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ORIGINAL ARTICLE

The anti-inflammatory effect of locally delivered nano-doxycycline gel in therapy of chronic periodontitis

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ABSTRACT

Background and objective: To date, various drugs as host modulating agents had been suggested as adjunctive treatment modality in the therapy of chronic periodontal disease. In this study, the anti-inflammatory effect of subgingivally delivered nanostructured doxycycline gel (nDOX) was evaluated and compared to conventional doxycycline gel (DOX) used as adjunct to scaling and root planning (SRP) in the treatment of moderate chronic periodontitis to reduce probing pocket depth.

Material and methods: Nanostructured doxycycline gel (nDOX) was prepared using spray-drying technique with chitosan (CH) as a matrix polymer, followed by dispersion in polyvinyl alcohol (PVA). The deepest periodontal pocket in 45 patients suffering from moderate chronic periodontitis was selected. The patients were divided into three groups following scaling and root planning (SRP); group I: SRP + nDOX, group II: SRP + DOX and group III: SRP + placeboCH. Plaque Index (PI), Gingival Index (GI), pocket depth (PD) and clinical attachment level(CAL), as well as ginigival crevicular fluid levels of (GCF) IL-6 and TNF-α were assessed at baseline, 1 and 3 months following local drug application.

Results: Group I showed significant reduction in probing depth and attachment gain compared with group II and III at one and three months period. The inflammatory mediators levels were significantly reduced in all treatment groups at one-month period. Except for group I, the reduced values were observed at three-month period.

Conclusion: The results suggest that treatment with nDOX gel as an adjunct to SRP had anti-inflammatory effect by improving both clinical parameters and inflammatory markers up to three months period.

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Introduction

Periodontal disease is defined as inflammation of gingival and adjacent supporting structures of the teeth that results in connective tissue attachment and alveolar bone loss. To date, it is well known that the major soft- and hard-tissue destruction seen in periodontal disease occurs as a result of host's immune inflammatory defence in the presence of periopathogens [1,2]. Even though the exact mechanism of the host inflammatory response is still unclear, it opens a whole new approach in periodontal therapy known as host-modulating therapy (HMT). HMT is one of the promising adjunctive treatment modality for the management of periodontal disease, which can be combined with conventional approaches (scaling and root planning/SRP, smoking cessation therapy). The newly introduced promising host response modifier in the treatment of periodontal disease is doxycycline [3]. Doxycyline (DOX) is tetracycline-derived synthetic antibiotic known to have cytoprotective properties, mainly the wide spectrum of anti-inflammatory effects, such as inhibition of host-derived tissue-destructive enzymes matrix metalloproteinases (MMPs), suppression of hydrolases (α-amylases, phospholipase A2) and cytokines (tumour necrosis factor-alpha/TNF-α, IL-1β, IL-6) and subsequent increase in collagen production, osteoblast activity and bone formation [3,4]. Furthermore, DOX also scavenge for and inhibit reactive oxygen metabolites (hypochlorous acid) and thereby prevent or reduce pathological tissue destruction seen in periodontal disease [5,6].

The available literature suggest that systemically delivered doxycycline hyclate can be successfully used for the management of periodontal disease in adjunct to SRP, resulting in statistically significant reduction in pocket-probing depth (PPD) and gain in clinical attachment level (CAL) [7–11]. However, in order to overcome the major concern of systemically delivered DOX (long-term administration that can lead to antibiotic resistance), a low subantimicrobial doxycycline dose (SDD) was introduced [7–11]. A SDD at 20 mg has been found to be a safe and effective adjunct when taken twice daily for at least three months and up to 24 months in randomized placebo-controlled clinical trials, with regards to PPD reduction and CAL gain [10–12]. SDD can be applied in various local delivery systems, such as fibres, films, injectable systems, gels, strips and compact vesicular systems [13–18].

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Local delivery systems have been found to have several benefits that include the ease of application, precise selective targeting of diseased sites that were unresponsive to conventional therapy and possibly enhanced treatment results at specific locations [13–17]. A recently developed concept in nanotechnology is nanodrug delivery in which drugs can be attached to specifically designed carrier for a cell-specific targeting [19]. Due to their small sizes (smaller than 100 nm, in at least one dimension), the nanostructures exhibit unique physicochemical and biological properties, favourable for biomedical applications [19]. Generally, nanocarrier systems provide advantages over conventional drug delivery, such as protection of the entrapped drug from enzymatic destruction, sustained drug release, reduction in daily drug doses and side effects as well as cell targeting [19,20]. Uptake of nanostructures has been reported to be 15–250 times greater than that of microparticles in the 1–10 μm range, with sustained release properties of nanoparticles [20]. Recently, chitosan is reported to be useful as a nanocarrier in many biomedical applications [21]. Chitosan is a biodegradable polysaccharide composed of randomly distributed linked D-glucosamine and N-acetyl-D-glucosamine [21,22]. Wide biomedical applications of chitosan are mainly due to its low cytotoxicity, biodegradability, antimicrobial activity and good haemostatic properties [21–23].

