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Efficacy of subgingivally applied minocycline in the treatment of chronic periodontitis

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Background: The use of adjunctive minocycline with mechanical debridement in treating periodontitis has been widely studied using different methods. However, the results from these studies are equivocal.

Objective: The purpose of this study was to clarify the efficacy of the adjunctive use of subgingival minocycline application plus scaling/root planing as compared with the results of one episode of scaling/root planing in the treatment of chronic periodontitis.

Methods: Fifteen patients were enrolled in this split-mouth clinical trial. Probing depth, clinical attachment loss, gingival index, and bleeding on probing were evaluated at the baseline before scaling/root planing and 6, 10, 14, and 18 weeks later according to a single-blind protocol. The amount of interleukin-1β (interleukin-1β pg/site) at each lesion was also simultaneously measured in gingival crevicular fluid in a parallel comparison design. After full-mouth baseline measurements and scaling/root planing, 78 lesions with a residual mean probing depth of 5 mm at anterior teeth were selected and equally distributed in either right or left sites based on a split-mouth symmetrical design and randomly assigned to one of two treatment groups (with or without minocycline administration, $n = 39$ for each group).

Results: Probing depth significantly decreased from the baseline (week 0) to week 6 after scaling/root planing ($p < 0.05$) in both groups, but there was no statistically significant difference between the two groups ($p > 0.05$). However, at weeks 10, 14, and 18, the experimental group showed significantly greater improvement in pocket reduction than the control group ($p < 0.05$). Similarly, both groups also showed significant decreases in gingival index scores from weeks 0–6 ($p < 0.05$), but gingival index reductions at weeks 10, 14, and 18 were statistically significant in favor of the experimental group ($p < 0.05$). The experimental group had more attachment gain than the control group at weeks 14 and 18 ($p < 0.05$). Values of interleukin-1β (pg/site) at the experimental sites were significantly reduced at weeks 10, 14, and 18, as compared to values at control sites ($p < 0.01$). Finally, the incidence of bleeding on probing showed no differences between the two groups for any time interval ($p > 0.05$).

Conclusions: In this 18-week clinical trial, the results suggested that scaling/root planing with adjunctive subgingival administration of minocycline ointment has a significantly better and prolonged effect compared to

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scaling/root planing alone on the reduction of probing depth, clinical attachment loss, gingival index, and interleukin-1β content, but not on bleeding on probing.

The initial treatment of chronic periodontitis consists of oral hygiene instructions and meticulously repeated supra- and subgingival mechanical debridement to remove plaque and calculus deposits, even though it is rarely effective in the complete removal of putative periodontal pathogens (1). For pockets deeper than 5 mm after initial treatment, surgical procedures have been suggested (2). In 1979, Goodson *et al.* (3) first proposed the local delivery of 25% tetracycline hydrochloride in the treatment of periodontitis. Several research groups focused on this local application for a number of years (4–6). It seems that the local administration of antimicrobials can reduce pocket depths and achieve more advantageous outcomes for probing attachment level and bleeding on deep probing with non-surgical periodontal treatment (7, 8). As a possible alternative to periodontal surgery, local delivery devices that provide effective drug concentrations during periodontal therapy have been developed. Recently, in two multicenter clinical trials, subgingival delivery of doxycycline demonstrated similar positive results to scaling and root planing for probing depth reduction and attachment level gain in the treatment of periodontitis (9). For patients undergoing supportive periodontal therapy, the locally delivered controlled-release doxycycline without concomitant mechanical debridement was also as effective as scaling and root planing over a 9-month period (10).

Other studies have also demonstrated equal clinical effects for tetracycline fibers compared to metronidazole gel and minocycline ointment (11, 12). In another study by Meinberg *et al.* (13), scaling and root planing with four subgingival administrations of minocycline resulted in greater probing depth reduction and less-frequent bone height loss compared to conventional periodontal maintenance.

