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Effect of ultrasonic debridement using a chlorhexidine irrigant on circulating levels of lipopolysaccharides and interleukin-6

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Abstract

Aim: Transient bacteraemia and endotoxaemia, and elevated levels of systemic cytokines have been reported following subgingival debridement. This study aimed to investigate the effect of chlorhexidine (CHX) solution on circulating levels of lipopolysaccharide (LPS) and interleukin-6 (IL-6) when used as an irrigant during ultrasonic debridement in patients with periodontitis.

Material and Methods: Eighteen patients with moderate to advanced chronic periodontitis were treated in a split-mouth, crossover, single-masked study. Irrigation with 0.02% CHX solution or water was used during treatment of two ipsilateral quadrants on two separate occasions 7 days apart, randomized as to order. Peripheral blood samples were collected for circulating levels of LPS and IL-6 at baseline, 5 and 120 min. after instrumentation commenced.

Results: Median concentrations of LPS were elevated from baseline to 5 min. into treatment with both CHX and control irrigant ($p < 0.05$). Median levels of IL-6 increased with both treatments from baseline to 120 min. ($p < 0.001$): CHX, 0.81–1.85 pg/ml; control, 0.78–1.78 EU/ml.

Conclusions: Ultrasonic instrumentation in patients with moderate to advanced periodontitis increases circulating levels of LPS after 5 min. and IL-6 120 min. after commencement of treatment, and is not affected by using 0.02% CHX as an irrigant instead of water.

Key words: chlorhexidine; IL-6; lipopolysaccharide; peridontal disease; treatment

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Several authors have described a transient bacteraemia following subgingival debridement (Lockhart 2000, Kinane et al. 2005, Forner et al. 2006, Lafaurie et al. 2007), and this may be accompanied by an increase in inflammatory mediators such as interleukin-1 (IL-1) (D'Aiuto et al. 2007) and interleukin-6 (IL-6) (D'Aiuto et al. 2004, Ide et al. 2004, Forner et al. 2006). Furthermore, Geerts et al. (2002) illustrated that even gentle mastication was capable of causing systemic dissemination of endotoxin to a greater degree in patients with more advanced periodontal breakdown, and previous work with endotoxin administration to volunteers has reported a response similar to that reported above for periodontal instrumentation (Fong et al. 1989, Krogh-Madsen et al. 2008, Mayr et al. 2008). The dissemination of either whole bacteria, their toxins or antigens alone, or in combination, has been suggested to be a possible mechanism by which the presence of periodontitis may lead to systemic inflammatory responses and other problems, including development of cardiovascular events.

Conflict of interest and source of funding statement

There are no reported conflicts of interest for any of the authors in relation to the above work.

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Chlorhexidine (CHX) is an antimicrobial agent that has been used to reduce the magnitude of bacteraemia from the oral cavity. It is commonly used as a mouthrinse (0.2%) before surgical procedures but has also been used as an irrigant in ultrasonic scaling devices at varying concentrations. Some studies have indicated a slight adjunctive effect with CHX (Taggart et al. 1990, 0.02%; Reynolds et al. 1992, 0.12%), while others have not (Allison et al. 1993, 0.12% and Chapple et al. 1992, 0.2%). One in vitro study suggests that concentrations >0.02% may lead to cytotoxicity to bone cells in a standard cytotoxicity model over the long periods associated with debridement (Patel et al. 2006). CHX at 0.02% concentration has been reported to have a 99.99% bactericidal effect in vitro (Hennessey 1973). As a result, it may be possible that the use of CHX as an irrigant acts to further the inflammatory stimulation associated with debridement as described above, by virtue of its bactericidal ability. However, it may be that the use of such an irrigant acts to promote inflammatory responses either by direct toxicity or by encouraging the release of extra lipopolysaccharide (LPS) from organisms within the pockets under treatment, following bacterial death.

Therefore, the aim of this study was to investigate the effect of CHX solution on circulating levels of LPS and IL-6 when used as an irrigant during ultrasonic debridement in patients with periodontitis.

Material and Methods

The study was a prospective single-masked, split-mouth, crossover interventional study. The study was approved by Guy's Hospital Ethics Committee. All participants gave informed consent. Nineteen participants with untreated chronic periodontitis were recruited from the Department of Periodontology, King's College London Dental Institute. All subjects were adults and had at least 20 standing teeth, with at least five sites per quadrant with a clinical probing depth of $\geqslant$5 mm and radiographic evidence of alveolar bone loss.

