

The clinical result of subgingivally delivered andrographis paniculata gel versus 10% doxycycline gel in the treatment of supportive periodontal therapy patients

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Objective: To evaluate and compare the clinical effects between locally delivered *Andrographis paniculata* gel (AP gel) and 10% doxycycline gel as an adjunct to scaling and root planing (SRP) in the treatment of chronic periodontitis subjects, who were involving on supportive periodontal therapy (SPT).

Materials and methods: Twenty nine systemically healthy subjects with chronic periodontitis, who were involving on supportive periodontal therapy, were recruited in this split mouth, single-blinded, randomized controlled clinical trial (RCT), 6 months study. The subjects had to have three non-adjacent single root teeth with probing depth (PD) \geq 5 mm and bleeding on probing (BOP), they had not received periodontal therapy within 6 months before study. The disease sites were randomly treated with; SRP plus AP gel, SRP plus 10% doxycycline gel, or SRP alone. Probing depth (PD), relative attachment level (RAL), gingival index (GI), plaque index (PI) and BOP were recorded at baseline, 3 and 6 months after treatments.

Results: Clinical changes were significantly improved in all treatment groups ($p < 0.05$). When compared the clinical outcomes among the treatments, found that the treated sites with SRP plus AP gel or SRP plus 10% doxycycline gel were similar. These two treatment modalities can increased more number of treated sites with RAL gain >2 mm and reduced GI better than SRP alone.

Conclusion: The local application of AP gel or 10% doxycycline gel as an adjunct to SRP showed better improvement of clinical parameters. These benefit may be reduce the need of periodontal surgery in some patients or reduce the frequency of periodontal maintenance visit.

Keywords: *Andrographis paniculata* gel, chronic periodontitis, doxycycline gel, supportive periodontal therapy, subtraction technique

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Introduction

Periodontitis is the most common oral disease that cause tooth loss in adult population. [1] This disease incur the inflammation of periodontium, loss of periodontal ligament (PDL) and alveolar bone. Which lead to tooth mobility and tooth loss eventually. Periodontitis is primarily driven by bacterial plaque. The toxicity of bacteria components (endotoxin) and

their by product (exotoxin), destruct periodontal tissue both directly and indirectly. [2, 3]

The treatment of periodontitis currently focus on eliminating the bacterial plaque. SRP is the basic of periodontal treatment. However, in some cases, SRP alone can not successfully eliminate bacteria. Due to the ineffectively function of the instrumentation in some areas [4, 5]. Thus the adjunctive using of antibiotic, in the form of local

subgingivally delivered drug has been suggested to overcome the limitations of this non-surgical treatment.

Doxycycline gel is a local drug delivery system which consists of doxycycline, a broad spectrum antibiotic. Doxycycline is a semi-synthetic tetracycline. There are several studies [6-9] reported that when used doxycycline gel in conjunction with SRP, yielded to promotion of periodontal tissue healing, such as reduced gingival inflammation, enhanced PD reduction and clinical attachment level (CAL) gain, better than SRP alone. Some studies also shown that the use of doxycycline gel alone in the treatment of periodontitis, could reduce gingival inflammation, reduce PD and promote CAL gain equivalent to SRP. [10] Furthermore, doxycycline gel is the only one of local drug delivery system, which was approved by the Food and Drug Administration of USA (FDA) that can be used as a single treatment (monotherapy) for the treatment of periodontitis. [3] However, this drug has high price because it must be imported from other countries. Thus, local subgingivally delivered drug had been developed from Thai herbal for replacing the drugs from abroad.

“Fah talayjone” is the herb commonly found in the equatorial region of Asia. The scientific name is *Andrographis paniculata* (AP) (Burm.f.) Nees, plants in the family Acanthaceae. This herb was the one of five herbal species which had been promoted from Thai economic and social development plan No. 6 to use in community hospitals for treated infections of the upper respiratory tract and relieved the symptom of acute diarrhea. [11] Active ingredients found in AP consists of andrographolide, dehydrographolide, neoanhydrographolide and deoxyanhydrographolide. [12] These ingredients have ability for anti-infective to *Staphylococcus aureus* [13], *Streptococcus mutans* (KPSK2 and GS-5 species) and *Porphyromonas gingivalis*. [14] These also have anti-inflammatory effect and can stimulate the host's immune system.

[13, 15] Subsequently this herb had been developed and used in dentistry in the form of gel (AP gel) for the treatment of periodontitis.

