # Differences between baseline and month 6 were significant ($p < 0.05$).

different compare to the control group. Papilla bleeding index in the minocycline group was decreased and significantly different in treatment group, with moderate to severe baseline CAL remaining stable for the 6 months of follow-up. These results are similar to two short-term, double-blind, parallel studies by Nakagawa *et al* (1991) and Van Steenberghe *et al* (1993) that evaluated the effect of subgingivally administered 2% minocycline in addition to mechanical debridement. Their studies showed that the treatment group had better response than patients in the placebo group. However, another study by Timmerman *et al* (1996) showed no statistically significant differences between test and control groups in probing depth and attachment level.

High quality radiographic imaging at the right time period is needed to evaluate the quality and quantity of alveolar bone after periodontal treatment (Van Steenberghe *et al* 1993). Among our patients who had bone density and low CAL ≤4 mm, minocycline significantly increased bone density after six months. Although not significantly different from the control group, the same pattern can be observed in patients who have low bone density with moderate to severe CAL ≥4 mm. Previous studies have stated that bone healing in chronic periodontitis patients took 4 to 6 months, while it might take 6 to 12 months to form mature bone (Ellis *et al* 2002, White *et al* 2005).

It should be acknowledge that our study had several weaknesses. Firstly, this study was

an open randomized trial without masking. This design was prone to observation bias that may have impacted the results. However, in this study we used hard clinical and radiological endpoints, thus the bias was reduced. Moreover, our effort to blind the statisticians who analyzed the results may help minimize the bias. Second, the study was conducted only in a single center located in a metropolitan city. Obviously, this impacted generalizability, as patient characteristics and severity of the disease in a metropolitan city may differ from other areas. However, patient characteristics in our study were similar with to the characteristics of chronic periodontitis populations in general.

Conclusion

Adjuvant minocycline HCl 2% gel to scaling and root planing has showed promising results. The use of minocycline HCl 2% might assist dentists in providing effective treatment options for their chronic periodontitis patients.

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