Local and Systemic Inflammatory Responses in Gingivitis Subjects: Clinical Trial of Topical Triclosan

Sheppard A. McKenzie IV

A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Science in the Department of Periodontology.

Chapel Hill
2008

Approved by:
David W. Paquette, DMD, MPH, DMSc

Salvador Nares, DDS, PhD

James Beck, PhD, MS

ABSTRACT

Sheppard A. McKenzie IV: Local and Systemic Inflammatory Responses in Gingivitis Subjects: Clinical Trial of Topical Triclosan

(Under direction of David Paquette, DMD,MPH, DMSc; Salvador Nares, DDS, PhD; James Beck, PhD, MS)

The objectives of this randomized clinical trial (RCT) were to evaluate the local pharmacodynamic effects of triclosan dentifrice on GCF concentrations of inflammatory mediators in subjects with moderate plaque-induced gingivitis, and to measure systemic inflammatory mediators and document trends in levels. Gingivitis subjects were randomized for: 1)triclosan dentifrice; 2)placebo bid over four weeks. GCF analyses of inflammatory mediators were performed on days 1, 8, 15, 22 and 29 (pre-dosing and post-dosing). Serum analyses of inflammatory mediators were performed each visit. IL-1β levels were significantly suppressed in the triclosan group short-term. The data indicate consistent short term reductions in local inflammatory mediators when using the triclosan dentifrice (in particular IL-1β and Rank-L ($p<0.05$). There were no differences in levels of serum inflammatory mediators over the course of 29 days. Nevertheless, the levels of CRP in this population were elevated to the range that confers moderate risk for future cardiovascular disease.

ACKNOWLEDGEMENTS

I would like to thank the following individuals and institutions for support in conducting this research project and completing this thesis:

David Paquette
Truphenia Kelley
Bryan Proctor
Katherine Tripp
Peggy Davis
Kevin Moss
David Barrow
Katherine McKenzie

General and Oral Health Center, UNC at Chapel Hill
The Colgate-Palmolive Company

TABLE OF CONTENTS

Page

LIST OF TABLES……..……...……...…..vi

LIST OF FIGURES…….……..…….……...vii

LIST OF ABBREVATIONS……..……...……...viii

Chapter

I. LOCAL INFLAMMATORY RESPONSE TO TOPICAL APPLICATION OF TRICLOSAN…….……...……...1

Abstract…….……...……..1

Introduction…..…….……...……..2

Material and Methods……..……...…….……..…3

Results…….……..…..7

Discussion…….…….....9

Conclusions…….…….……15

II. ASSESMENT OF SERUM INFLAMMATORY MEDIATORS IN GINGIVITIS POPULATION…….……..……...16

Abstract……..…….……..…16

Introduction……..17

Materials and Methods…….…….……..…….....18

Results……..…….....22

Discussion……..……..23

Conclusions……..……27

III. REFERENCES……..…….…..…39

LIST OF TABLES

Table 1. Demographics……..28

Table 2. Mean Clinical Measures by Day and Group (ANCOVA).....28

Table 3. GCF Mean and Median Values Day 1…….……29

Table 4. GCF Mean and Median Values Days 8,15, 22, 29……..30

Table 5. GCF Mean and Median Values Days 8,15, 22, 29……..31

Table6. Mean and Median Serum Marker Values by Day and Group……..32

Table 7. Mean Serum Markers by Day and Group (ANCOVA)……...……33

LIST OF FIGURES

Figure 1. Mean Percent Change in GCF IL-1β from Pre-dosing Day 1……....34

Figure 2. Mean Percent Change in GCF IL--1β from Pre-dosing Days 8-29……..….34

Figure 3. Mean Percent Change in GCF IL-6 from Pre-dosing Day 1……..…..…….35

Figure 4. Mean Percent Change in GCF IL-6 from Pre-dosing Days 8-29…….....35

Figure 5. Mean Percent Change in GCF RANK-L from Pre-dosing Day 1…….…..…36

Figure 6. Mean Percent Change in GCF RANK-L from Pre-dosing Days 8-29……..….36

Figure 7. Mean Percent Change in GCF PGE_2 from Pre-dosing Day 1…….…...37

Figure 8. Mean Percent Change in GCF PGE_2 from Pre-dosing Days 8-29……...…..37

Figure 9. Mean Percent Change in GCF 8-iso-$PGF_2\alpha$ from Pre-dosing Day 1……..…...38

