

A randomized-controlled trial of low-dose doxycycline for periodontitis in smokers

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Abstract

Background/Aim: Tobacco use reduces the effect of non-surgical periodontal therapy. Host-modulation with low-dose doxycycline (LDD) might favour repair and promote an improved treatment response. The aim of this study was to investigate the effect of LDD in smokers on non-surgical periodontal therapy.

Material and Methods: This was a parallel arm, randomized, identical placebo-controlled trial with masking of examiner, care-giver, participant and statistician and 6 months of follow-up. Patients received non-surgical therapy and 3 months of test or control drug. Statistical analysis used both conventional methods and multilevel modelling.

Results: Eighteen control and 16 test patients completed the study. The velocity of change was statistically greater for the test group for clinical attachment level -0.19 mm/month (95% CI = $-0.34, 0.04$; $p = 0.012$) and probing depth 0.30 mm/month (95% CI = $-0.42, -0.17$; $p < 0.001$). However, no differences were observed for absolute change in clinical or biochemical markers at 6 months.

Conclusions: This study does not provide evidence of a benefit of using LDD as an adjunct to non-surgical periodontal therapy in smokers.

Key words: dental scaling; periodontal diseases; randomized-controlled trial; smoking; tetracycline

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Tobacco use has a major influence on periodontal therapy. A reduction in clinical benefits in smokers following non-surgical periodontal therapy is a consistent finding across many studies (Preber & Bergstrom 1986, Bostrom et al. 1999, Palmer et al. 1999a, Kinane & Chestnutt 2000, Labriola et al. 2005). The sug-

gested mechanisms for this finding include inflammatory, immunological, microbiological and wound-healing phenomena (Palmer et al. 1999b, Kinane & Chestnutt 2000).

Upregulation of inflammation has been strongly implicated in the impaired response with increased gingival levels of the pro-inflammatory cytokine tumour necrosis factor- α (TNF- α) in smokers compared with non-smokers (Bostrom et al. 1999, Palmer et al. 1999b, Kinane & Chestnutt 2000). In addition, lower levels of regulators of tissue breakdown have been found such as protease inhibitors (e.g. α -1-antitrypsin and α -1-macroglobulin) in the gingival fluid of smokers than non-smokers (Kinane & Chestnutt 2000). Whichever arm(s) are activated, the net result will be an increase in matrix metalloproteinases, leading to periodontal destruction and this might be further exacerbated by

the finding of increased levels of serum-soluble inter-cellular adhesion molecule-1 (sICAM-1) in smokers. The few studies that have used objective markers of smoking exposure have demonstrated a significant correlation between serum cotinine (COT) levels and both sICAM-1 levels and attachment loss (Palmer et al. 1999b).

Biochemical measures of tobacco use have developed from concerns that self-reported tobacco data may give inaccurate information on the magnitude of ‘‘tobacco exposure’’ (Jarvis et al. 1987). Biochemical analyses of tobacco use include nicotine metabolites (e.g. in saliva) and exhaled carbon monoxide (CO) (Jarvis et al. 1987, Barnfather et al. 2005), although no gold standard currently exists (Needleman et al. 2006).

While quitting tobacco use is important for general and oral health, only a minority of tobacco users will quit at

Conflict of interest and source of funding statement

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any one time (Needleman et al. 2006). In view of this consistent finding, developing methods to improve the periodontal treatment response of tobacco users unable to quit is important as the prevalence of both tobacco use and periodontitis remains high. If smoking enhances connective tissue breakdown in periodontitis, one novel therapeutic approach might be to inhibit metalloproteinase (MMP) activity as has been demonstrated for tetracyclines (Golub et al. 1997). Well-evaluated markers of collagen turnover, such as the pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP), have been used to investigate changes in bone breakdown and bone turnover (Sarmant et al. 2006). ICTP was reduced in patients with periodontitis following administration of low-dose doxycycline (LDD) and with no effect on non-treated subjects.

Clinical trials of LDD in humans have shown an improved healing following non-surgical periodontal therapy (Caton et al. 2000). A subgroup analysis of smokers, combining data from two trials of LDD, has suggested improved clinical healing in smokers receiving the active drug compared with placebo (Preshaw et al. 2005). However, these analyses were not direct comparisons based on the original randomization scheme and may therefore be subject to bias. Clarification of this hypothesis is therefore needed.

With respect to designing periodontal clinical trials, multiple data points per outcome for each subject at each visit are collected. It is unreasonable to treat observations for each periodontal site as independent, statistically speaking, which is why patient-based opposed to site-based analyses have been recommended (Imrey 1986). However, such analytical methods lose valuable information where more than one site per subject is studied. Methods such as multilevel modelling may be used to analyse the behaviour of repeated measures of individual sites while acknowledging that these are grouped by teeth and nested within subjects (Gilthorpe et al. 2000, 2001). This allows for a much deeper understanding of the site-specific changes induced by therapy (Gilthorpe et al. 2003).

Therefore, the aim of this study was to investigate the effect of a novel approach to modulating the healing response of smokers to periodontal therapy using LDD as an adjunct to non-

surgical therapy in smokers unable to quit tobacco use. The hypothesis was that host modulation might improve the clinical outcome of treatment. A further aim was to examine the use of multi-level modelling as a tool in the analysis of periodontal clinical trial data.

