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Periodontal therapy: a novel non-drug-induced experimental model to study human inflammation

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Background: Chronic periodontitis causes a low-grade systemic inflammatory response; its standard treatment, however, induces an acute inflammatory response. The aim of this study was to describe the systemic inflammatory reactions to an intensive periodontal treatment regimen.

Methods: Fourteen otherwise healthy subjects suffering from severe chronic periodontitis were enrolled in a 1 month pilot single-blind trial. Intensive periodontal treatment, consisting of full-mouth subgingival root debridement delivered within a 6-h period, was performed. Periodontal parameters were recorded before and 1 month after completion of treatment. Blood samples were taken at baseline and 1, 3, 5, 7 and 30 days after treatment. Interleukin-1 receptor antagonist (IL-1Ra), Interleukin-6 (IL-6) and C-reactive protein (CRP) serum concentrations were determined by enzyme-linked immunosorbent assay (ELISA). Complete blood counts were also performed.

Results: One day after treatment, mild neutrophilia and monocytosis ($p < 0.05$) and lymphopenia ($p < 0.01$) were accompanied by a sharp increase in inflammatory markers (IL-1Ra, IL-6, $p < 0.01$). A 10-fold increase in CRP ($p < 0.001$) was detected on day 1 and its kinetics followed a pattern of a classical acute phase response (significantly raised concentrations up to 1 week, $p < 0.01$). At 3–7 days after treatment, subjects presented also with a mild tendency towards a normocytic anaemic state ($p < 0.01$) and a degree of lympho-thrombocytosis ($p < 0.05$). The observed changes were similar to those expected following the well-characterized endotoxin-challenge model of inflammation.

Conclusions: Intensive periodontal treatment produced an acute systemic inflammatory response of 1 week duration and might represent an alternative to classic endotoxin-challenge or drug-induced models to study acute inflammation in humans.

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Experimental models to study *in vivo* acute inflammation have been developed over the years; they utilize a variety of stimuli capable of inducing a systemic inflammatory response. Among them the endotoxin model (parenteral injection of lipopolysaccharide in healthy subjects) represents perhaps the most known and adopted, although the physical training model (strenuous exercise) and the vaccination model have been equally useful (1–9).

In the reported models, the systemic inflammatory responses to the stimuli

are measured as changes in concentrations of well-recognized serum markers (C-reactive protein: CRP; interleukin-6: IL-6; interleukin-1: IL-1). Some of these mediators are thought to exert direct defensive functions (like CRP), whereas others are involved in the amplification/regulation of the local stimulus and induction of a systemic response to it (10).

The concept that periodontitis and its treatment could represent a useful model to study human inflammation originates from growing evidence that periodontal infections are associated with and contribute to the systemic inflammatory burden of affected individuals. Several reports have confirmed that moderate to severe forms of periodontitis are associated with a mild systemic inflammatory response, as defined by raised serum concentrations of inflammatory markers (CRP, IL-6) (11–14). Furthermore, standard periodontal treatment has been shown to reduce the level of systemic inflammation, thus giving strength to the concept that periodontitis contributes to the systemic inflammatory burden of the individual (15).

Periodontitis is a prototype chronic low-grade infection. It is caused primarily by anaerobic Gram-negative bacteria organized in a protected biofilm in the subgingival portion of the root surface. Its treatment relies upon the mechanical removal of the biofilm and subgingival calculus deposits in order to reduce bacterial load and thus local inflammation (16). Mechanical instrumentation of the subgingival environment, however, results in an intense transient bacteraemia as well as significant soft tissue damage at the level of the periodontium (17, 18). The fact that periodontitis is caused by a mixed anaerobic Gram-negative flora would suggest that bacteraemia following periodontal instrumentation may challenge the systemic inflammatory response in a similar fashion with respect to endotoxin injection. Since mechanical periodontal therapy in the absence of administration of antimicrobials represents the standard of care delivered to the 10–15% of the adult population affected by chronic severe periodontitis, periodontal therapy may represent a readily available experimental model to study inflammation.

The aims of this pilot investigation were (i) to describe the changes in systemic inflammatory parameters consequent to an intensive periodontal treatment regimen and (ii) to determine the kinetics of changes in inflammatory markers and mediators (CRP, IL-6, IL-1 receptor antagonist: IL-1Ra) in the early days following the treatment-associated bacteraemia.