Figure 10. Mean Percent Change in GCF 8-iso-$PGF_2\alpha$ from Pre-dosing Days 8-29…...38

LIST OF ABBREVIATIONS & SYMBOLS

8-iso-PGF2α	Total 8-iso-prostaglandin F2 α
μg	Microgram
μg	Microliter
μM	Micromolar
°C	Degrees Celsius
AE	Adverse event
ANCOVA	Analysis of covariance
APR	Allergic patch reaction
AUC	Area under the curve
Bid	Twice daily
BMI	Body mass index
BOP	Bleeding on probing
C	Concentration
CAL	Clinical attachment level
CRP	C-reactive protein
ELISA	Enzyme linked immunosorbant assay
FDA	Food and Drug Administration
G	Gram
GCF	Gingival crevicular fluid
GI	Gingival Index
GM	Gingival margin
IC	Inhibitory concentration

IL	Interleukin
IRB	Institutional Review Board
kg	Kilogram
L	Liter
M	Meter
mg	Milligram
ml	Milliliter
mm	Millimeter
OD	Optical density
oz	ounces
ng	nanogram
PD	Pocket depth
PGE_2	Prostaglandin E_2
PI	Plaque Index
PPM	Parts per million
RANK-L	Receptor activator of nuclear factor kappa B ligand
RPM	Revolutions per minute
TNF-α	Tumor necrosis factor α
UNC-15	University of North Carolina periodontal probe

LOCAL INFLAMMATORY RESPONSE TO TOPICAL APPLICATION OF TRICLOSAN

ABSTRACT

Triclosan is a broad spectrum antimicrobial agent that has been shown to reduce dental plaque, gingivitis and calculus. The primary objective of this study was to evaluate the local pharmacodynamic effects of 0.3% Triclosan/copolymer dentifrice on gingival crevicular fluid (GCF) concentrations of inflammatory mediators in subjects with moderate plaque-induced gingivitis. **Methods:** This single center, double blinded, randomized, controlled trial had two arms: 1) gingivitis subjects using 0.3% Triclosan/copolymer/fluoride dentifrice (bid); 2) gingivitis subjects using placebo dentifrice (bid). GCF samples were collected on days 1 (pre-dosing, 1, 2, 3 and 6 hours post-dosing), 8, 15, 22 and 29 (pre-dosing and 2 hours post-dosing). Periodontal probing exams were performed at baseline (prior to randomization) and day 29. GCF samples were analyzed for specific inflammatory biomarkers (PGE_2, IL-1β, IL-6, 8-iso-$PGF_2\alpha$ and RANK-L) using multiplex immunoassay and enzyme-linked immunosorbant assay (ELISA) techniques. **Results:** A statistically significant suppression of IL-1β at 2 hours post-dose on day 1 (p=.0042) was observed in the triclosan group. In addition, analysis showed significant suppression of 8-iso-$PGF_2\alpha$ in the triclosan group at day 29 at 2-hours post-dose (p=.008). Further analysis showed a reduction of IL-1β within the triclosan group at 1 hour post-dose (p=0.012). In general, there were consistent elevations of inflammatory mediators in the placebo group versus the triclosan group. **Conclusions:**

Data indicate that there is short-term *in vivo* suppression of inflammatory mediators associated with periodontal disease after topical application of triclosan in gingivitis subjects.