Participants were required to have had no form of periodontal treatment in the past 6 months or antimicrobial therapy in the past 3 months. Exclusion criteria included any chronic inflammatory or immunological condition, risk of infective endocarditis, any anti-inflammatory, steroid, immunosuppressive or anticoagulant as a regular medication in the past 6 months, diabetes and anyone who was pregnant, planning a pregnancy or lactating. All participants were never smokers or ex-smokers who had stopped at least 12 months before recruitment.

A medical, dental and social history was obtained and a complete periodontal examination was performed, recording plaque, bleeding and probing depth at six sites per tooth. The visible plaque was recorded using an O'Leary chart (O'Leary et al. 1972), from which a percentage plaque score per subject could be calculated. Probing depths were assessed to the nearest millimetre using a Williams 14W graduated manual periodontal probe. Subjects were allocated to an order of treatment using a computer-generated list. The operator (M. K. L.) was blinded, as far as possible, to which solution was used at each visit. While the CHX irrigant may have had a mint smell; it was assumed that the combination of a facemask and visor worn by the operator during treatment would have limited this. In addition, we felt that adding a mint flavouring to the control irrigant would have introduced a further variable that may have influenced the results obtained. The upper and lower right quadrants were instrumented at the first appointment in all subjects. A local anaesthetic (2% lidocaine and adrenaline 1:80,000; Astra Zeneca, Macclesfield, UK) was administered in all subjects where the probing depth sites were 4 mm or more via infiltrations in the upper arch and inferior dental nerve block in the lower arch.

The instrumentation was performed using an ultrasonic scaling device (Dentsply® Bobcat™ ultrasonic scaler Type G115-E, Dentsply International, York, PAUSA) setting 2, with a fitted irrigation unit (Dentsply® Cavitron® Dual Select™ irrigation unit; Model number DISP 118, Dentsply International, Long Island, NY, USA) set at 20 ml/min. rate, using the ultrasonic scaler universal tip (TF-1000; 25K). This tip is widely used within the UK and at the study site.

The irrigation solution was administered via two irrigation bottles (500 ml/bottle), which were connected to the irrigation unit by a person other than the operator to ensure that the operator was masked to which solution was used. The irrigation solutions used were either a colourless soluble CHX (0.02%) solution (Corsodyl 0.2% mint mouthwash, 1:10 dilution, GlaxoSmithKline, Brentford, UK) or sterile water (Baxter SA, B-7860, Baxter healthcare, Norfolk, UK). Instrumentation was carried out for 60 min., starting at the most distal point in each quadrant with the ultrasonic scaling device. No teeth were removed at this phase of treatment. One week later, instrumentation of the left upper and lower quadrants using the alternative solution was undertaken.

At each treatment session, three venous blood samples were taken. An indwelling cannula (Y-can cannula with a syringe valve, 23G-YC23SY, Beldico, Belgium) was placed in the ante cubital fossa before baseline samples were taken. The venepuncture was performed following local guidelines for skin disinfection. Blood samples were taken at baseline, 5 and 120 min. after instrumentation commenced. Using an aseptic technique, 4 ml of venous blood was collected in a syringe at each time point and 2 ml was transferred to plain tubes for serum IL-6 analysis. The 2 ml sample to be analysed for LPS was placed in heparinized tubes, concentration 25 IU/ml of blood (N201 Cambex with sodium Heparin Monoparin, Guy's Hospital Pharmacy, UK), and stored over ice while being transported to the laboratory. All blood sampling equipment was reported by the manufacturers to be pyrogen-free. The risk of atmospheric LPS contamination was minimized by thorough skin preparation before sampling, carefully combined high- and low-volume aspiration during treatment and the use of a long sampling system. Furthermore, it was assumed that there would be no residual contamination at baseline or at 120 min. Between sample collections, 1 ml of intravenous 0.9% sodium chloride infusion (BP, Product code: 362 7667, B. Braun Melsungen AG, Germany) was used to flush the cannula. The first 2 ml of venous blood collected for the second and third samples was discarded to avoid contamination of the sample from the previous saline flush. The venous blood samples were transferred to the laboratory to undergo analysis.