Methods

Study population and design

The study was a prospective single-blind intervention trial with 1 month follow-up. Participants were recruited from subjects referred to the Department of Periodontology of the Eastman Dental Hospital, University College London. Subjects presenting with severe (probing pocket depths greater than 6 mm and marginal alveolar bone loss greater than 30%), generalized (at least 50% of teeth affected) periodontitis were invited to participate in the study. Exclusion criteria included: (i) known systemic diseases, (ii) history and/or presence of other infections, (iii) systemic antibiotic treatment in the preceding 3 months, (iv) treatment with any medication known to affect the serum level of inflammatory markers and (v) pregnant or lactating females. All patients gave written informed consent; the study had been reviewed and approved by the Eastman/UCLH joint ethics committee.

A baseline visit was conducted by a blind calibrated examiner who collected a complete medical history and standard clinical periodontal parameters (presence of plaque, bleeding on probing, probing pocket depths and recession of the gingival margin). Subjects thereafter received an intensive session of subgingival mechanical instrumentation under local anaesthesia (within a 6-h period). Instrumentation was performed using a piezoelectric instrument (EMS, Nyon, Switzerland) equipped with fine tips for access in the subgingival environment. Extraction of hopeless teeth was performed during the same session according to standard clinical practice. Periodontal outcomes were re-assessed 1 month following completion of periodontal therapy.

Inflammatory markers and blood counts analyses

Serial blood samples were drawn into vacutainer tubes at baseline, 1, 3, 5, 7 and 30 days after periodontal therapy. Serum was obtained by centrifugation at 2000 *g* for 15 min at 4°C and stored at −70°C until analysis in a standardized blind fashion. CRP levels were assessed by an automated immunoturbidimetric high-sensitivity assay (Cobas Integra, Roche AG Diagnostics, Mannheim, Germany; detection limit of 0.25 mg/l) essentially as described (15); IL-6 and IL-1Ra were measured with high-sensitivity two-site enzyme-linked immunosorbent assay (ELISA) kits (Quantikine HS, R & D System, Minneapolis, MN, USA; detection limit 0.04 pg/ml and 14 pg/ml, respectively) (15, 19). Differential blood counts were performed using standard clinical haematology procedures on an automated analyser.

Statistical methods

Data are reported as means ± standard deviation (SD) for variables normally distributed or median and interquartile ranges (IQR). Non-parametric statistical comparisons were applied. All statistical comparisons between visits were performed with the Friedman ANOVA and the Wilcoxon signed rank-sum test for post-hoc comparisons. Concentrations of inflammatory markers were skewed and therefore log transformed values were used in all graphical representations for ease of interpretation. The alpha value was set at 0.05.

Results

Fourteen subjects (mean age 48 ± 6 years) were included in the trial. With the exception of the presence of severe, generalized periodontitis, all subjects were medically healthy. Eight

patients were females, 10 were Caucasians, two subjects were smokers and one was a former smoker. They had an average body mass index of 26 ± 4 kg/m^2. During the study period, all patients remained stable and there were no changes in lifestyle issues and habits including exercise, diet, smoking and medications.

In terms of periodontal parameters participants presented with high levels of gingival inflammation (full-mouth bleeding scores of 65 ± 14%) and severe widespread periodontitis (average of 67 ± 23 periodontal pockets 5 mm or deeper per subject with an average clinical attachment level loss of 4.8 ± 0.9 mm).

Significant improvements in all clinical parameters were observed ($p < 0.0001$, paired *t*-test) 1 month after completion of treatment (Table 1). Subgingival instrumentation was performed for an average of 200 ± 27 min over a 6-h period. A mean of 3 ± 3 hopeless teeth were extracted at the same time. At day 1 all participants reported a series of symptoms occurring the evening after the treatment, including headache, rise in body temperature, tiredness, chills and general malaise. No significant changes were observed in participants' body temperature between the various time points (data not shown).

Box and whiskers plots of the log transformed concentrations of inflammatory markers (IL-1Ra, IL-6 and CRP) at baseline and 1, 3, 5, 7 and 30 days after treatment are displayed in Fig. 1. IL-1Ra kinetics showed a significant increase of its concentrations only 1 day after treatment (917.94 ± 569.33 pg/ml compared with 572.91 ± 248.89 pg/ml at baseline, $p < 0.001$ Wilcoxon rank-sum test). No changes were observed at later time points for this early marker.