Introduction

The current model of the pathogenesis of periodontal diseases, including gingivitis and periodontitis, places emphasis on the bacterial biofilm as the primary etiology; however, it is the host inflammatory response, which plays a central role in the severity and progression of disease.[1] Accordingly, patients with periodontal disease exhibit local elevations in inflammatory mediators such as prostaglandin E_2 ($PGE_{2)}$ and interleukin-1β (IL-1β) in periodontal tissues and gingival crevicular fluid (GCF).[2-4] Cohort studies and clinical trials indicate that controlling inflammation remains a principal strategy for preventing initiation or progression of periodontal diseases.[5-9] Similarly, cumulative evidence from humans and animal models demonstrates that host modulatory agents can inhibit local inflammatory mediator expression and improve periodontal status independent of any plaque biofilm effects.[10-12]

The majority of preventive and therapeutic approaches for periodontal disease focus on reducing or altering biofilm. Triclosan (2,4, 4′, trichloro-2′-hroxydiphenyl ether) is a broad spectrum antimicrobial, which has been used for decades in consumer products. It is currently the active agent in a dentifrice (Total™; Colgate-Palmolive; Piscataway, NJ) approved for the reduction of dental plaque, gingivitis, calculus and dental caries in human patients.[13-15] Clinical trials have shown, when coupled with a copolymer delivery system (polyvinylmethylether maleic acid), that triclosan is highly

substantive and provides effective concentration in the oral cavity for 12 hours following tooth brushing.[16]

In addition to the substantiated antimicrobial effects of triclosan, recent *in vitro* investigations demonstrate that triclosan also has host modulatory or anti-inflammatory effects. Triclosan significantly blocks prostaglandin and IL-1 production in human gingival fibroblasts challenged with cytokines.[17, 18] Laboratory data also demonstrate that the agent inhibits several host enzymes in the arachidonic acid cascade including cyclooxygenase-1 and 2, 5-lipoxygenase and 15-lipoxygenase.[19, 20] Triclosan has a favorable partial coefficient that allows the agent to readily penetrate epithelial barriers like gingiva and skin.[20] Transmission electron microscopy using gingival fibroblasts and C-labeled-triclosan shows that the agent crosses cell membranes and localizes in the nuclei for potential alteration in intracellular signaling pathways related to inflammation.[21] In addition, triclosan significantly decreases *in vivo* histamine-induced skin inflammation in humans and the allergic patch reaction (APR) to 1% nickel sulfate in sensitized subjects.[22]

Despite the proven antiplaque and antigingivitis effects of triclosan in human trials, no clinical study to date has investigated the direct anti-inflammatory effects of triclosan on local periodontal inflammatory mediators in human subjects. The primary aim of this randomized, controlled clinical trial was to measure and document the *in vivo* local anti-inflammatory effects of topical triclosan in humans with gingivitis.

Materials and Methods

Patient Population and Study Design

This was a double-blind, randomized, single-center clinical trial conducted at the University of North Carolina School of Dentistry. The protocol was approved by the Biomedical Institutional Review Board (IRB), and informed consent was obtained from each subject at screening. Clinical measurements were taken by two examiners who were assessed for intra- and inter-examiner reliability prior to study initiation; inter- and intra-examiner reliability scores were greater than 95% for probing depths within 1mm.

Forty-eight subjects meeting inclusion criteria were recruited for this study. Subjects were adult males or females, 18 to 60 years old. Subjects presented with generalized, moderate to severe plaque-associated gingivitis, which was defined as having at least four teeth with 4-5mm probing depth and 30% of sites bleeding to gentle probing. Subjects presented with at least 20 teeth in the functional dentition excluding third molars. Subjects denied having chronically used triclosan dentifrice within 6 months prior to enrollment. Participants also denied having been treated (i.e., two weeks or more) with any medication known to affect inflammation or periodontal status (e.g., aspirin, nonsteroidal anti-inflammatory drugs, steroids, statins, phenytoin, calcium antagonists, cyclosporin and coumadin) within one month of the screening examination. All other medications for chronic medical conditions were initiated at least three months prior to enrollment. In addition, subjects did not have gross oral pathology, widespread untreated caries, periodontitis (periodontal pocketing $\geq$ 6 mm at screening), or a history of necrotizing periodontal diseases. Subjects did not currently smoke or report using tobacco products within one year of screening. Finally, no subjects had orthodontic appliances or removable partial dentures.

The study design was parallel (two arms) and featured a 29-day treatment period.