Among those studies described above, the effect of scaling/root planing significantly reduced probing attachment levels (14–17) and pocket depth (17) between experimental and control groups. However, in some studies the locally delivered antibiotics after scaling/root planing failed to show any reduction of pocket depth and gain of attachment level as compared with control groups. Even microbiological evaluations using DNA probe technology showed no obvious differences between minocycline ointment and vehicle control groups (15, 17).

Therefore, the aim of the present study was to compare the clinical effect of subgingivally applied 2% minocycline hydrochloride plus scaling/root planing vs. a non-vehicle control (single episode of scaling/root planing) on four periodontal clinical parameters and the expression of a proinflammatory cytokine (interleukin-1β) in gingival crevicular fluid for the treatment of chronic periodontitis.

Materials and methods

Patient eligibility

Fifteen patients with generalized moderate to severe periodontitis were selected from the dental clinic population of the Department of Periodontics at Taipei Medical University Hospital. Patients were included if they met the following criteria: (i) were 20 years or older and in good general health; (ii) had more than 16 natural teeth; (iii) had test teeth that still had both mesial and distal neighboring teeth; and (iv) had periodontal pockets with more than 6-mm deep residual pockets and bleeding on probing. Exclusion criteria included: (i) being pregnant and nursing; (ii) taking contraindicated medications and/or having a compromised medical condition, such as diabetes, hepatitis, or hematotic disorders; (iii) allergic to minocycline and/or tetracycline antibiotics; (iv) received antibiotic treatment 2 weeks prior to or during the study; (v) the clinic used drugs with anti-inflammatory properties and/or 0.12% chlorhexidine mouth rinse on a routine basis; and (vi) received periodontal surgery, restorative work, or tooth extraction adjacent to either of the test areas in the previous 3 months. The study was explained to patients, and all participants signed informed consent at enrollment.

Study design

This study protocol was designed as a split-mouth clinical trial of 18-weeks duration simulating the treatment flow of conventional periodontal therapy until the first 3-month visit. After qualifying for the study entry, 15 patients (eight males, seven females; age range, 33–62 years; mean 43 years) were enrolled in this study. Five clinical variables were evaluated at the baseline (week 0) by one examiner who recorded all measurements in a single-blind design, including probing depth, attachment loss, bleeding on probing, gingival index, and interleukin-1β pg/site in gingival crevicular fluid. Full mouth scaling/root planing was performed at the baseline appointment following the initial examination.

Six weeks after the initial scaling/root planing (week 6), the above measurements were repeated at the first recall. Those single root teeth still bleeding on probing and with a mean probing depth of 5 mm measured at six sites were randomly assigned to one of two treatment groups (with or without minocycline hydrochloride administration). Thirty-nine sites for each group (totally 15 patients) were approximately depth-matched with a split-mouth

design and included in this study. Two percent minocycline gel (Periocline, Sun Star, Osaka, Japan) was applied to the pocket base of experimental sites by gently inserting the tip of a specially designed applicator and withdrawing it in a zigzag motion until the paste flowed over the gingival margin. The patient was instructed not to drink or eat for 30 min after drug administration. Over the following 3 weeks, the experimental sites received additional subgingival minocycline administration once a week, whereas the control sites received only supragingival plaque control. During appointments at weeks 6, 10, 14, and 18 (3 months after initial treatment), all periodontal measurements, including the measurement of interleukin-1β content in gingival crevicular fluid, were repeated. Data were collected and analyzed to compare the treatment effect between the test and control sites.

Clinical measurements

Probing depth — Probing depth was measured from the free gingival margin to the base of the periodontal pocket with a slight manual force (of 0.25 N) using a UNC #15 periodontal probe calibrated in 1-mm intervals. Measurements were taken at six sites per tooth at the baseline appointment, and at weeks 6, 10, 14, and 18 for all 78 sampled teeth.

Clinical attachment loss — Clinical attachment loss was measured from the cemento-enamel junction, or from the margin of a fixed restoration, to the bottom of the periodontal pocket. Six sites per tooth were measured for all selected teeth of both groups.

Gingival index — The Löe and Silness gingival index was used. Inflammation was measured at six sites around each of the 78 sampled teeth.