Material and Methods

This was a parallel arm, randomized, identical placebo-controlled trial with masking of examiner, care-giver, participant and statistician, with 6 months of follow-up. The study examined the effect of host-modulation by LDD as an adjunct to non-surgical periodontal therapy in current smokers.

Study sample size

The primary outcome was clinical attachment level (CAL). Study sample size calculations were based on subject-level analyses (*t*-tests of aggregate mean scores at a single follow-up) as the study randomized individuals. Under the alternative hypothesis, a mean difference in the observed CAL between groups of 1 mm, with a standard deviation (SD) of 1 mm, would require 16 or 22 individuals in each group to detect a significant difference at the 5% level with 80% or 90% power, respectively. With multiple follow-up measures and the intention-to-analyse sites within a multilevel framework, study-size estimates based on subject-level *t*-tests were anticipated to be overly conservative. Consequently, while the study sought to recruit up to 22 individuals per group, achieving 16 and 18 in the treatment and control groups, respectively, was deemed satisfactory. Recruits were patients referred to the Unit of Periodontology, UCL Eastman Dental Institute, from September 2002 until August 2003.

Patient selection

The inclusion criteria were as follows: age 30–70 years, at least 16 teeth present, diagnosis of chronic periodontitis, at least two teeth with probing depth (PD) of ≥ 6 mm and at least 30% bone loss in at least two quadrants excluding third molars, current smoker of at least 10 cigarettes per day for a minimum of 1 year. Exclusion criteria were: allergy to tetracycline, antibiotic prophylaxis required before periodontal therapy, dai-

ly consumption of non-steroidal anti-inflammatory drugs or antibiotics and pregnancy or lactation and any health condition potentially affecting the response to periodontal therapy (e.g. diabetes mellitus).

All potentially eligible subjects were given brief smoking cessation interventions including advice about the negative effect of smoking on general and periodontal health and the importance of stopping smoking to achieve periodontal health. Individuals were asked whether they were planning to quit in the next 6 months and those declining to quit were invited to participate in the study. The study was approved by the Ethics Committee of the Eastman Dental Hospital (Reference 01/E012), and informed consent was obtained from all patients. The study was prospectively registered on the Current Controlled Trials Register in October 2002 (ISRCTN11033714).

Treatment allocation

Assignment to test (LDD) or control (inactive identical placebo) group was random. The sequence was computer generated in blocks of four by the research coordinator. Randomization was concealed by the use of sequentially numbered, identical containers containing active or identical-appearing placebo medication. The randomization code was held centrally by the research coordinator remote from the study and was not broken until completion of the data analyses.

Outcome assessment

The primary outcome was change in the CAL. CAL was recorded from the cemento-enamel junction (CEJ) to the base of the probing pocket using a graduated UNC-15 probe (Hu Friedy, Chicago, IL, USA). Where the CEJ was absent or unclear, an alternative landmark was selected and used for all subsequent recordings. A single trained and calibrated examiner, an experienced dentist (GSG), was used throughout the trial and performed all clinical measurements. The calibration included duplicate full-mouth assessment of 10 patients with disease severity similar to those included in the trial. For PPD, 98.4% of sites were within 2 mm and the corresponding values for gingival margin (GM) and CAL were 97.5% of sites and 99.3% of sites.

Gingival crevicular fluid (GCF) was sampled for ICTP (terminal carboxytelopeptide of type 1 collagen) at the eight mesiobuccal sites per patient with the initially deepest PDs with the same sites sampled throughout the study. GCF ICTP was collected on standardized filter paper strips (Periopaper, OraFlow, Plainview, NY, USA). Strips were gently inserted into the gingival crevice until slight resistance was felt and left for 15 s. Samples were kept on ice before transfer to a freezer at -20°C . Samples were stored and then transferred to the University of Michigan for analysis. Frozen samples were thawed at room temperature and the proteins were then eluted through centrifugation $5 \times$ in 12×75 ml polypropylene tubes at 2060 g for 5 min. with $20 \mu\text{l}$ phosphate-buffered saline (pH 7.4) containing 15 nM aprotinin, 1 mM PMSF and 0.1% of human serum albumin as described previously (Giannobile et al. 1995). GCF ICTP levels were quantified using a radioimmunoassay (RIA) (DiaSorin Inc., Stillwater, MN, USA) as described previously (Risteli et al. 1993). ICTP was determined as total amount/time of collection (pg/site/patient).

Secondary outcomes included PD, gingival recession and bleeding on probing (BoP) recorded at six sites per tooth with the manual probe and ICTP.

Explanatory variables that were recorded were plaque (presence or absence to a probe at the GM at six sites per tooth) and measures of smoking exposure, assessed in the following ways: self-reported smoking history, number of cigarettes smoked per day, and number of years smoked, exhaled CO (Smokerlyser, Bedfont Scientific Ltd, Rochester, Kent, UK) and salivary COT. For salivary COT, a dental cotton roll was placed in the mouth until saturated with saliva. It was then placed in a 10-ml syringe and the saliva was expressed into a 5 ml vial. The vial was stored at -20°C . Salivary COT was assayed by the gas-liquid chromatography method (detection limit 0.1 ng/ml) (Feyerabend & Russell 1990). This assay has been used widely in previous studies (Jarvis et al. 1987, Smith et al. 1998).