IL-6 concentrations were sharply increased at day 1 (6.98 ± 1.60 pg/ml compared with 1.78 ± 1.67 pg/ml at baseline, $p < 0.001$ Wilcoxon rank-sum paired test) and remained higher than baseline for the first week.

Clear significant changes in CRP concentrations were observed between baseline and 1, 3, 5 and 7 days after treatment ($p < 0.0001$ Friedman ANOVA). From an initial 1.61 ± 2.11 mg/l, participants showed a significant increase of CRP concentrations at day 1 of 12.49 ± 2.63 mg/l ($p < 0.0001$ Wilcoxon signed rank-sum test). CRP concentrations remained significantly higher than baseline 3, 5 and 7 days after treatment (7.31 ± 2.40, 3.63 ± 1.99, 2.59 ± 1.81, respectively, $p < 0.001$ Wilcoxon rank-sum paired test).

With respect to haematological parameters, early changes in differential leukocyte counts were observed at day 1: circulating neutrophils significantly increased ($p < 0.01$ Wilcoxon test compared with baseline). An increase in the numbers of circulating monocytes was also observed at days 1 and 3 ($p < 0.05$ and $p < 0.01$, respectively). Lymphocyte numbers fell ($p < 0.01$) at day 1 and then increased 7 and 30 days after treatment ($p < 0.05$) (Table 2). Interestingly a reduction in total leukocyte counts was detected 1 month after completion of treatment ($p < 0.05$ Wilcoxon rank-sum paired test).

At 5 and 7 days after treatment, erythrocyte numbers, haematocrit and haemoglobin concentration decreased (Table 2). The number of platelets was significantly increased on day 5 ($p < 0.05$).

Table 1. Changes in clinical parameters after periodontal therapy

	Baseline	30 days
NPPD	66.80 ± 23.02	21.33 ± 12.03*
PPD (mm)	4.12 ± 0.76	2.95 ± 0.31*
REC (mm)	0.70 ± 0.46	1.30 ± 0.51*
CAL (mm)	4.82 ± 0.87	4.26 ± 0.62**
FMPS	49.07 ± 22.27	13.79 ± 11.73*
FMBS	65.40 ± 14.60	17.29 ± 7.96*

CAL, clinical attachment levels; FMBS, full-mouth bleeding score; FMPS, full-mouth plaque score; NPPD, number of probing pocket depths ≥ 5 mm; PPD, probing pocket depth; REC, recession of gingival margin.

Values are expressed as mean ± SD, *$p < 0.0001$, **$p < 0.01$ Wilcoxon post-hoc rank-sum paired test vs. baseline.

Discussion

This report described the effects of an intensive periodontal treatment regimen (full-mouth subgingival instrumentation delivered within a 6-h period) (20) on biochemical and haematological parameters in the days following therapy. Transient alterations in complete blood counts (mild neutrophilia and monocytosis and relative lymphopenia) were observed 1 day after treatment. At 2–6 days thereafter a tendency towards a mild normocytic anaemic status developed. Significant changes in inflammatory markers accompanied these cellular responses: CRP, a prototype acute phase marker, increased nearly 10-fold after 1 day and returned to baseline values only 1 month after treatment. Its concentration changes followed the pattern of a classic acute phase marker with a rise within 24 h. Data indicated that IL-6 followed the same pattern with a significant increase on day 1 and slower decreases over 1 week. Serum IL-1Ra levels, however, followed a different pattern. A relatively modest increase was detected only on day 1 after therapy, possibly reflecting the tail of a rapid acute response resolved within the first 24 h.

Given the evidence of significant ingress of bacteria into the systemic circulation following periodontal instrumentation (17, 18, 21–23), the present data describe the systemic inflammatory changes occurring after periodontal treatment. As bacteraemia and tissue damage are produced, local and systemic production of pro-inflammatory mediators (such as tumour necrosis factor α and IL-1β) has been described as a component of host defenses (8, 10, 24). In order to modulate the process, anti-inflammatory molecules (such as IL-1Ra, IL-6) appear on the scene to control inflammation and stimulate the hepatic synthesis of acute phase proteins (CRP) (25). Changes in leukocytes and erythrocytes numbers become transiently