Investigational Treatments, Procedures, and Parameters

On day 1 of treatment (up to 21 days following screening), vital signs were taken, concomitant medication changes were recorded, and an oral soft tissue examination performed. The dental examiner clinically measured the Plaque Index (PI), Gingival Index (GI), percent bleeding on probing (BOP), probing depth (PD) and clinical attachment level (CAL) for all existing teeth (excluding third molars) at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, distolingual).[23, 24]

The dental examiner collected 16 GCF samples for inflammatory mediatory analyses (Day 1, predosing) from the following sites in four quadrants: mesiobuccal of second molars, distobuccal of first molars, mesiobuccal of second premolars and distobuccal of first premolars. If one of the listed teeth was missing, the rule was to sample from the next anterior (mesial) tooth. Samples were collected onto filter paper strips (Pro Flow, Inc., Amityville, NY), and the volume was determined using a Periotron 8000® (Harco Electronics Limited, Winnipeg, MB, Canada). Samples were wrapped in aluminum foil and sealed in cryovials that were labeled and bar-coded with unique identifiers for study, subject and specimen identifier. Cryovials were immediately placed into liquid nitrogen chair-side and stored at -180°C.

Subjects were stratified by gender and randomized into one of two treatment arms:

Placebo – Control dentifrice containing 0.243% (1100 PPM) sodium fluoride used twice daily over 29 days.

Experimental - Commercially available dentifrice containing 0.3% triclosan, 2% copolymer and 0.243% (1100 PPM) sodium fluoride used twice daily over 29 days.

Study dentifrices were packaged in masked, encoded 221g tubes, and one randomized tube was given to each subject. The patient was instructed to administer the randomized dentifrice twice daily via the modified Bass brushing technique for three minutes.[25] In addition, a subject diary was given to the patient to record adverse events or changes in medication.

A three-minute supervised administration of study dentifrice was performed at baseline, and GCF sample collection was repeated at 1, 2, 3, and 6-hours post-dosing.

On days 8, 15, 22, 29 subjects returned to the research clinic. Vital signs and changes in medications were recorded. Oral soft tissue examinations were preformed as well. The dental examiner again collected GCF samples for inflammatory mediator analyses (pre-dosing), and the study dentifrice was subject-applied for three-minutes. GCF samples were re-collected at two-hours post-dosing.

Additionally on Day 29, the examiner re-measured clinical indices. Study diaries were collected, and the returned unused dentifrice was weighed.

Laboratory Analyses

GCF samples were analyzed for specific inflammatory biomarkers using multiplex immunoassay and enzyme-linked immunosorbent assay (ELISA) methods previously described.[2, 3, 26, 27] Four samples were analyzed for each visit, representing each quadrant, and the remaining twelve samples were archived. Biomarkers included PGE_2, IL-1β, IL-6, 8-iso-prostaglandin$F_2\alpha$ (8-iso-$PGF_2\alpha$), and receptor activator of nuclear factor kappa B ligand (RANK-L). The multiplex immunoassay allowed simultaneous measurements of IL-1β and IL-6 in each GCF sample. The remaining mediators were analyzed with standard ELISA techniques. Crevicular fluid

concentrations of PGE_2, IL-1β, TNF-α, IL-6, RANK-L total are reported in pg/ml, and 8-iso-$PGF_2\alpha$ is reported in mg/l.

Statistical Plan

The *a priori* analytical plan for this trial followed the intent-to-treat principle such that all randomized subjects were included in the primary pharmacodynamic comparisons. Patient demographics, clinical parameters and baseline (day 1, pre-dosing) mediator levels were compared overall between the groups using t-tests for continuous variables and chi-square, Fisher's exact tests, or analysis of covariance (ANCOVA) for categorical variables. Treatment comparisons for percent inhibition or change from pre-dosing in GCF were based on analyses using the logarithmic base ten scale to normalize the right-tailed skewed distribution of original mediator values. An ANCOVA was used for inter-group comparisons of inflammatory markers for baseline pre-dosing only. Paired t-tests were used to examine intra-group differences for each inflammatory mediatory pre-dosing and post-dosing for each visit. Finally, unpaired t-tests were used to evaluate inter-group comparisons for changes in GCF concentration at each study visit.