The aim of the present study was to evaluate and compare the anti-inflammatory effect of a newly formulated nanostructured doxycycline (nDOX) periodontal gel to conventional doxycycline gel (DOX) used as adjunct to scaling and root planning (SRP) in the treatment of moderate chronic periodontitis to reduce probing pocket depth.

Materials and methods

Study design

- Patients from the Faculty of Dentistry, Alexandria University, Alexandria, Egypt diagnosed with moderate chronic periodontitis enrolled in this study (45 patients from both gender) were selected according to the following criteria:
- aged between 20 and 45 years,
- presence of PPD 4–6 mm in at least three permanent teeth,
- systemically healthy patients with no history of antibiotics or any other medication used in the previous 6 months,
- non-smokers,
- non-pregnant or lactating females,
- no history of allergy on doxycycline,
- no history of previous periodontal surgical treatment in the last 12 months.

The study protocol was reviewed and approved by the Ethics committee of Alexandria University. All participants were informed about the research protocol in details and signed informed consent.

All recruited patients underwent full mouth supra- and subgingival scaling using both hand and ultrasonic instruments. Patients' were instructed and motivated to perform strict personal oral hygiene. One week later was considered the baseline, SRP were performed to the deepest periodontal pocket (more than 4 mm) in each patient and randomly distributed into the three treatment groups as follows:

Group I: Nano-DOX chitosan periodontal gel/nDOX (n =15)
Group II: Conventional DOX periodontal gel/DOX (n = 15)
Group III: Placebo nano-chitosan periodontal gel/CH (n = 15)

Preparation of placebo- and doxycycline-loaded CH nanoparticles

Placebo nano-CH particles were obtained by spray drying of 1.5% w/w CH solution in 1% w/w acetic acid solution using a mini-spray dryer (B-290, Buchi, Flawil, Switzerland) equipped with a high performance cyclone, with inlet temperature of 135 °C, outlet temperature of 90 °C, aspiration air of 90%, feed flow of 5 mL/min, spraying pressure of 5.0–5.8 mbar and air flow rate of 320 L/h. The same procedure was applied for DOX-loaded nanoparticles by dissolving the drug in the feed solution at a 20% w/w concentration.

Characterization of nanoparticles and microsized particles

Particle size and zeta potential

Particle size of the reconstituted spray-dried nanoparticles was measured using a dynamic light scattering (DLS) technique with the Zetasizer (Malvern, UK). The system is equipped with a 4 mW He–Ne laser (633 nm wavelength) and measures the particle size with the non-invasive backscattering technology at a detection angle of 173° after an at least 200-fold dilution with purified water. The electrophoresis mobility was measured and the zeta potential was calculated by the Dispersion Technology Software provided by Malvern.

Preparation of doxycycline gel

The method of preparation was slightly modified from that discussed in previous article [24]. PVA (10%w/w) was dispersed in water using a magnetic stirrer at 90 °C. After complete dispersion, PVP (5% w/w), glycerol (10%) and PEG 400 (5%v/v) were added. The mixture was left to cool at room temperature and DOX was then added at 1% concentration when the temperature was about 40 °C. For nanostructured DOX gels, an amount of the nanoparticles equivalent to 1% DOX was incorporated into the gel upon reaching 40 °C. In the case of placebo chitosan gel, an equal amount of the placebo nano-CH was used.

Drug content in the prepared systems

An accurately weighed sample of each of the prepared gel equivalent to 2 mg DOX was dissolved in 1% acetic acid. The doxycycline content was determined by UV spectrophotometry. For doxycycline-loaded nanoparticles, the sample equivalent to 2 mg doxycycline was digested in 1% acetic

acid and the drug content was assayed spectrophotometrically. The percentage-loading capacity (% LC) and incorporation efficiency (% IE) were calculated using the following equations:

$$\%\ LC = (\text{mass of the drug}/\text{mass of recovered particles}) \times 100$$

$$\%\ IE = (\text{mass of the drug}/\text{mass of the drug added in formulation}) \times 100$$

Treatment and periodontal clinical parameters

After partial isolation, the selected site was dried with air. The tested gel was applied in the pocket by using 29-gauge needles assuring that the tip reaches deep inside the pocket. The needle was then slowly removed from the pocket when gel overflow appeared at the gingival margin. A periodontal pack was then placed over the tooth with the tested pocket for one week.