Previous studies [16, 17] compared the efficacy of clinically using AP gel and the other local drug delivery system from abroad (metronidazole gel and minocycline ointment), as an adjunct to non-surgical treatment in the initial phase of periodontal therapy. The results showed that AP gel could promote healing of periodontal tissue equivalent to the drugs from abroad. However, no study investigated the clinical outcomes of AP gel when use in the periodontitis patient, who involved on SPT. In addition, there are no study compared the clinical outcomes between AP gel and doxycycline gel, when used in conjunction with SRP.

The objective of this study was to compare the clinical effect of SRP with the adjunctive subgingivally administration of AP gel or 10 % doxycycline gel in the treatment of chronic periodontitis patient, who involved on SPT in 6 months trial.

Materials and Methods

This research protocol had been approved by Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Institutional Review Board, Bangkok, Thailand. (MU-DT/PY-IRB 2012/035.2408).

Study Population

The number of sample size was calculated from the result of previous study. [17] A total of 29 chronic periodontitis patients were included in the study according to the following criteria: 1) had at least three non-adjacent, single-rooted teeth with PD ≥ 5 mm which bled upon probing. 2) radiographic evidence of alveolar bone loss. 3) involving on SPT (recall interval 6 months to 1 year). 4) had not received periodontal treatment for at least 6 months prior to the study 5) signed

informed consent form. The exclusion criteria were: 1) systemic diseases/conditions that affect the periodontal tissue. 2) history of antibiotic therapy within 3 months before the study. 3) smoking patient.

Gel preparations

The AP gels were compounded by the Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. [18] The doxycycline gels were imported from USA (TOLMAR Inc.). The components of drug systems were reported as in the previous studies. [7, 10, 18] The AP and doxycycline gels were stored at 4 °C throughout the study.



Figure 1 The doxycycline (left) and AP gels (right)

Examination Variables

The following variables were assessed at baseline, 3 and 6 months : PI [19] ; GI [20] ; gingival bleeding on probing (GBP) [21] ; PD and RAL. Both PD and RAL were recorded by the use of stent as the guide for periodontal probe (UNC-15, Hu-Friedy, Chicago, IL) placement. The examiners were blinded for treatment group (single-blinded). Intra & inter-examiner reproducibility test were performed prior the study, shown excellent reproducibility (kappa value 0.95-0.98).

Randomization & Treatment procedures

This was split mouth design study, randomed by computer-generated table for three treatment options, SRP alone , SRP with doxycycline gel (SRP+ Doxy gel) and SRP with AP gel (SRP+AP gel).

After baseline examination, the subjects were given proper oral hygiene instruction (OHI). Full mouth SRP was done by ultrasonic scaler

(Piezon Master 400 with A and PS tips, EMS, Nyon, Switzerland), Universal and Gracey curette (Hu-Friedy, Chicago, IL) under local anesthetics. The 3 test groups, after SRP, the roots were irrigated with 0.9 % sodium chloride 2 ml and subgingivally applied Doxy gel or AP gel (figure 2). In the AP gel group, AP gel were re-administrated at 1, 2, 3 weeks after the first application. In addition, Doxy gel and AP gel were re-applied again at 3 months. Subjects were not allowed to use floss, interproximal brush or brushing with modified Bass technique for a week after each visit of drug application.



Figure 2 Subgingivally application of doxycycline (left) and AP gels (right)

Data analysis

Primary outcome variables were PD and RAL. This study focused both changing volumn and frequency of site of PD and RAL change. Subject was unit of statistical analysis. Each tooth in each treatment group had at least one site, therefore clinical data was calculated to average value from each subjects in 3 periods. All data was analyzed by SPSS for Windows Release 16.0 computer software. All tests were significance at $p<0.05$. Examiner reproducibility was assessed by Cohen's Kappa. Repeated measure ANOVA and Bonferroni test were used for comparing between group and within group for all clinical parameters.

Results

Twenty-nine subjects (male 12, female 17) were included in this study, all subjects attained through the end of study. The subject's age range

was 35-66 years with mean age of 51.38 ± 8.8 . No side effects were reported from all of subjects. Because some subjects had experimental teeth more than one tooth per group. Overall number of teeth included in this study was 111 teeth (200 sites). The detail of number of teeth and sites for each treatment group for one subjects were shown in table 1. At baseline examination, there was no any difference of all parameters between group. All parameters improved significantly after treatment when compared with baseline data.