Bleeding on probing — A dichotomous bleeding score was measured by probing with the same slight manual force at six detected sites around each sampled tooth. Bleeding on probing was recorded as '0' if no bleeding occurred within 30 s, and '1' if there was bleeding within 30 s of the time of conventional probing.

Interleukin-1β content — Gingival fluid samples were collected from the mesiobuccal and mesiolingual surfaces of selected teeth with Periopaper strips (HARCO Electronics, Irvine, CA, USA) of standard dimensions. Prior to sampling, the test area was air-dried and isolated with gauze. The first paper strip was slipped into a gingival pocket 1 mm subgingivally for 5 s and discarded immediately in order to avoid contamination by saliva. A second paper strip was inserted in the selected site for another 30 s. The remaining paper strip was then dipped in a 200-μl phosphate-buffered saline container and stored at −80°C for further interleukin-1β analysis. A Periotron HAR-6000 (Periotron 6000, HARCO Electronics) was used to accurately estimate gingival fluid volume by daily adjustment, and this value was adjusted in a calibration function using human serum as the standard. Results were calculated as the mean of triplicate measurements in each sample taken from each site at each time point.

The total amount of interleukin-1β for each sample was measured using an R & D (Minneapolis, MN, USA) Quantikine interleukin-1β-specific ELISA kit. The 250-pg/ml stock standard solution was first prepared by adding 5 ml of RD6C calibrator diluents into interleukin-1β standard powder and waiting for 15 min. Equal volumes of stock standard solutions and RD6C were then mixed and diluted in order to obtain serial dilutions of interleukin-1β with different concentrations (62.5, 31.2, 15.6, 7.8, and 3.9 pg/ml). All samples were tested for the total amount of interleukin-1β following the protocol provided by the manufacturer.

Data analysis and statistical methods

Computer software (SPSS 10.0 for Windows) was used for data analysis. Mean values and standard deviations for probing depth, clinical attachment loss, gingival index, bleeding on probing, and interleukin-1β content for both treatment groups and at different time intervals were calculated. For between-group detection of statistically significant differences, paired *t*-test and Student's *t*-test for probing depth, clinical attachment loss, and interleukin-1β content; Wilcoxon signed-rank test for gingival index; and McNemar's test for bleeding on probing were utilized. Results were regarded as statistically significant when $p < 0.05$.

Results

Probing depth

Before performing the study, calculations of the minimal sample size for the single-blind study by using variances indicated that approximately nine sites for each group would allow a mean detectable difference in probing depth change with 90% power and $\alpha = 0.05$ (one-tailed). In total, 39 sites for each group were selected from 15 patients in this study. In addition, an intraexaminer κ-value of 0.82 was determined based on the measurement of 15 periodontal pockets in duplicate by a single-blinded operator. The strength of agreement was judged as being almost perfect.

The measurement of pocket depth showed no differences between the experimental and control groups at the baseline (week 0, Fig. 1). At week 6, both groups showed statistically significant probing depth reduction when compared to respective baseline ($p < 0.05$), but no statistical differences were found between the two groups ($p > 0.05$). However, the experimental group showed significantly greater improvement than the control group at weeks 10 (4.21 ± 1.13 vs. 4.85 ± 1.20 mm), 14 (3.92 ± 1.01 vs. 4.92 ± 1.20 mm), and 18 (3.92 ± 1.24 vs. 5.15 ± 1.27 mm) ($p < 0.05$).

In the experimental group, the 5-mm pocket reduction rate of the probing depth ($\leq$ 4 mm) at 18 weeks was measured 74.36% (29 sites/39 sites). However, the reduction rate of control group was only 28.21% (11sites/39 sites) as compared to that of baseline data.