Serum samples were frozen at -80°C until required for analysis. IL-6 was assayed using a high-sensitivity immunoassay kit (Quantikine HS Immunoassay kit, R&D Systems Europe Ltd, Abingdon, UK) according to the

manufacturer's instructions. The average value for circulating IL-6 in the blood of a healthy individual is 1 pg/ml. The minimum detection level for the IL-6 assay ranged from 0.016 to 0.110 pg/ml, with a mean minimum detection of 0.039 pg/ml.

Plasma samples was analysed using a kinetic chromogenic limulus amebocyte lysate (LAL) assay for LPS in the baseline and 5 min. samples. The LAL assay has been described previously by Ide et al. (2004). The minimum detection level of the LAL kit used was 0.1 EU/ml.

Statistical analysis

Sample size was determined using previous IL-6 data generated by our group (Ide et al. 2004). This indicated that 15 subjects would provide 80% power to show a 50% reduction in the elevation of IL-6 after 120 min.

The data collected were analysed using a statistical software programme (Stata 9, Stata Co, TX, USA). Some variables were not normally distributed and all are described using median and inter-quartile range. Initial analysis of variance was performed on the biochemical data, following transformation to the negative reciprocal of the square root, using a specific repeated measures analysis of variance designed for use in pharmaceutical crossover trials (Stata 9 PK analysis). Subsequent comparisons within and between irrigants were performed using the Wilcoxon matched-pairs, signed-ranks test. Statistical significance was inferred at $p < 0.05$.

Results

One participant failed to provide one blood sample at the second visit, and the data for this subject were excluded from the analysis. The demographic and clinical variables for the remaining 18 participants are presented in Table 1. Table 2 shows the concentrations of LPS and IL-6 at baseline and 5 min. after commencing treatment, together with IL-6 concentrations 120 min. after commencing treatment. No statistically significant sequence, crossover or period effect was observed, but treatment with both irrigants was associated with a significant percentage increase in LPS after 5 min. of treatment. A decrease in circulating IL-6 levels from baseline was observed at 5 min., and a significant percentage increase after 120 min.

Table 1. Demographic and clinical characteristics of the participants

	Median (interquartile range)
Age (years)	39 (35–44)
Gender (male/female)	9/9
Ethnicity (Afro-Caribbean/Asian/Caucasian)	5/3/10
Number of teeth	28 (27–31)
Sites with plaque (%)	37 (23–60)
Sites bleeding on probing (%)	47 (27–60)
Mean probing depth (mm)	3.66 (3.12–4.07)
Sites probing >4 mm (%)	28 (22–35)

Table 2. Median (interquartile range) of circulating concentrations of IL-6 (pg/ml), and LPS (EU/ml) before (0 min.), during (5 min.) and 120 min. following treatment with CHX or control irrigant

	Irrigant	Time (min.)		
		0	5	120
IL-6	CHX	0.81 (0.62–1.15)	0.58^{**} (0.46–0.99)	1.85^{***} (1.17–2.45)
	Water	0.85 (0.62–1.24)	0.75^{*} (0.60–1.12)	1.73^{***} (1.15–3.51)
LPS	CHX	0.046 (0.022–0.276)	0.079^{**} (0.031–0.151)	–
	Water	0.034 (0.020–0.069)	0.081^{*} (0.016–0.176)	–

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.
CHX, chlorhexidine; IL-6, interleukin-6; LPS, lipopolysaccharide.

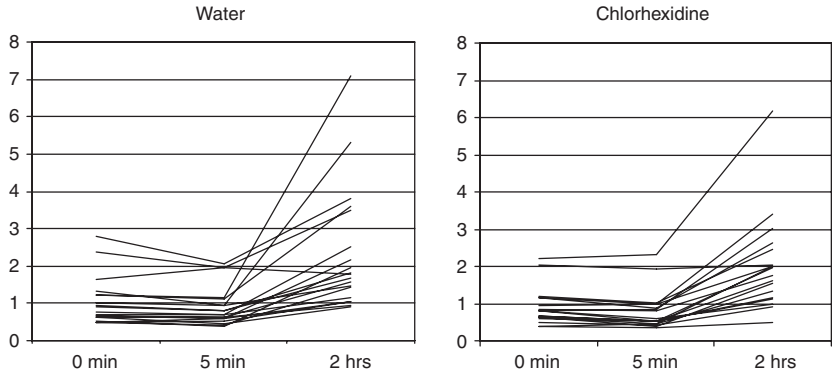

Fig. 1. Changes in IL-6 levels at baseline, 5 and 120 min. after scaling with water irrigation and chlorhexidine (CHX) irrigation.