PD reduction and RAL gain.

After treatment, there was no difference in PD reduction between group but RAL gain. We could find that in the first 3 months of study, RAL of test groups were improved significantly, while the improvement of control group was significance at six months, as shown in table 2.

When determined to the frequency of site of PD and RAL change, we found that at the end of study, the local drug groups notified more frequency of site had PD reduction and RAL gain >2 mm than control group (table 3)

Gingival inflammation.

From table 4, we could find that after treatment, all groups had GI improvement but there was no difference between groups. GI was significantly decreased both three and six months evaluation in local drug groups but only three months evaluation in control group.

Plaque index.

All groups had significant PI improvement from baseline for all time points with no significant difference between groups. (table 5).

Table 1 Number of teeth and sites in one subject for each treatment group.

	Control	Doxy gel	AP gel
Number of teeth	$1.31 \pm 0.54(1-3)$	$1.28 \pm 0.96(1-6)$	$1.28 \pm 0.46(1-2)$
Number of sites	$2.34 \pm 1.59(1-8)$	$2.24 \pm 3.14(1-18)$	$2.28 \pm 1.87(1-10)$

Table 2 Mean \pm SD of PD and RAL of each treatment group in three examination periods.

Tx. group	PD			RAL		
	Baseline	3 months	6 months	Baseline	3 months	6 months
Control	5.567 ± 0.76	$4.10 \pm 0.94^*$	3.39 ± 0.73	15.09 ± 1.97	$14.79 \pm 1.92^*$	14.46 ± 1.74
Doxy gel	5.767 ± 1.25	$3.85 \pm 1.13^*$	3.15 ± 0.98	15.58 ± 2.66	$14.59 \pm 2.73^*$	14.08 ± 2.61
AP gel	5.76 ± 0.99	$4.08 \pm 1.19^*$	3.30 ± 1.16	15.27 ± 2.19	$14.27 \pm 2.18^*$	13.79 ± 2.01

*significant differences ($p < 0.05$)

Table 3 Frequency of site (%) of PD and RAL change at the end of study.

Tx group	PD change				RAL change			
	Increase	No change	Decrease		Loss	No change	Gain	
	≤ 2 mm	0 mm	≤ 2 mm	>2 mm	1 mm	0 mm	≤ 2 mm	>2 mm
Control	3.0	4.5	60.6	31.9	6.0	42.4	47.1	4.5
Doxy gel	1	-	50.0	50.0	-	13	63	24
AP gel	1.4	4.3	47.2	47.1	5.72	11.43	61.4	21.45

*significant differences ($p < 0.05$)

Table 4 Mean \pm SD of GI and GBP of each treatment group in three examination periods.

Tx. group	GI			GBP		
	Baseline	3 months	6 months	Baseline	3 months	6 months
Control	1.88 \pm 0.37	1.49 \pm 0.62*	1.27 \pm 0.49*	70.00 \pm 17.01	48.50 \pm 21.81*	33.43 \pm 13.36*
Doxy gel	2.01 \pm 0.31	1.61 \pm 0.48*	0.78 \pm 0.70*	62.99 \pm 24.54	49.67 \pm 25.96*	18.33 \pm 17.68*
AP gel	1.91 \pm 2.26	1.63 \pm 0.53*	0.66 \pm 0.65*	72.17 \pm 28.43	50.33 \pm 24.11*	17.53 \pm 18.42*

*significant differences ($p<0.05$)**Table 5** Mean \pm SD of PI of each treatment group in three examination periods.

Tx. group	PI		
	Baseline	3 months	6 months
Control	1.67 \pm 0.54	1.05 \pm 0.57*	0.56 \pm 0.47*
Doxy gel	1.70 \pm 0.76	1.16 \pm 0.76*	0.67 \pm 0.59*
AP gel	1.47 \pm 0.64	1.01 \pm 0.61*	0.45 \pm 0.44*

*significant differences ($p<0.05$)

Discussion

The results of this study revealed that subgingivally application of doxycycline gel or AP gel in SPT patients provided the additional improvement in decreasing gingival inflammation, increasing frequency of site with RAL gain >2 mm better than SRP alone. From this six months study, every patient did not report side effect from the treatments.