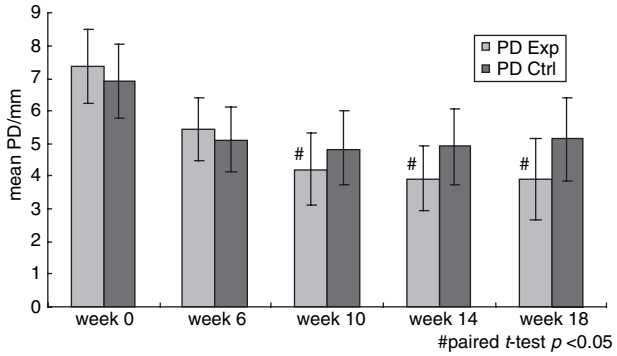

Fig. 1. Probing depth (PD, mean ± SD) of the experimental (PD Exp) and control (PD Ctrl) groups measured following a parallel-design protocol. Statistically significant for intergroup comparison at $p < 0.05$.

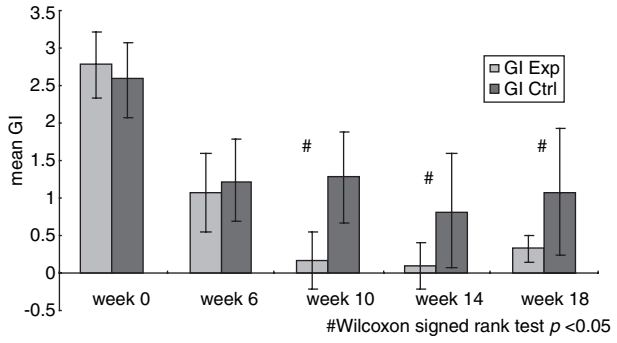

Fig. 2. Gingival index (GI, mean ± SD) of the experimental (GI Exp) and control (GI Ctrl) groups measured at the initial appointment and subsequent study periods. # Statistically significant for intergroup comparison at $p < 0.05$.

Gingival index

The baseline gingival indices were similar between the experimental and control groups (week 0, Fig. 2). At week 6, when compared to their respective baselines, both groups showed significant decreases in the gingival index scores in the intragroup comparison after scaling/root planing ($p < 0.05$), but no statistical differences were found between the two groups ($p > 0.05$). Reductions in the gingival index at weeks 10, 14, and 18 were statistically significant in favor of the experimental group ($p < 0.05$).

Clinical attachment loss

Figure 3 shows statistically significant differences in results for weeks 14 (6.54 ± 1.54 vs. 7.13 ± 2.10 mm) and 18 (6.40 ± 1.54 vs. 7.58 ± 2.00 mm) ($p < 0.05$) between the two groups, with the experimental group experiencing more attachment gain than the control group.

Bleeding on probing

Figure 4 summarizes changes in bleeding on probing in the present study. All sites of both groups bled on probing at the baseline (week 0). At week 6, the incidence of bleeding on probing was significantly reduced within groups when compared to the respective baselines ($p < 0.05$). There were no significant differences between the two groups at any time interval ($p > 0.05$).

Interleukin-1β amount/site

The mean interleukin-1β content values in gingival crevicular fluid before scaling/root planing were 103.9 ± 31.5 and 106.2 ± 29.9 pg/site for the experimental and control groups, respectively (Fig. 5). Both groups presented a significant reduction in interleukin-1β content in gingival crevicular fluid after scaling/root planing treatment. However, after four consecutive applications of 2% minocycline, the experimental group showed a significant difference over the control group in the total amount of interleukin-1β content in gingival crevicular fluid at weeks 10 (37.0 ± 14.8 vs. 55.3 ± 18.4 pg/site), 14 (34.0 ± 15.1 vs. 50.0 ± 14.1 pg/site), and 18 (36.9 ± 15.6 vs. 63.1 ± 24.3 pg/site) ($p < 0.05$).

Discussion

The present study was a randomized, single-blind study in which sample sites were selected to simulate the clinical evaluation of periodontal lesions with moderate to severe probing depth after scaling/root planing. Results showed significant improvement in experimental lesions treated with minocycline ointment by the greater probing depth reduction, gingival index reduction, and more attachment gain. The results are similar to those reported in earlier multicenter studies, which demonstrated improvements in probing depth and attachment level (18, 19). However, no outcome differences in bleeding on probing between the experimental and control groups were found in the current study.