At baseline, 1 and 3 months following therapy, clinical evaluations were carried out using full mouth Silness and Löe Plaque Index (PI) [25], Loe and Silness Gingival Index (GI) [26], PPD and CAL for evaluated sites. A pre-calibrated examiner (M. M.) carried out the clinical and double assessments for the clinical examination. Intra-examiner evaluation revealed minor differences between the two readings; all measurements were within 5% of the initial measurements [27].

Gingival crevicular fluid sample collection and IL-6 and TNF-α assays

For gingival crevicular fluid (GCF) sample collection sterile filter papers were pre-cut into strips 2 × 8 mm. The participants were instructed not to eat for a minimum of two hours before sampling. The selected tooth was isolated with cotton rolls, supragingival plaque was removed without disturbing the soft tissues, and the tooth was dried with a gentle stream of air for 5 s [28]. Filter paper strips were used for GCF sampling [29]. They were placed into the defect with the greatest pocket depth in each patient till mild resistance was felt. They were left for 60 s to collect the resting GCF [30]. The strips contaminated with blood or saliva were discarded. Then, the strips were put in sterile plastic Eppendorf tube containing 150 µl phosphate-buffered saline (PBS; Sigma Aldrich, St. Louis, MO). The Eppendorf tubes were then centrifuged at 11.000 rpm. The concentration of IL-6 and TNF-α markers in the samples was determined by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (eBioscience Inc, San Diego, CA) in accordance with the manufacturer's instructions.

Statistical analysis

Sample size was estimated using the following assumptions: alpha error = 5%, power = 80%, percentage reduction in pocket depth in control group using scaling and root planning alone = 12.5%, using DOX agent = 25% and using nanoDOX = 37.5% with standard deviation = 11 [12,31]. The least required sample size was calculated to be 13. To make up for potential loss to follow up of 10%, the sample size was increased to 15 per group.

All results were expressed as the mean ± standard deviation (SD). Percentage reduction was calculated as follows:

$$[(\text{PD after treatment} - \text{PD before treatment})/\text{PD before treatment}] \times 100$$

Statistical comparisons between three treatment groups were performed using ANOVA test, and Fisher's *least significant difference,* as *post hoc* test. Statistical analysis was performed using SPSS 20.0 statistical software (SPSS Inc, Chicago, IL). $p < .05$ was considered to indicate a statistically significant difference.

Results

Demographic and periodontal clinical parameters

The demographic characteristics were summarized in Table 1. Table 2 provides comparative *p* values between the healthy and therapy groups (multiple comparisons to provide insight into the differences in periodontal clinical parameters between treatment groups). The PI showed improvement in all groups at 1 and 3 months follow-up; however, no significant difference was observed between groups (Table 2). Regarding GI, there was no statistically significant difference between all groups at the baseline; however, at one month and three months, there was statistically significant difference between mean GI in group I when compared to both, group II and group III. There was also a statistically significant difference between mean % reduction in GI after three months in relation to group I and group II when compared to group III (Table 2). As expected, PI and GI data demonstrate clearly that dental prophylaxis improved the clinical measures of gingival health during follow-up, in such way that the measured periodontal parameters shifted toward health.

Regarding PPD, there was a statistically significant PPD reduction in group I when compared to both, group II and group III after one and three months period (Table 2). As for CAL, there was no statistically significant difference at the baseline in all groups; however, there was a statistically significant gain in CAL level in relation to group I at one-month period when compared to group III. Also, at three months, a significant difference was observed between both groups I and II compared to group III (Table 2). None of the individuals under therapy complained of gingival pain, oedema or any adverse effect during the follow-up period.

Table 1. The demographic data for each group.

	Group I nDOX	Group II DOX	Group III CH	*p* value
Age: mean (SD)	34.3 (1.5)	35.1 (1.3)	33.9 (1.9)	.22
Gender				.56
Male: *n* (%)	5 (33.3)	7 (46.7)	6 (40)	
Female: *n* (%)	10 (66.7)	8 (53.3)	9 (60)	

Group I (nDOX), Group II (DOX) and Group III (CH).

Table 2. Clinical parameters of nDOX, DOX and CH groups .