Results

Table 1 summarizes the demographics for all subjects enrolled in the trial. There were 48 recruited subjects. The mean age was 39.9 years old for the placebo group and 34.0 years for the experimental group. The gender of the subjects was predominantly female with 70.1% in placebo group and 66.6% in the experimental group. There were 18 African Americans, 1 Hispanic, and 6 Caucasian subjects in placebo group and 9 African American, 1 Hispanic, and 14 Caucasian subjects in the experimental group. The Body Mass Index (BMI) was 26±5.7 kg/m^2 for placebo group and 25.2±7.0 kg/m^2. There

were no significant differences for any demographic criteria between the two groups using chi-square tests and t-tests; however, race was trending toward significance (p=.09).

All patients tolerated study dentifrices, and no severe adverse events (AEs) were reported. Seventeen non-severe AEs were reported over the course of the trial (nine with the placebo dentifrice and eight with the triclosan dentifrice). All AEs were categorized as Grade I according to the CTCAE v3.0 scale.[28] Overall, AEs were judged as "unlikely related" or "not related" to study treatments. The most frequently reported AEs were classified as "headache" or "cold-like symptoms" (eight with the placebo dentifrice and six with the triclosan dentifrice). One placebo-treated subject reported "tooth sensitivity" over the treatment period, and two triclosan-treated subjects reported "oral/gum soreness" or a "cold sore". All AEs resolved by the conclusion of the trial.

Review of the study diaries and tube weights determined that subjects consistently displayed good compliance with bid dosing of dentifrice (data not shown).

Baseline and post-treatment (day 29) mean and extent clinical measures are reported in Table 2. The baseline mean PD for placebo group and triclosan group were 2.52mm and 2.54 respectively. The baseline mean GI was 1.05 and 1.10 for placebo group and triclosan group respectively. The baseline mean PI was .96 for placebo group and .99 for the experimental group. There were no significant differences in the clinical measurements when using an ANCOVA analysis.

The mean and median inflammatory mediator values for baseline are reported in Table 3 and for the subsequent days in Table 4 and Table 5. Unpaired t-tests were conducted to examine differences between groups for change in GCF concentration 2-

hours post-dosing for days 1, 8, 15, 22, and 29; they are documented in Figures 1-10. On day one, 2 hours post-dosing, IL-1β was significantly depressed in the triclosan group (p=.0042) compared to control. In addition, 8-iso-$PGF_2\alpha$ was statistically lower in the test group when percent change in GCF concentration 2-hours post dosing was compared to the control at day 29 (p=.008). In general, there was consistently greater mean percent reduction post-dosing in the triclosan group versus placebo although this did not always reach statistical significance.

Paired t-tests on the log transformed inflammatory mediators were used to examine within group differences pre-dosing to post-dosing in placebo group and triclosan group. Placebo group had exhibited significant increases for RANK-L between pre-dosing at Day 15 (p=.03) and Day 29 (p=.04) at 2 hour post-dosing. The placebo group also had statistically significant increases in levels of PGE_2 at Day 2 (p=.01) between pre-dosing and post-dosing. The placebo group also showed an increase in IL-6 between pre-dosing Day 1 (p=.006) and Day 29 (p=.03) and post-dosing. There was a decrease in the placebo group for IL-1β on Day 2 (p=.005) between pre-dosing and post-dosing. The experimental group exhibited an increase for IL-6 (p= .02) on Day 1 between pre-dosing and post-dosing. In contrast, the experimental group did have a statistically significant decrease in IL-β on day 1 between pre-dosing and 1 hour-post-dosing. On average the inflammatory mediators in the placebo group were elevated compared to the triclosan group, and this elevation in the placebo group reached statistical significance multiple times.

Discussion

The inflammatory markers explored in this study have been implicated in the local host inflammatory response associated with periodontal disease progression/pathogenesis. Local elevations of these markers signify a local inflammatory response, and reduction of these local inflammatory markers has been associated with reduction of disease.