The minocycline ointment utilized for subgingival application consisted of a bioresorbable delivery system loaded with 2% minocycline HCl. The matrix was a mixture of hydroxyethyl-cellulose, aminoalkyl-methacrylate triacetine, and glycerinum. Magnesium chloride was used to modify the release properties. The elimination half-life based on the assumption of a single-compartment open model was estimated to be 3.9 h, and the total time of effective antimicrobial activity was expected to be approximately 1 day (20). As judging from a study of minimal inhibition concentration of 2% minocycline against periodontopathic bacteria, the curve obtained from values measured up to 72 h after administration in 97 patients with

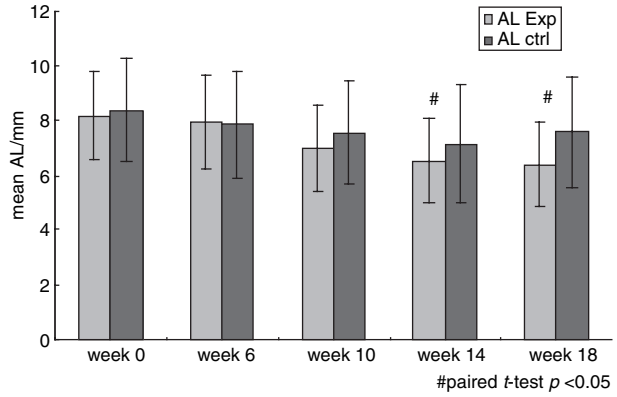

Fig. 3. Attachment loss (AL, mean ± SD) of the experimental (AL Exp) and control (AL Ctrl) groups measured following the study protocol. The intergroup difference was examined by paired *t*-test and showed the experimental group experiencing more attachment gain than the control group in week 14 and week 18.

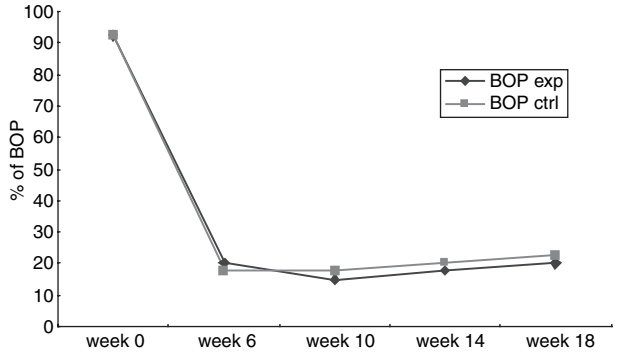

Fig. 4. Bleeding on probing (% of BOP) of both groups showing significant reductions after scaling/root planing (week 6). There was no statistical difference between the experimental and control groups after subgingival minocycline application (McNemar's test).

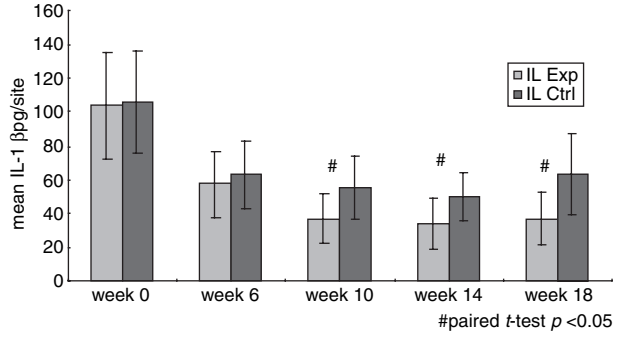

Fig. 5. Experimental groups (IL Exp) showing a significant reduction of IL-1β (pg/site, mean ± SD) at weeks 10, 14, and 18 after undergoing 2% minocycline treatment. # Statistically significant for intergroup comparison at $p < 0.05$. IL, interleukin.

periodontitis showed that the effective concentration was maintained for approximately 7 days (20). Under the above conditions, a regimen using 1.25–2.5 ml of minocycline ointment to treat a 5- to 7-mm pocket including four administrations at weekly intervals was suggested.