		Group I nDOX	Group II DOX	Group III CH	*p*
PI	Baseline	0.83 ± 0.12	0.83 ± 0.20	0.84 ± 0.21	.886
	1 month	0.66 ± 0.11	0.67 ± 0.11	0.70 ± 0.11	.692
	3 months	0.68 ± 0.08	0.68 ± 0.07	0.73 ± 0.12	.127
	Reduction % after 1 month	12.63 ± 21.50	18.75 ± 13.96	17.23 ± 13.62	.700
	Reduction % after 3 month	25.67 ± 14.27	27.35 ± 10.68	20.37 ± 8.38	.371
GI	Baseline	2.43 ± 0.56	2.44 ± 0.36	2.49 ± 0.30	.890
	1 month	1.23 ± 0.14	1.44[a] ± 0.15	1.52[a] ± 0.25	.005*
	3 months	0.55 ± 0.17	0.73[a] ± 0.13	0.91[a,b] ± 0.22	<.001*
	Reduction % after 1 month	42.98 ± 19.12	35.87 ± 8.19	34.22 ± 14.18	.245
	Reduction % after 3 month	72.50 ± 15.37	68.20[a] ± 5.62	57.97[a,b] ± 7.52	.001*
PD	Baseline	5.21 ± 1.25	5.33 ± 0.67	5.31 ± 0.67	.698
	1 month	3.40 ± 0.48	4.31[a] ± 0.48	4.62[a] ± 0.82	<.001*
	3 months	3.90 ± 0.34	4.10[a] ± 0.54	4.40[a] ± 0.84	<.001*
	Reduction % after 1 month	27.50 ± 30.48	17.33 ± 15.78	11.33[a] ± 9.84	.026*
	Reduction % after 3 month	35.50 ± 31.49	17.33[a] ± 15.78	17.0[a] ± 11.27	.005*
CAL	Baseline	3.82 ± 0.42	3.73 ± 0.53	3.83 ± 0.42	.259
	1 month	3.0 ± 0.12	3.30[a] ± 0.48	3.60[a] ± 0.52	.011*
	3 months	2.80 ± 0.32	3.30[a] ± 0.51	3.80[a,b] ±0.41	<.001*
	Gain % after 1 month	20.0 ± 10.54	4.17[a] ± 17.68	5.0[a] ± 10.54	.021*
	Gain % after 3 month	44.17 ± 9.66	4.17[a] ± 17.68	0.0[a] ± 0.0	<.001*

Clinical examination data (PI: Plaque Index; GI: Gingival Index; PD: pocket depth; CAL: clinical attachment level.
[a]significant with group I.
[b]significant with group II.
*statistically significant).

Table 3. Gingival crevicular fluid (GCF) IL-6 values for nDOX, DOX and CH groups.

	Baseline	1 month	3 months	p_1
Group I nDOX	3.23 ± 0.39	1.71[b,c,]# ± 0.15	1.63[b,c,]# ± 0.33	<.001*
Group II DOX	3.02 ± 0.28	2.21[a,c,]# ± 0.41	3.01[a] ± 0.42	<.001*
Group III CH	3.08 ± 0.36	2.61[a,b,]# ± 0.32	3.16[a,]# ±0.21	<.001*
p_2	.392	<.001*	<.001*	–

Gingival crevicular fluid (GCF) IL-6 values (p_1: *p* value for *F*-test (ANOVA) with repeated measures for comparing between different periods, p_2: *p* value for *F*-test (ANOVA) for comparing between the different studied groups.
[a]significant with group I.
[b]significant with group II.
[c]significant with group III.
#significant with baseline.
*Statistically significant).

Table 4. Gingival crevicular fluid (GCF) TNF-α values for nDOX, DOX and CH groups.

	Baseline	1 month	3 months	p_1
Group I nDOX	6.58 ± 2.66	1.22[b,c,]# ± 0.63	1.20[b,c,]# ± 0.41	<.001*
Group II DOX	6.60 ± 3.04	2.91[a,c,]# ± 0.42	6.15[a,c] ± 0.40	<.001*
Group III CH	6.68 ± 2.59	4.87[a,b,]# ± 0.45	6.54[a,b] ± 0.51	<.001*
p_2	.984	<.001*	<.001*	

Gingival crevicular fluid (GCF) TNF-α values (p_1: *p* value for *F*-test (ANOVA) with repeated measures for comparing between different periods, p_2: *p* value for *F*-test (ANOVA) for comparing between the different studied groups.
[a]Significant with group II.
[b]Significant with group I.
[c]Significant with group III.
#Significant with baseline.
*Statistically significant.

Inflammatory markers

Regarding the GCF IL-6 values in the three studied groups, at baseline, there was no statistically significant difference between IL-6 values in all groups. A statistical significant decrease in IL-6 values in all groups at one-month period was observed. However, statistically significant decreased value was continued only in relation to group I, being insignificant in group II while it was significantly increased in relation to group III (Table 3). Regarding the GCF TNF-α values in the three studied groups, at baseline, there was no statistically significant difference between TNF-α in all groups. A statistical significant decrease in TNF-α values in all groups at one-month period was observed. However, after three months, the values increased and were insignificant to the baseline for group II and III. Regarding group I significant difference at three months was still observed in relation to group II and III, as well as to baseline (Table 4).