Patient compliance and adverse events were also recorded at each visit.

Treatment

Non-surgical periodontal therapy was provided to all patients by an experienced periodontist (RT). Therapy con-

sisted of up to four visits (total 4 h) of oral hygiene instructions (including modified Bass toothbrushing, inter-dental cleaning with dental floss or inter-dental brushes as appropriate) and scaling and root planing of all supragingival and subgingival deposits of plaque and calculus, using both hand and ultrasonic instruments and local anaesthetic as needed. There was no limit to time for debridement during the 4 h of available appointment time, although the duration of debridement was not recorded. At the first treatment visit, test drug or placebo was provided and the need for adherence with both oral hygiene and drug use was emphasized. The test drug was 20 mg doxycycline (Periostat, CollaGenex Pharmaceuticals, Newtown, PA, USA) twice daily for 3 months. The placebo was identical, with the exception of omission of doxycycline. Enough drug was dispensed for 1 month and re-supplied at follow-up visits. Adherence to drug use was examined by asking the participants and also by the return of drug containers.

Follow-up

At each recall visit, supportive periodontal therapy was provided including monitoring and advice in oral hygiene and removal of all supra- and subgingival deposits. All clinical, biochemical and smoking exposure parameters were recorded at each visit. Patients were recalled following completion of therapy at 1, 3 and 6 months.

Statistical methods

All data were entered into an *Excel* spreadsheet, and then imported into the statistical package *R* (version 2.1.1) for validation (meticulously trawled for transcription errors and inconsistencies against a range of criteria), manipulation (for structures appropriate for subsequent analyses) and summary/aggregate analyses [e.g. analysis of covariance (ANCOVA) of the subject-level means], before exporting to the multilevel software package *MLwiN* (version 2.02) for multilevel modelling. Only sites with initial $\text{PD} \geq 5$ mm were included in the statistical analyses, as shallower sites did not receive root planing, although they were treated with subgingival scaling.

ANCOVA was used for the aggregate data analyses as randomization ensured sufficiently balanced subject-level baseline mean outcomes. As sites were not

randomized, and there were substantial site-level differences in means between groups, multilevel ANCOVA analysis was not deemed appropriate. A multilevel time-series analysis was undertaken instead, whereby site-level outcomes from each occasion were modelled as curvilinear ‘trajectories’ over time. These trajectories were then examined in relation to several covariates. In addition to *time* (both *linear* and *quadratic* components to reflect the curvilinear nature of the outcome trajectories), the covariates considered were *group* (treatment/placebo), smoking history and interactions between these covariates and the *time* covariates. The interactions between *group* and *time* determined whether treatment effects over time differed between groups (i.e. exhibited different trajectories).

Results

Baseline comparisons

Thirty-five subjects were recruited, although one withdrew before allocation of treatment group and was therefore not included in the study (Fig. 1). Consequently, 34 subjects were included in the analysis (18 control, 16 test), with an age range of 32–58 years at baseline. Four subjects did not complete the study (two in each group). Three subjects did not return due to personal problems and one due to loss of interest in the study. An intention-to-treat analysis used a last observation carried forward.

The age range of the participants was 32–50 years. The placebo group was slightly younger, with a mean birth year of 1957.8 [standard deviation (SD) = 6.1], than the Periostat group, with a mean birth year of 1960.3 (SD = 7.6), although both sexes were similarly aged with males’ mean birth year being 1959.8 (SD = 6.6) and females’ mean birth year being 1958.1 (SD = 7.3). Baseline characteristics are summarized in Table 1. Subjects within groups were generally well matched at baseline, with measures of tobacco exposure being similar except for salivary COT, which was slightly higher in the placebo group. Clinically, a 10% difference for BoP was also observed.

Effect of treatment

At 6 months, aggregate analyses (ANCOVA comparing subject-level mean values of treated teeth) revealed that there were