The results of van Steenberghe *et al.* (15) revealed that four consecutive applications of minocycline combined with scaling and root planing proved significantly better in terms of decreasing probing pocket depths at 1 and 3 months, but no significant differences were observed in terms of changes in clinical attachment level or bleeding index. In the present study, a dichotomous bleeding score (bleeding on probing) was used to evaluate the clinical effect of the antimicrobial agent. The incidence of bleeding on probing was significantly reduced within groups when comparing week 6 to the baseline, but there were no differences between the two groups at any time interval. Bleeding on probing has been reported to have a high false-positive rate among those prognostic indicators (21). A graded bleeding index, combining criteria of rapidity and extent of bleeding, is highly recommended as a better prognosticator for clinical trials. In this study, bleeding on probing is not sensitive enough to detect the difference of scaling/root planing and scaling/root planing adjunct with subgingival minocycline application in the 6–18-week follow-up period.

A split-mouth technique was used in this study to investigate the clinical effect of subgingivally applied minocycline. It has been suggested that a split-mouth design may induce a carry-over effect of subgingival antibiotic administration due to wash-out of antimicrobial agents and boosting of systemic responses (22). However, it was concluded that gingival crevicular fluid is relatively isolated from saliva (23). Considerable evidence has also shown that antibiotic mouth rinses do not penetrate the periodontal pocket (18). Results of our study show that probing depth, gingival index, and the content of interleukin-1β in the gingival crevicular fluid of the experimental group were significantly reduced as compared to those of the scaling/root planing group at 10, 14, and 18 weeks. This implies that the systemic boosting effect of topical minocycline application may have been negligible in our clinical trial. The design of split-mouth match in this study may define the statistical unit for analysis as qualifying sites instead of using the mean values for each patient. A considerable influence of behavior factors,

environmental factors, and fluctuant individual susceptibility on the measurement of gingival crevicular fluid interleukin-1β level were also avoided simultaneously (24).

There are some contradictions among the results of studies using subgingival minocycline application with a placebo vehicle. The long-term efficacy and safety of locally applied minocycline in adult periodontitis patients were investigated in an 18-month, randomized, double-blind, parallel, comparative study with local application of 2% minocycline hydrochloride-gel vs. placebo-gel in patients with moderate to severe chronic adult periodontitis (17). Results showed a statistically significant improvement in all clinical parameters, including probing depth, attachment loss, and papillary bleeding index, irrespective of the treatment modality. Another controlled study using a 2% minocycline formulation also showed a non-significant difference in attachment loss and the bleeding index between the active drug group and vehicle gel group (15). Both studies demonstrated gains in probing attachment level and reductions in the bleeding index over their control groups. A similar placebo effect was also evident in some vehicle-designed studies of subgingival irrigation with antibacterial agents on periodontal inflammation (25, 26). Even the use of saline irrigation in a placebo design might introduce a statistically significant improvement in most clinical parameters and a transient therapeutic effect on the subgingival microflora.

In contrast to the control studies of Timmerman *et al*. (17) and van Steenberghe *et al*. (15), a study done by Jones *et al*. (16) without a placebo design showed that the mean probing depth reduction with adjunctive minocycline was significantly greater at 3 months. Furthermore, Williams *et al*. (27), when treating periodontitis by local administration of minocycline microspheres, concluded that scaling and root planing plus minocycline microspheres were more effective than scaling and root planing alone in reducing probing depths in periodontitis patients. Those reports demonstrated improvements related to probing depth reduction that coincided with the results of the current study. The differences in reductions in probing depth and gains in clinical attachment loss between these studies may have arisen from their different designs, sample sizes, and methodologies. It also infers that using a placebo in comparative studies of subgingival minocycline administration might perturb the ecosystem of the subgingival plaque in the periodontal pocket and induce transient therapeutic actions on the control group. Therefore, no placebo vehicle was used in the control group in order to avoid introducing confounding influences of a placebo design into this subgingivally antibiotic study.