Figure 1 shows the changes in IL-6 circulating levels for all the participants with both water and CHX irrigation.

Discussion

The results from the study suggest that ultrasonic instrumentation in patients with moderate to advanced periodontitis increases circulating levels of LPS after 5 min. and IL-6 concentrations 120 min. after commencement of treatment, and that these effects are not affected by using 0.02% CHX as an irrigant instead of water.

The median concentrations of LPS at baseline and 5 min. were relatively low compared with the mean concentrations in previous studies. Geerts et al. (2002) detected a mean LPS level of 1.33 EU/ml at baseline and 2.4 EU/ml after 5–10 min. of chewing, while Ide et al. (2004) reported mean concentrations of 0.024 EU/ml at baseline and 0.27 EU/ml after 15 min. of scaling. However, LAL assays for detecting LPS concentrations are notoriously variable from study to

study and between laboratories. In addition, data are frequently skewed and means can be misleading when compared with medians. The concentrations of IL-6 were also lower than reported in previous studies where baseline levels of 1–6 pg/ml have been reported (D'Aiuto et al. 2004, Ide et al. 2004, Forner et al. 2006). However, it was apparent that the severity of disease was higher in two of these studies. D'Aiuto et al. (2004) reported severe periodontitis with higher bleeding on probing (65% of sites) and plaque (49% of sites), while Ide et al. (2004) described more extensive disease with >20% of sites having probing depths over 7 mm, and greater plaque (70% of sites) and bleeding (76% of sites). Few studies have investigated the short-term effect of treatment on circulating IL-6 levels, and these generally showed an increase in plasma IL-6 levels of a greater magnitude than this study detected. This may be due to differences in the detection levels of the assay or different levels of disease present (Mengel et al. 2002). The present study had just over 11% of subjects with probing depths over 7 mm. Mengel et al. (2002) have suggested that IL-6 levels are correlated with disease severity, although Takahashi et al. (1994) found similar levels in healthy and periodontally involved patients.

IL-6 has a major role in the mediation of inflammatory and immune responses and may be initiated by infection, injury or other stresses (Ershler & Keller 2000). The elevation of IL-6 after treatment occurred in both groups and may be explained by a combination of these mechanisms. Infective material may have been released into the circulation and trauma may have occurred as a direct result of instrumenting the periodontal pockets. Either or both may have stimulated an increase in cytokine response and the present results do not provide any indication of the proportional contribution to elevated IL-6 concentrations from bacteraemia or injury associated with debridement.

In this study, a statistically significant increase in both LPS and IL-6 occurred in both groups but no differences were seen between the two groups. This would suggest that 0.02% CHX had no influence on LPS or IL-6 levels when used as an irrigant. The lack of effect on LPS in this study may have been due to the low concentration of CHX solution used compared with widely used products in Europe and the USA. However, recent studies have observed that CHX does not inactivate LPS in experimental animal studies (Tanomaru et al. 2003, Silva et al. 2004). It is possible that the use of a universal rather than slimline scaler tips (which may penetrate subgingivally with more ease) may have affected the results obtained. However, we cannot be sure that this would be the case, and have followed the protocol used in previous studies, in order to allow comparisons with previous data. Even so, there is the possibility that trauma from instrumentation may have affected the results obtained.

In conclusion, this study indicates that ultrasonic instrumentation in patients with moderate to advanced periodontitis increases circulating levels of LPS after 5 min. and IL-6 120 min. after commencement of treatment, and is not affected by using 0.02% CHX as an irrigant instead of water.

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Clinical Relevance

Scientific rationale for the study: To determine whether the use of CHX solution as an ultrasonic irrigant influences the level of circulating endotoxin and host response associated with periodontal treatment.

Principal findings: Elevations in circulating LPS and IL-6 concentration observed after treatment were not influenced by choice of water or CHX solution as an irrigant.

Practical implications: Irrigation with 0.02% CHX may not be effective as a means to reduce the levels of circulating endotoxin, and possibly bacteraemia, or systemic inflammatory response associated with ultrasonic subgingival debridement.