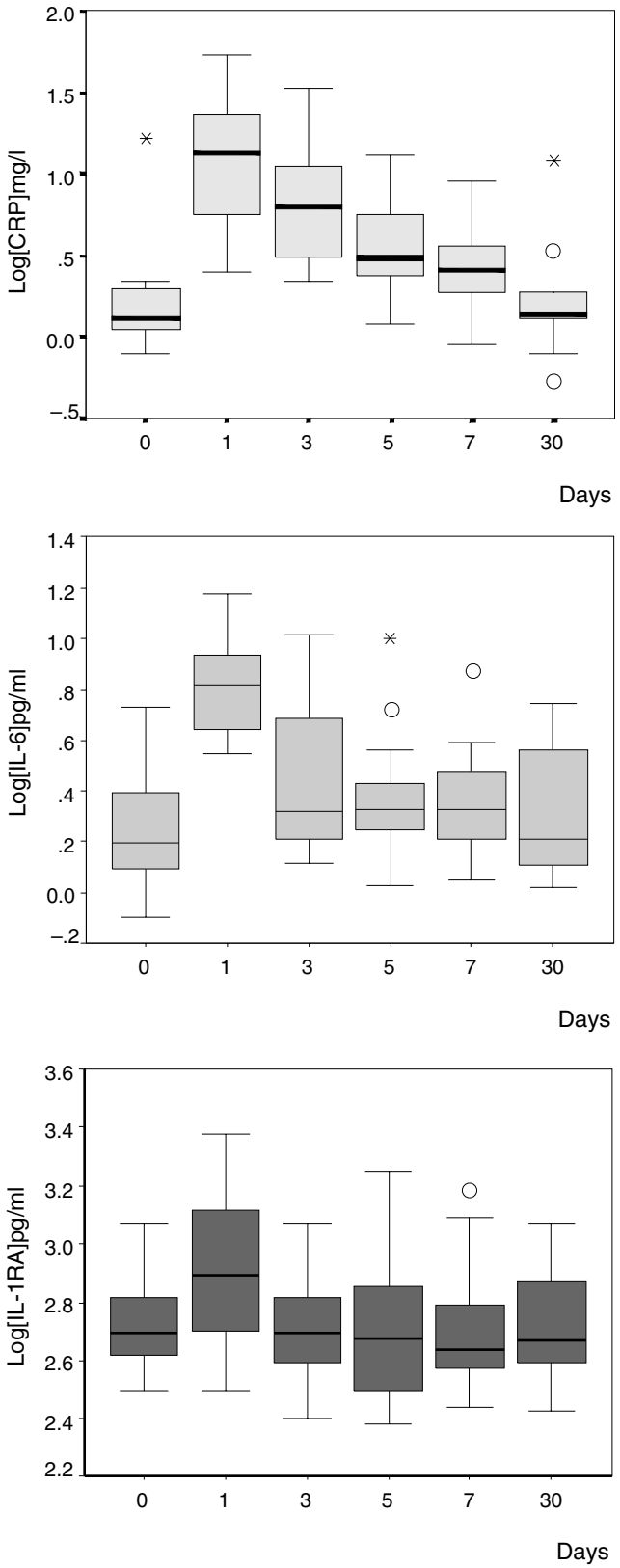

Fig. 1. Box and whiskers plots showing changes in Log[CRP], Log[IL-6], Log[IL-1Ra] before and after periodontal therapy. The box refers to the 25th (bottom) and 75th (up) percentiles and the median is the horizontal line inside. Open circles represent outliers whereas asterisks stand for extreme observations. CRP, C-reactive protein; IL-6, interleukin-6; IL-1Ra, interleukin-1 receptor antagonist.

apparent in the first few days to a week (10).

The observed results, although different in magnitude of changes, are in agreement with those reported in several models of human inflammation. Intravenous endotoxin administration results in a systemic syndrome of several hours with fever and constitutionals symptoms. A rapid decrease in leukocyte counts due to peripheral vascular margination is observed and it is subsequently followed by a degree of neutrophilia. Production of pro-inflammatory mediators (tumour necrosis factor α accompanied by modest IL-1β increase) amplify the reaction against endotoxin, while anti-inflammatory cytokines (IL-6, IL-1Ra) trigger the hepatic response that within 24 h results in raised concentrations of acute phase proteins in order to contain and clear the microbial insult (8, 10).