Most of the previous studies applied the local drug system in the initial phase of periodontal treatment. However, it is well known that the periodontal inflammation can be subsided after SRP alone [22] and practically, the using of alternative treatment such as systemic or local route of antibiotics usually was considered after initial treatment had already done. [23] Therefore, the target subjects of this study were the maintenance patients who had already treated initial periodontal therapy for at least six months. [24]

Although, we did not find the difference of PD reduction between group but the results showed that the RAL gain in test groups was

occurred faster than site of control groups and the frequency of site which RAL gain >2 mm were reported in test groups more than the control group as well (table 3). Thus this result could interpret that PD reduction from control group was occurred by gingival recession but CAL gain was found in test groups. The authors suggested that the reasons of the superior outcome in test groups became from the properties of doxycycline gel which provides antimicrobial, anti-collagenase, immuno-stimulant, inhibiting bone resorption and inducing attachment of fibroblast. [25] These properties have been found from AP gel as well. [26]

Because the authors expected to see the pure effect of local drugs by eliminated confounder such as healing from SRP. Therefore, we included only SPT patients which had not received periodontal treatment for at least 6 months prior to the study [24], but the results showed teeth in the control group were still improved in PD reduction and RAL gain. We speculated that these improvements were caused by the subject's plaque control at baseline was not good enough (PI 1.47-1.70) then after received treatment and proper plaque control, the clinical parameters were improved, according to the result from the previous studies. [20, 27, 28] However, the additional clinical improvement was possible of repeated instrumentation. [29]

When compared the result of our doxycycline group with the previous studies, [30-32] which had the similar group of patient (SPT patient or the patient who passed initial periodontal treatment), we found that the PD reduction and CAL gained from our study was more superior. That may resulted from our baseline subject's gingival inflammation

(GI, GBP) was more severe, as previously reported about the correlation between the site severity and the improvement after non-surgical periodontal therapy. [33] However, we found the identical results with the other studies which applied doxycycline gel or AP gel in initial phase of periodontal therapy, that had the same subject's baseline gingival inflammation (GI, GBP). [6, 7, 16, 17, 34]

The advantages of split mouth study design were the reduction of biological variants between subjects but there was the caution about cross-over effect, which was occurred by the interaction between drugs. [36] This effect was controlled by the assignment of experimental teeth between group to have one tooth apart. [37, 38] From the pharmacokinetic study of AP gel [39] showed that the active ingredients of drug after application, were found in saliva only first half an hour, which was below the MIC of periodontal pathogenic bacteria. And they did not detect the active ingredients of AP gel in serum. The studies of doxycycline gel also reported that doxycycline also was not found in serum, but it was detected in saliva in the first two hours. [40] However we expected that this ingredients in saliva cannot effectively penetrate into the periodontal pocket. [41] In addition, many studies of doxycycline gel did not report cross-over effect even they were split mouth design studies. [6, 7, 9, 30]

The results of this study showed that GI in test groups was reduced in every examination periods while GI in control group was reduced only in the first three months period. The authors speculated that they were caused by the local drug in test groups had antimicrobial and anti-inflammatory effect but control group had not. [12, 14, 15, 25]

This result could be interpreted under the limitations as, first the authors could not include only good oral hygiene subjects because the difficulty of recruitment patients according to our study's criteria. Second, because the baseline PD of teeth in this study was moderate depth (5.6-5.8 mm) which may not deep enough for

inspection the difference between groups. As the other studies found the correlation between baseline PD and the PD reduction, CAL gain after treatment. [33, 35] Third, there was possibility that the pocket wall of teeth in this study may less inflamed than the periodontal pocket which had never been treated, made the drug less penetrate in pocket wall.

From the ability of decreasing gingival inflammation and increasing site of RAL gain of AP gel doxycycline gel and doxycycline gel group as aforementioned. The subgingivally delivered AP gel or doxycycline gel might reduce the necessity of periodontal surgery in some patients and reduce the frequency of maintenance visit. However, doxycycline gel which must be imported from abroad, has high cost. The AP gel which can extract from thai herbal and has low cost, might be alternative treatment option for the using in SPT. The authors suggest for further study that should include only patients with good baseline oral hygiene, and the experimental teeth should present vertical bony defect, for exploration the regenerative effect of local drugs.

In summary, SRP adjunct with AP gel or doxycycline gel provided the similar results in PD reduction, RAL gain and reducing gingival inflammation. In addition, both treatments provided faster onset of RAL gain, more sites with RAL gain >2 mm and reduced gingival inflammation better than SRP alone.

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