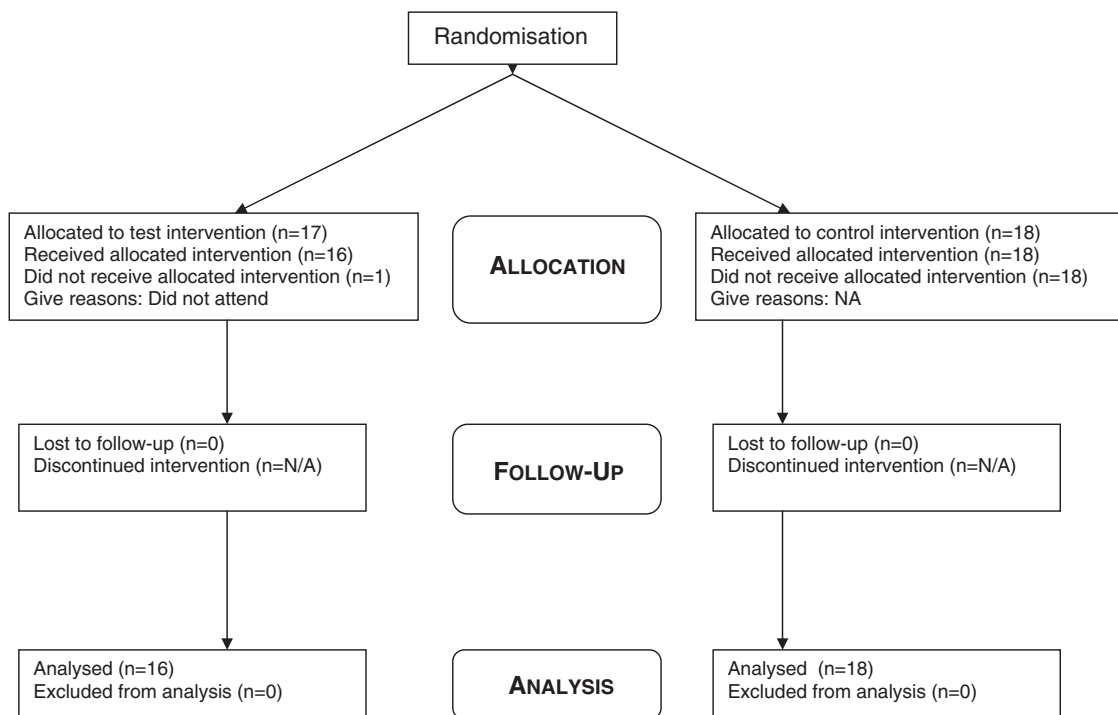


Fig. 1. Patient flow through study.

no significant differences in the clinical improvements observed between groups (Table 2). However, when these same data were analysed using multilevel time-series methods, there were significant differences between groups (Table 3).

For all outcomes, the modelled trajectories were curvilinear, i.e. outcome = intercept + linear component \times time + quadratic component \times time², where the linear component indicates change in the outcome per unit change in time (velocity), and the quadratic component indicates change in the outcome per unit squared in time (acceleration).

For the primary outcome of CAL and subsidiary outcomes of PD, recession and BoP, there were significant differences between the mean trajectories for the test and placebo groups. For instance, considering the interaction of *group* with *velocity* (i.e. the *linear time* interaction), CAL consistently reduced more within the test than the control group (Fig. 2), irrespective of whether or not adjustment was made for smoking exposure (either baseline or occasion-specific); Models 1 and 2: *difference in mean velocity of CAL* = -0.19 mm/6 month (95% CI = -0.34 , -0.04 ; $p = 0.012$). The velocity of change was thus greater in the treatment group than the control group. However, divergence

Table 1. Baseline characteristics of subjects

	Subject means (95% CI)		
	placebo (N = 18)	LDD (N = 16)	difference
Subjects			
Smoking (years)	26.1 (13.0, 39.1)	24.4 (8.0, 40.9)	1.6 (−3.6, 6.8)
Cigarettes (per day)	18.3 (7.0, 29.5)	16.7 (3.2, 30.2)	1.6 (−2.7, 5.9)
CO* (% volume)	5.0 (−0.3, 10.2)	4.6 (0.0, 9.2)	0.4 (−1.3, 2.1)
CO* (ppm)	27.7 (−0.8, 56.1)	25.3 (−2.4, 52.9)	2.4 (−7.2, 12.1)
Cotinine (ng/ml)	379.6 (0.0, 771.6)	327.2 (19.5, 634.9)	52.4 (−69.9, 174.7)
Treated teeth/sites			
PPD (mm)	5.79 (4.93, 6.65)	5.96 (4.92, 7.01)	−0.17 (−0.51, 0.16)
REC (mm)	−0.12 (−2.13, 1.89)	0.28 (−1.82, 2.39)	−0.41 (−1.12, 0.30)
CAL (mm)	5.67 (3.52, 7.82)	6.25 (3.99, 8.50)	−0.58 (−1.34, 0.18)
BoP* (%)	76.3 (52.5, 100.1)	65.8 (21.7, 109.8)	10.5 (−2.1, 23.1)
Plaque* (%)	76.8 (42.9, 110.8)	79.5 (37.6, 121.4)	−2.7 (−16.0, 10.6)
ICTP† (pg/site)	2.25 (0.00, 4750.76)	0.81 (0.00, 9279.67)	1.44 (−1.95, 3.99)

*Not normally distributed hence means and 95% CIs have limited meaning.

†Geometric mean (i.e. natural logarithm taken before mean is calculated and inverse logarithm applied afterwards).

CI, confidence interval; ICTP, carboxyterminal telopeptide of type I collagen; BoP, bleeding on probing; CAL, clinical attachment level; CO, carbon monoxide; LDD, Low Dose Doxycycline.

between groups slowed, revealed by the positive interaction of *group* with *acceleration* (i.e. the *quadratic time* interaction). The rate of divergence was thus not maintained and the reduction in CAL was slowing for the treatment group compared with the control group, *difference in mean acceleration of CAL* = 0.38 mm/6 month² (95% CI = -0.07 , 0.83 ; $p = 0.097$). When

adjustment was made for occasion-specific smoking exposures (Model 3), the interaction between *group* and *velocity* was slightly more negative (-0.22 mm/6 month; 95% CI = -0.41 , -0.03 ; $p = 0.027$) and the interaction between *group* and *acceleration* was more positive (0.79 mm/6 month²; 95% CI = 0.21 , 1.36 ; $p = 0.008$), indicating that for