The mild tendency towards an anaemic status following intensive periodontal therapy reported in this study merits some discussion. Previous studies have indicated that following acute inflammatory stimuli (minor surgery or strenuous exercise) a post-operative/trauma anaemia develops with the characteristics of a functional iron deficiency (26, 27). A clear shift of the metal from the erythropoiesis and circulation to the reticulo-endothelial system might represent the pathogenetic mechanism (28). During a systemic inflammatory reaction (as in this and other experimental models) increased production of cytokines (IL-1, IL-6) might directly affect iron metabolism and inhibit the maturation and synthesis of the erythroid progenitor cells (29–32). Furthermore a specific IL-6-induced hepatic mediator (hepcidin) could be responsible for iron sequestration in macrophages and a reduced iron absorption in the small intestine (33). Patients suffering from periodontitis already manifest signs of anaemia of chronic diseases when compared with healthy controls (34). Further investigations are necessary to gain insight into the nature of the association between periodontitis and anaemia and the mechanisms of these observations.

Table 2. Differential blood counts before and after periodontal therapy

		Days after periodontal therapy					
	Baseline	1	3	5	7	30	*p*†
WBC (10^9/l)	6.3 (1.8)	7.0 (2.2)	6.1 (1.8)	5.8 (2.3)	6.0 (1.3)	**5.8 (1.4)***	0.029
Lymp (10^9/l)	554 (189)	**346 (222)****	587 (279)	586 (541)	**637 (242)***	**578 (354)***	< 0.0001
Neutr (10^9/l)	887 (75)	**996 (183)****	970 (278)	927 (219)	900 (223)	916 (225)	0.04
Mon (10^9/l)	114 (39)	**138 (28)***	**150 (66)****	136 (62)	109 (37)	137 (37)	0.012
Baso (10^9/l)	5 (5)	**4 (2)***	5 (8)	6 (6)	7 (5)	8 (2)	0.01
Eosin (10^9/l)	25 (33)	20 (14)	35 (24)	37 (33)	37 (26)	40 (17)	0.007
RBC (10^{12}/l)	4.5 (0.9)	4.7 (0.5)	4.5 (0.6)	**4.4 (0.6)***	**4.3 (0.9)****	4.5 (0.7)	< 0.0001
HB (g/dl)	13.7 (2.5)	14.0 (1.5)	**13.5 (2.7)***	**13.4 (2.1)****	**13.3 (2.5)****	13.8 (2.8)	< 0.0001
MCV (fl)	94.1 (7.6)	94.0 (4.3)	93.5 (6.7)	94.4 (8.0)	94.2 (7.0)	94.0 (6.7)	NS
HCT (l/l)	0.42 (0.1)	0.43 (0.04)	0.42 (0.1)	**0.41 (0.04)***	**0.40 (0.1)****	0.42 (0.1)	< 0.0001
MCH (pg)	31.6 (2.5)	**30.6 (2.8)****	30.8 (3.3)	31.0 (2.5)	31.1 (3.5)	**30.3 (2.8)****	0.033
MCHC (mg/dl)	33.1 (1.4)	**32.3 (1.0)****	33.0 (1.5)	32.6 (1.2)	32.8 (2.0)	37.2 (1.2)	0.08
PLTS (10^9/l)	290 (85)	289 (87)	284 (78)	**309 (65)***	291 (59)	286 (53)	0.005

Baso, basophils; Eosin, eosinophils; HB, haemoglobin; HCT, haematocrit; Lymp, lymphocytes; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; Mon, monocytes; Neutr, neutrophils; PLTS, platelets; RBC, red cell count; WBC, white cell count.
†Friedman ANOVA.
*$p < 0.05$, ** $p < 0.01$ Wilcoxon post-hoc rank-sum paired test vs. baseline.

Due to the lack of observations in the early post-treatment hours, this study offers no direct evidence on the peak of the inflammatory response generated by periodontal therapy. Comparisons with other inflammatory models, however, would suggest that the peak of intensity could occur within the first 2–5 h after treatment. This interpretation is consistent with the observation that rapid inflammatory markers such as IL-1Ra had returned to baseline levels within the first 3 days. Further studies focusing on the early inflammatory responses (up to first 6–8 h) are needed.

On the basis of these results, it is concluded that intensive periodontal treatment induced an acute systemic inflammatory response. Since the observed response seems to share many of the features of the well-characterized endotoxin, vaccination and strenuous exercise models, periodontal therapy may represent a useful non-drug-induced model to study human inflammation. Its relevance may be particularly evident when researchers wish to study the functional relevance of specific genetic variants or the effect of pharmacological interventions that require relatively large sample sizes and when the use of the other established models (such as the endotoxin challenge) maybe impractical or unethical.

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