IL-1β, a cytokine that possesses pro-inflammatory properties is induced by microbial challenge, inflammatory agents, and antigens. [29]IL-1β is mainly produced by monocytes/macrophages, but is also produced by bone cells and fibroblasts.[30, 31] IL-1β stimulates T-lymphocytes and lymphokines, increases production of B-lymphocytes, prostaglandin release, promotes release of matrix metalloproteinases, and promotes osteoclast formation and bone resorption.[29] IL-1β can also affect neutrophil chemotaxis and activation.[32] Levels of IL-1β have correlated closely with levels of periodontal disease severity, and increased levels of IL-1β may serve as a marker for periodontal destruction.[33, 34] Recently, Zhong and coworkers demonstrated that elevated levels of GCF IL-1β were associated with increased PD and BOP.[35] An IL-1 gene cluster has been identified that is associated with periodontal disease severity.[36]

Similarly, IL-6 is a cytokine that recruits macrophages and activates the acquired immune response. In general, it is released in response to IL-1 or other cytokines as opposed to a microbial challenge; therefore, it is considered to represent a second wave of inflammatory mediators. IL-6 can cause tissue destruction by enhancing expression of matrix metalloproteinases.[37] Offenbacher and coworkers recently showed that patients with gingivitis had increased levels of GCF IL-6 compared to healthy controls.[26] In

addition, the same group showed that periodontal therapy resulted in a decrease in serum IL-6 levels in pregnant subjects with periodontitis.[38]

RANK-L, a member of the Tumor Necrosis Factor (TNF) family, is considered a marker of bone resorption due to its effects on the upregulation of osteoclasts. Recent clinical investigations have shown an increase in GCF RANK-L in periodontitis patients versus healthy patients.[39, 40]

PGE_2 is a lipid end-product of the arachidonic acid cascade, which is stimulated by microbial challenge and is central to the destructive host response in periodontal disease.[41] PGE_2 has been shown to be a potent stimulator of bone resorption, and prostanglandin levels are elevated in gingival tissues and crevicular fluid from periodontitis patients relative to healthy controls.[42] Local concentrations of PGE_2 have also been shown to be a sensitive marker of attachment loss.[43] Recently, elevations in PGE_2 were positively related to increased PD and increased BOP.[35]

8-iso-$PGF_2\alpha$ is a prostaglandin-like compound which is generated at the site of a free radical attack of the arachidonate component of cell membranes; it can serve as a marker for oxidative stress, lipid peroxidation, and low levels of inflammation. Recently, PD greater than or equal to 4mm with third molars was associated with increased serum levels of 8-iso-$PGF_2\alpha$ cross-sectionally.[44]

Triclosan has shown efficacy against oral bacteria *in vitro*.[45] In addition, clinical trials have consistently supported the antiplaque and antigingivitis activity of triclosan. Lindhe and coworkers showed a reduction in plaque and gingivitis in subjects over a 6-month trial when using a triclosan dentifrice.[46] Lindhe and coworkers have remarked that triclosan appears to reduce gingivitis beyond that anticipated by plaque reduction alone.[47]

For instance, in one trial the investigators observed less clinical inflammation for the same amount of plaque in the triclosan-treated group versus gingivitis subjects receiving the control dentifrice. Another clinical trial by Rosling and coworkers showed that patients who with destructive periodontitis showed less frequency of deep pockets as well as a reduced number of sites with additional probing attachment when using a triclosan dentifrice versus placebo.[48] These clinical trials suggest that a triclosan dentifrice may reduce inflammation independent of the biofilm effects.

To explore a reduction in inflammation not concomitant with plaque reduction, Modeer and coworkers explored the effect of triclosan on prostaglandin biosynthesis. Modeer challenged gingival fibroblasts with IL-1β and a simultaneously applied triclosan. The stimulatory effect of IL-1β on PGE_2 formation was reduced in a dose-dependent manner. In addition, triclosan reduced the PGE_2 production from gingival fibroblasts stimulated by TNFα.[17] Similarly, Mustafa and coworkers challenged gingival fibroblasts with inflammatory cytokines.[18] The gingival fibroblasts were treated simultaneously with triclosan and TNFα; the stimulation of TNFα on IL-1β production was reduced by triclosan. *In situ* hybridization showed that the TNFα induced expression of IL-1β mRNA was significantly reduced by triclosan; however, the triclosan did not reduce mRNA expression or production of the IL-6 challenged by TNFα. The *in vitro* data from Modeer and Mustafa suggest that triclosan can inhibit the production of inflammatory cytokines produced by gingival fibroblasts.