Table 2. Results from a subject-level analysis of covariance of primary and subsidiary outcomes

	Change in subject-level means (95% CI)			
	placebo (N = 18)	LDD (N = 16)	ANCOVA	p-value
PPD (mm)	-0.98 (-2.17, 0.21)	-1.40 (-2.69, -0.11)	-0.33 (-0.71, 0.05)	0.103
REC (mm)	0.58 (-0.40, 1.55)	0.76 (-0.24, 1.76)	0.12 (-0.20, 0.45)	0.461
CAL (mm)	-0.40 (-1.20, 0.39)	-0.65 (-2.13, 0.84)	-0.23 (-0.64, 0.18)	0.282
BoP (%)	-27.2 (-64.8, 10.4)	-21.8 (-59.7, 16.1)	-2.0 (-12.2, 8.1)	0.699
Plaque (%)	-19.3 (-67.0, 28.4)	-21.7 (-60.1, 16.7)	-1.5 (-15.5, 12.5)	0.834
ICTP (ln pg/site)	0.01 (0.00, 4307.44)*	0.02 (0.00, 5379.19)*	0.13 (-3.78, 3.51)†	0.944

*Geometric mean change.

†Difference on the natural logarithm scale.

CI, confidence interval; ANCOVA, the mean difference in subject-level mean outcome at 6 months between treatment and placebo groups estimated by analysis of covariance; ICTP, carboxyterminal telopeptide of type I collagen; BoP, bleeding on probing; CAL, clinical attachment level; LDD, Low Dose Doxycycline.

Table 3. Results of multilevel time-series analysis of primary and subsidiary outcomes

	Model 1		Model 2		Model 3	
	diff (95% CI)	p-value	diff (95% CI)	p-value	diff (95% CI)	p-value
CAL						
Velocity	-0.19 (-0.34, -0.04)	0.012	-0.19 (-0.34, -0.04)	0.012	-0.22 (-0.41, -0.03)	0.027
Acceleration	0.38 (-0.07, 0.83)	0.097	0.38 (-0.07, 0.83)	0.097	0.79 (0.21, 1.36)	0.008
PD						
Velocity	-0.30 (-0.42, -0.17)	<0.001	-0.30 (-0.42, -0.17)	<0.001	-0.38 (-0.53, -0.24)	<0.001
Acceleration	-0.16 (-0.54, 0.22)	0.417	-0.16 (-0.54, 0.22)	0.417	0.71 (0.27, 1.15)	0.002
REC						
Velocity	0.10 (-0.01, 0.22)	0.085	0.10 (-0.01, 0.22)	0.085	0.18 (0.03, 0.33)	0.021
Acceleration	0.54 (0.19, 0.89)	0.003	0.54 (0.19, 0.89)	0.003	0.06 (-0.39, 0.51)	0.806
ICTP						
Velocity*	-1.16 (-2.62, 0.30)	0.119	-1.16 (-2.62, 0.30)	0.119	-1.61 (-3.34, 0.11)	0.067
Acceleration	5.93 (1.54, 10.31)	0.008	5.93 (1.54, 10.31)	0.008	10.24 (4.99, 15.49)	<0.001
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
BoP						
Velocity†	1.51 (1.08, 2.10)	0.016	1.49 (1.07, 2.08)	0.017	1.39 (0.97, 1.99)	0.075
Acceleration†	0.65 (0.25, 1.70)	0.380	0.66 (0.25, 1.71)	0.389	1.39 (0.48, 4.07)	0.546
Plaque						
Velocity†	1.15 (0.79, 1.69)	0.464	1.14 (0.79, 1.65)	0.484	1.65 (1.07, 2.57)	0.025
Acceleration†	1.55 (0.53, 4.51)	0.423	1.50 (0.53, 4.26)	0.442	6.28 (1.80, 21.84)	0.004

*N = 3208 (not 8428) as GCF data are not available for all treated teeth.

†Estimates are odds ratios.

Diff, estimated overall differences between treatment and placebo groups across the study period; ICTP, carboxyterminal telopeptide of type I collagen as ln(pg/site); OR, estimated overall odds ratio in the differences between treatment and placebo groups across the study period; CI, confidence interval; velocity, difference between groups in change in outcome per month; acceleration, difference between groups in rate of change in outcome per month; Model 1, no adjustment for smoking exposure; Model 2, adjustment for baseline smoking exposure (number of cigarettes per day, number of years as a smoker); Model 3, adjustment for occasion-specific baseline smoking exposure (cotinine, CO%); BoP, bleeding on probing; CAL, clinical attachment level.

Model 3 divergence was initially greater but then slowed more than observed for Models 1 and 2.

PD reduced with greater velocity within the test than the control group (Fig. 3), irrespective of whether or not adjustment was made for baseline smoking exposure; Models 1 and 2: difference in mean velocity of PD = -0.30 mm/month (95% CI = -0.42, -0.17; p < 0.001). Where adjustment was made for occasion-specific smoking exposure (Model 3), the interaction

between group and acceleration revealed a significant slowdown of the divergence between groups; Model 3: difference in mean acceleration of PD = 0.71 mm/month² (95% CI = 0.27, 1.15; p = 0.002). However, this finding was not consistent with the models that either adjusted for baseline smoking exposure (Model 2) or where no adjustment for smoking was made (Model 1).