It is well known that interleukin-1β plays a role in immune responsiveness, chronic infection, and bone resorption. The observation of interleukin-1β-producing cells presented in higher numbers in tissue from periodontal lesions suggests that interleukin-1β and these inflammatory cells may be related to periodontal pathogenesis (28). Many studies have reported that gingival crevicular fluid interleukin-1β levels are significantly elevated and associated with gingival inflammation (29, 30). Several studies also confirmed an association between elevated gingival crevicular fluid interleukin-1β levels and different severities of periodontal disease (31–34). The total interleukin-1β activity was found to be a sensitive indicator reflecting clinical status of periodontal disease. However, several studies have reported that no correlation occurs between interleukin-1β concentration and clinical parameters included probing depth, plaque index and bleeding index or attachment loss (35, 36). We calculated the total amount of interleukin-β in our sample instead of using interleukin-β concentration because the gingival crevicular fluid collected by paper strips is a combination of residual fluid and inflammatory exudates present in the gingival sulcus (37); the total volume of gingival crevicular fluid could be dynamically affected by inflammatory exudates, which might concomitantly dilute the concentration of any biomediators in gingival crevicular fluid (38). The volume of the gingival crevicular fluid became a factor of dependent variance, as did the concentration of interleukin-1β in the gingival crevicular fluid.

Furthermore, it has been estimated that the fluid present in a 5-mm periodontal pocket is replaced approximately 40 times/h (18). Such high clearance is the result of a low resting volume (0.5 μl) and a comparatively high flow rate (20 μl/h) (39). Therefore, an independent parameter for evaluation of the expected therapeutic performance of local periodontal delivery devices is the expected length of time in measuring the total amount of cytokines in the gingival crevicular fluid of the periodontal pocket. In our study, the resting volume of the gingival crevicular fluid was initially discarded in order to avoid possible contamination by saliva. A second paper strip was harvested for measuring the total content of interleukin1-β in a constant time period (30 s). The original concentration of inflammatory mediators in gingival crevicular fluid no longer existed theoretically.

Although bleeding on probing is not sensitive enough to differentiate the effect of subgingival minocycline administration on the status of gingival inflammation, it was clearly demonstrated at weeks 10, 14, and 18 when comparing the interleukin-1β content of the experimental sites to that of the control group. This implies that the clinical effect of subgingival application of minocycline microspheres on the control of periodontal inflammation, in addition to the reduction of probing depth and clinical attachment loss, may be sustained for at least 12 weeks.

National Health Insurance has been launched in Taiwan since 1995. The dental health care providers and the general public appeared to have successfully adapted to the new policy during these years, and the general satisfaction rate has been greater than 70% (40). However, it does not mean that Taiwan's National Health Insurance is flawless and will last for a long time in its current format. Financial balance has always been one of the major concerns of Taiwan's National

Health Insurance since its debut (41). To secure financial balance has been ranked high in the authority's priority list, and the strategy of global budget has been regarded as the best solution to this goal. Most of the therapeutic dental services are covered by the National Health Insurance, but under the dental global budget surveillance, repeated scaling/root planing within 180 days is not allowed. Therefore, the other purpose of the present study is try to find out another resolution in the treatment of residual pockets ≥5 mm in order to cope with the dental global budget in Taiwan's National Health Insurance system. In the present short-term clinical trial, it was indicated that most of the residual pockets would be reduced (≤ 4 mm, 74.36%) significantly in experimental sites without the necessity of surgical approach. Subgingival minocycline administration is an another option for the controlling of dental cases with moderate to severe chronic periodontitis.

In conclusion, within this 18-week clinical trial, our data suggest that scaling and root planing combined with subgingival administration of minocycline ointment have a significantly better and prolonged effect compared to scaling/root planing alone on the probing depth, clinical attachment loss, gingival index, and interleukin-1β content, but not on bleeding on probing. The local application of minocycline can be effective as an alternative adjunct to mechanical therapy in sites that respond poorly to a single episode of scaling/root planing.

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