Discussion

Periodontal disease is chronic inflammatory pathologic condition that, if left untreated, leads to both soft- and hard-tissue destruction. Degree of inflammation associated with the initiation and progress of periodontal disease can be measured through inflammatory biomarkers, such as IL-6 and TNF-α, which are produced as a result of bacterial lipopolysaccharide presence [32–36]. The significant decrease in GCF levels of IL-6 and TNF-α in the present study could be partially explained as a result of decreased inflammatory process associated with tissue destruction in periodontal pockets. Previous studies reported decreased collagenase activity, inflammatory mediators, such as IL-6 and reducing biomarkers of bone resorption in periodontal pocket following short term regimen of subantimicrobial doxycycline dose SDD [37,38]. Soory et al. [39] stated that doxycycline possessed anti-inflammatory effects through inhibiting MMPs, nitric oxide synthesis, reactive oxygen species formation [6], inhibiting apoptosis and stimulating bone formation. Therefore, IL-6 and TNF-α value decrease in the present study could be also attributed to the non-antibacterial effect of doxycycline in reducing the inflammatory burden. The results of the present study showed there was a statistically significant reduction in the mean of GCF TNF-α and IL-6 level in relation to both group I and group II at one month and three months period when compared to group III. This is in accordance with

Jin et al. [40] who evaluated the biocompatibility and therapeutic effects of doxycycline nano-liposome slow-release gel on experimental periodontitis, presented by the decrease of MMP-8 level compared with the conventional and control groups.

Similarly, Gad et al. [18] observed a significant improvement in the clinical as well as the microbiological parameters in periodontitis patients after using microparticles encapsulating doxycycline hydrochloride. Also, Rao et al. [41] found a significant decrease in probing depth and attachment loss after using locally delivered doxycycline microspheres with scaling and root planning. Moreover, porphyromonas gingivalis cell count was significantly decreased after 3 and 6 months.

Machion et al. [12] evaluated the effect of 10% locally delivered doxycycline with scaling and root planing on PD and CAL in smokers. They observed a greater PD reduction up to 2 mm after six months in DOX group than SRP alone.

Similar to our findings, Emingil et al. [42] evaluated the effect of SDD (20 mg per capsule; two times/day) for three months in chronic periodontitis on the cytokine-chemokine levels in GCF. The results showed that SDD, as an adjunct to non-surgical periodontal therapy could stabilize the inflammatory response by promoting the suppression of pro-inflammatory cytokines including TNF-α and IL-6, and increasing the pro-inflammatory cytokines as IL-6. The absence of inflammation in the present study as well as the long-time efficacy of nano-groups may be directly related to: firstly, the use of chitosan carrier which is known to be safe natural produced material and secondly due to the criteria of nano-drug and carrier form that help increasing the solubility and bioavailability of drugs [21,22]. Nanoparticles with diameter less than 200 nm could help intracellular drugs delivery. The advantages of using nanoparticles as a drug delivery system has been found to include the controlled and sustained release of the drug at the site of localization [43].

Chitosan-doxycycline gel, as local delivery system is very promising due to its ease of application and insertion dimensions, which suits well with the dimensions of the pocket, the ability to reach the depth of the pocket and minimum pain with insertion. In the present study, a newly formulated nano-doxycycline (nDOX) periodontal gel was evaluated in the management of moderate chronic periodontitis.

Doxycycline has been shown to be less hepatotoxic than tetracycline. Their application, as an adjunct to periodontal treatment, is relatively short term and less likely to induce toxicity or microbial resistance [44]. Suzuki et al. [45] observed no adverse effect on cell survival and protein expression of human gingival fibroblasts, epithelial cells and periodontal ligament fibroblasts when doxycycline was applied in higher concentrations than the clinically applied dose. Thus, local application of doxycycline has no adverse effect on the surrounding tissues. The American Academy of Periodontology reported that locally applied controlled release doxycycline gel may relatively neutralize the adverse effect of smoking on periodontal healing [46]. Moreover, a recent systematic review showed that the adjunctive use of local antimicrobial to SRP had a significant improvement in PPD reductions and CAL gains when compared with the control groups [47].

Conclusions

The use of subgingivally delivered nano-doxycycline periodontal gel as an adjunct to SRP was safe and improved both clinical parameters and inflammatory markers up to three months period.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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