For recession, during the study period there was no significant difference in outcome velocity between treatment and

control groups, whether or not adjustment was made for baseline smoking exposure; Models 1 and 2: difference in mean velocity of REC = 0.10 mm/month (95% CI = -0.01, 0.22; p = 0.085). However, this initially modest difference in velocity grew significantly; Models 1 and 2: difference in mean acceleration of REC = 0.54 mm/month² (95% CI = 0.19, 0.89; p = 0.003). When adjustment was made for occasion-specific smoking exposure (Model 3), the velocity of change in recession was

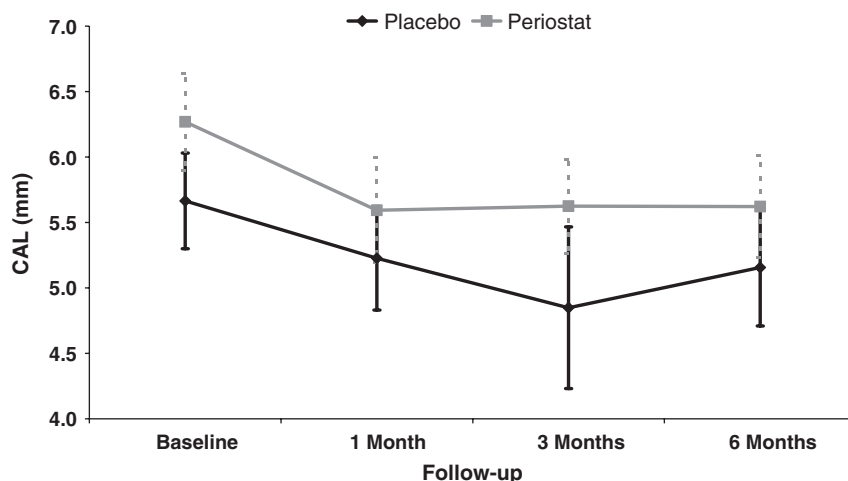


Fig. 2. Change in clinical attachment level with time (mean+SD).

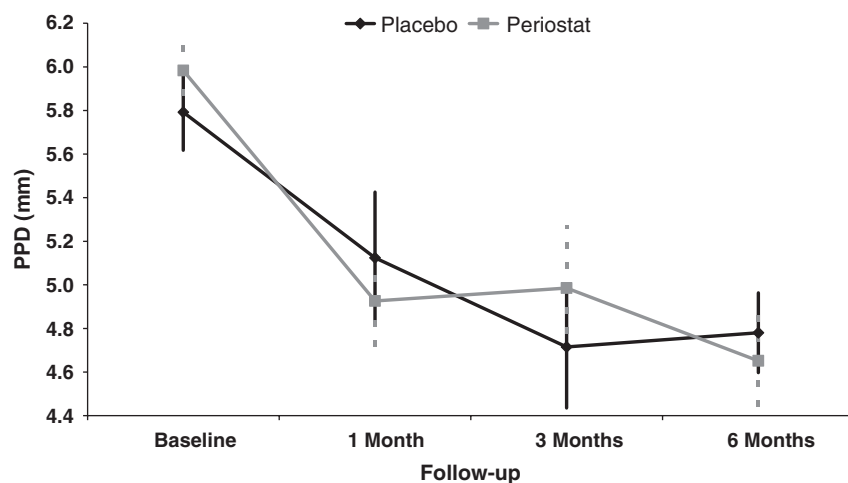


Fig. 3. Change in probing depth level with time (mean+SD).

significantly greater in the treatment group; Model 3: *difference in mean velocity of REC* = 0.18 mm/month (95% CI = 0.03, 0.33; $p = 0.021$). However, there was no notable *acceleration* of this divergence; Model 3: *difference in mean acceleration of REC* = 0.06 mm/month² (95% CI = -0.39 0.51; $p = 0.806$).

Similar trends were observed for ICTP as for REC, with patterns of change in the opposite direction. That is, with or without adjustment for baseline smoking exposure, ICTP showed a slightly greater *velocity* of reduction in the treatment group compared with the control group, although not formally significant; Models 1 and 2: *difference in mean velocity of ln(ICTP)* = -1.16 mm/month² (95% CI = -2.62, 0.30; $p = 0.119$). However, divergence between groups was growing and sig-

nificant; Models 1 and 2: *difference in mean acceleration of ln(ICTP)* = 5.93 mm/month² (95% CI = 1.54, 10.31; $p = 0.008$). When adjustment was made for occasion-specific smoking exposure (Model 3), the *velocity* of change between groups became borderline significant; Model 3: *difference in mean velocity of ln(ICTP)* = -1.61 mm/month (95% CI = -3.34, 0.11; $p = 0.067$). Furthermore, the divergence was accelerating even more rapidly; Model 3: *difference in mean acceleration of ln(ICTP)* = 10.24 mm/month² (95% CI = 4.99, 15.49; $p < 0.001$).

For BoP and plaque, logistic models gave rise to model coefficients interpreted as odds ratios (ORs), which are on a non-linear scale, as the OR is multiplicative (not additive). Thus, for BoP, there were significantly elevated odds of BoP in the treatment group

compared with the control group, with or without adjustment for baseline smoking experience (Models 1 and 2), and although the interaction between *group* and *acceleration* was not significant, the model nevertheless suggests that divergence between groups was increasing. When adjustment was made for occasion-specific smoking exposure (Model 3), the contrast between groups is attenuated towards the null. There were generally greater odds of plaque among the treatment group than the control group, although this was not statistically significant unless adjustment was made for occasion-specific smoking exposure.

These findings indicate substantial differences in the estimated differences in the patterns of change in the various outcomes (i.e. increasing or decreasing divergence between groups) when adjusting for occasion-specific exposures of smoking (*COT*, *CO*) compared with either no adjustment or adjustment for baseline smoking exposures (*number of cigarettes per day*, *number of years as a smoker*). Adjustment for baseline smoking exposures has virtually no impact on the estimated differences between treatment and control groups in the changes in outcome throughout the study period.

Adverse events

Adverse events were reported by five subjects, all in the placebo group: two were at 1 month follow-up and 3- at 6-month follow-up. These were judged not to be adverse reactions related to the medication and included pulpal problems in three subjects and periodontal problems in two subjects. Data on adverse events were missing from two patients who did not return following treatment (one in each group) and from six patients at one study visit.

Compliance

Full data were available for 27 subjects (Fig. 4). Those missing 10 or less tablets out of a total of approximately 180 tablets were 11/13 patients in the placebo group and 12/14 in the test group.

Discussion

Statement of principal findings

This study has shown that an intervention aimed at host modulation may

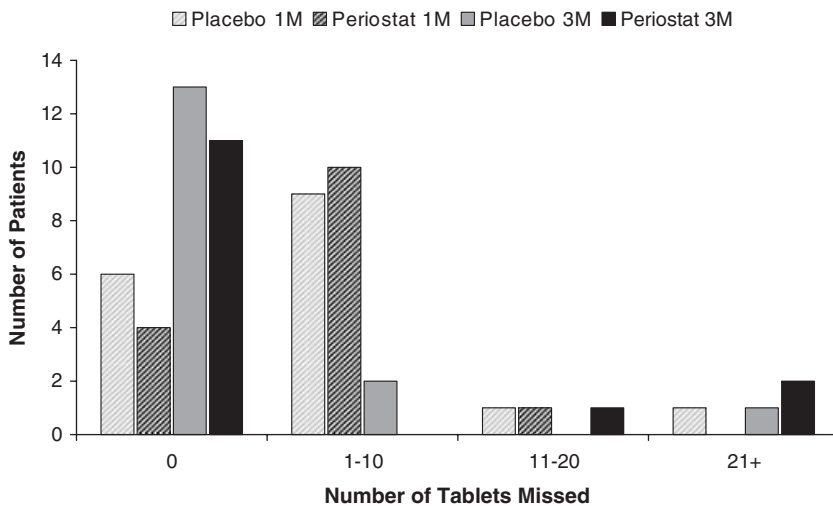


Fig. 4. Distribution of compliance with study medication.

effect clinical changes following non-surgical periodontal therapy in smokers. The final improvements were no different between the test and control groups, but multilevel modelling (MLM) revealed different trajectories for clinical changes. In other words, the endpoint of clinical healing was similar for both experimental groups; however, the rate of improvement was greater for the test group. No differences regarding absolute CAL change or GCF ICTP were evident.

Conventional statistics and MLM have very different methodologies and ask different questions of the data. The MLM approach was not selected as an attempt to find a difference between groups at all costs. Rather, the use of MLM was selected, a priori, to examine whether accounting for differences between sites within patients and at different visits might reveal differences in response to the intervention.

The contrast between the multilevel time-series models either with or without adjustment for baseline smoking exposures compared with adjustment for occasion-specific smoking exposures could be a chance finding, although it is consistent across all outcomes modelled except CAL and ICTP. Therefore, it is likely that baseline smoking exposure assessments had little or no impact, whereas occasion-specific smoking exposure assessments did. This might be because the baseline assessments of *number of cigarettes per day* and *number of years as a smoker* are too imprecise or are too inaccurate (error-prone, where self-report is unreliable and may be biased), or both. In contrast, the

occasion-specific measures of *COT* and *CO* may yield a more precise reflection of smoking habits and are free from responder bias. We are currently examining relationships between self-reported smoking status and biochemical values and these data will be reported in a further publication.

No difference could be shown for GCF ICTP levels between groups in this study. Previous investigations have demonstrated that LDD potently reduces ICTP levels in patients with previously untreated, severe disease and in patients receiving surgery combined with the drug (Golub et al. 1997, Gapski et al. 2004). However, in patients with previously treated periodontitis receiving SRP with or without locally delivered minocycline (another TCN analogue similar to doxycycline), minimal and transient reductions in GCF ICTP were demonstrated (Al-Shammari et al. 2001, Oringer et al. 2002). These data suggest a need to continue medication indefinitely if suppression of ICTP levels is desirable. The lack of effect in this study might indicate that smoking affects the pathways involved in bone turnover, reducing such targeting. It is interesting that another study, also exclusively in smokers, did not find an effect on ICTP when using the antibiotic azithromycin as an adjunct to non-surgical periodontal therapy (Mascarenhas et al. 2005). While ICTP levels in this study appeared to be lower than in other studies (Al-Shammari et al. 2001, Gapski et al. 2004, Mascarenhas et al. 2005), the differences may be more apparent than real. The geometric mean was selected as the appropriate statistic to

summarize the data as it was highly skewed. This representation results in low summary values although the same protocol for collection, storage and assay was used compared with other studies. For comparison, the arithmetic mean values (95% CI) for ICTP at baseline were: test group 50.45 ng/site (–56.66, 157.56), control group 63.66 ng/site (–106.49, 233.81).

It is interesting to note the high proportion of sites with BoP in smokers throughout the study. While it has been suggested that smoking reduced BoP, a systematic review of the effects of smoking on the response to non-surgical therapy could not find a difference in levels of the parameter (Labriola et al. 2005).

Strengths and weaknesses of the study

The study failed to show any significant differences between treatment arms using aggregate (standard subject-based) analyses because the observed treatment effects were smaller than originally anticipated when powering the study. In contrast, it was anticipated and indeed demonstrated that a multilevel (site-based) analysis would have sufficient power for the study of this size and thus be able to reveal statistically significant treatment effects (whether clinically significant or not). Although optimum statistical power is generally achieved using ANCOVA, the multilevel extension to this was questionable in view of subjects and not teeth or sites being randomized, as this brought into question the reliability of balanced baseline mean outcomes. Multilevel analyses might therefore yield greater power than aggregate analyses, although care must be exercised in favouring the multilevel time-series approach to a multilevel ANCOVA approach when randomization is not applicable to the lowest-level unit (sites in this instance).

Strengths and weaknesses in relation to other studies

A strength of this study was that it was designed from the outset to investigate the effect of the experimental intervention on smokers. This contrasts with other studies that have used analysis of smoking subgroups in clinical trials of non-surgical therapy with LDD where both smokers and non-smokers were recruited (Preshaw et al. 2005). Subgroup analyses can be misleading due

to the presence of unknown confounders and the possibility of chance of statistically significant results (Brookes et al. 2001). Therefore, the results of this study should provide more reliable evidence.

A further strength of this study was the minimization of bias. Allocation was concealed from those recruited for a study, thereby eliminating selection bias. The patient, examiner, caregiver and statistician were all masked to allocation until the analysis was complete. Regarding withdrawals, all subjects randomized to treatment contributed to the analysis using intention-to-treat methods. To minimize treatment-related effects, compliance with medication was reinforced and monitored. Compliance was good, although the data are based on self-reported data. Studies on smokers have shown increased losses to follow-up (Nasry et al. 2006), and the use of long-term medication for chronic conditions has been problematic (Roter et al. 1998). As a result of minimization of bias, the estimate of treatment effect should be reliable.

A limitation of this study was the relatively small number of participants. The study was powered adequately for the primary outcome, but may not have been adequately powered for secondary outcomes. The efficiency gained by using ML should have compensated for this especially as the N for MLM was estimated as >8000. However, more studies are needed to explore the use of ML on clinical trials in periodontology.

Meaning of the study: possible explanations and implications for clinicians and policymakers

This study does not provide evidence to support the use of LDD as an adjunct to non-surgical periodontal therapy in smokers. While differences in trajectory between the test and control groups were shown for clinical parameters, the value of this difference to clinical practice is not known currently (Nevins et al. 2005).

Upregulation of inflammation remains a compelling hypothesis to explain, at least in part, the reduced healing response in smokers (Palmer et al. 1999b, Kinane & Chestnutt 2000). The failure to show an adjunctive effect in this study might indicate that either the intervention was ineffective in achieving host modulation in smokers or

that other pathways or mechanisms must also be targeted to achieve a therapeutic effect. Further research to investigate mechanisms to explain the impaired healing response in smokers is therefore warranted and it is hoped that this will lead to improved strategies for managing periodontal disease in smokers. In addition, tobacco use cessation should continue to be of paramount importance in such individuals.

Conclusions

Non-surgical periodontal therapy in smokers can produce a substantial improvement in periodontal health. However, there is no evidence to support the use of LDD as an adjunct to non-surgical therapy in smokers. Quitting tobacco use continues to be of fundamental importance in improving the periodontal health of these individuals. Multilevel modelling shows great promise as an analytical tool for periodontal clinical trials and should be examined in a wide variety of trial designs for its contribution to data analysis.

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Clinical Relevance

Scientific rationale for the study: Periodontitis is less responsive to non-surgical therapy in smokers than non-smokers and this may be partly due to increased inflammation in smokers. The rationale for this study was to test whether chemically

modifying inflammation and tissue breakdown using LDD at the same time as non-surgical periodontal therapy in smokers might be beneficial. *Principal findings:* Despite improvements in periodontal health, no additional benefit was demonstrated comparing those subjects who received

the test drug with the identical placebo. *Practical implications:* Conventional periodontal therapy improves the periodontal health of smokers. Quitting tobacco use continues to be a high priority.