Gaffar and coworkers have also explored the anti-inflammatory properties of triclosan *in vitro*.[19] Their experiments focused on the enzymes which are important for arachidonic acid metabolism: cyclo-oxygenase 1 (Cox-1), cyclo-oxygenase 2 (Cox-2),

5-lipoxygenase, and 15-lipoxygenase. Their laboratory data demonstrate that the triclosan inhibits the following enzymes: Cox-1 and 2 (IC-50=43 and 227 μM respectively), 5-lipoxygenase (IC-50=43 μM) and 15-lipoxygenase (IC-50=61 μM). These *in vitro* data suggest that relatively low concentrations of triclosan may be able to reduce inflammatory mediators from the arachidonic acid pathway.

ANCOVA for GCF inflammatory mediators in the placebo versus triclosan group at baseline (pre-dosing, day one) showed no statistical significance differences. This analysis indicates comparability between the levels of local inflammatory mediators prior to the intervention with either the triclosan or placebo dentifrice.

The inter-group comparison, analyzed with unpaired t-tests, revealed that the percent change of IL-1β levels between pre-dosing and 2-hours post-dosing was significantly reduced in the triclosan group versus the control group on day one. In addition, the percent change of 8-iso-$PGF_2\alpha$ between pre-dosing and 2-hours post-dosing were statistically significantly reduced in the triclosan group versus the control group on day 29. This data indicates that there was a significant reduction of two of the inflammatory markers analyzed two hour post-dosing when comparing the test dentifrice with the control dentifrice. When comparing the change in local inflammatory mediators (2 hours post-dose) between the two groups, there was an overall tendency for there to be an elevation in the control group versus the triclosan group.

A paired t-test analysis was performed to see if either of the dentifrices caused suppression of the inflammatory markers between pre-dosing and post-dosing. Interestingly, the inflammatory markers were elevated in the control group for three inflammatory markers (RANKL, PGE_2, and IL-6) over 5 different time points when

comparing pre-dosing levels to 2 hours post-dosing levels. In the test group there was an elevation of only one inflammatory marker (8-iso-$PGF_2\alpha$) at one time point when comparing pre-dosing levels to 2 hour post-dosing levels. Application of the test and control dentifrices was carried out through a toothbrush and the modified Bass technique. It is possible that the process of topical application causes local trauma or bacteremia resulting in local increases of inflammatory markers. The data for our clinical trial indicate that were more elevations in local inflammatory mediators (RANKL, PGE_2, and IL-6) in the control group versus the triclosan group.

The paired t-test analysis also showed that there was a statistically significant reduction in IL-1β levels in the test group on Day 1 between pre-dose and 1-hour post-dose. Although there were reductions of IL-1β levels at each additional visit (Days 8, 15, 22, 29) between pre-dosing and 2-hours post-dosing, these results did not reach statistical significance. One hour post-dosing measurements were only taken on Day 1. It is possible that topical nature of triclosan application only provides adequate anti-inflammatory effects for a short period of time. During these same visits that IL-1β levels in the triclosan group were trending toward a decrease after topical application of triclosan, 60% of visits (Days 1, 8, 22) in the control group showed a trend towards elevation of the IL-1B levels. Consistently, there was a reduction of local inflammatory mediators (especially IL-1β) after dosing with triclosan dentifrice compared to control.

These clinical data appear to be in agreement with Mustafa and coworkers *in vitro* work, which showed that challenged gingival fibroblasts treated with triclosan reduced IL-1β production.[21] These clinical data suggest there was a reduction of IL-1β, which was significant for a within group comparison at 1 hour post-dosing on day 1. These data

also show that there was a significant difference between the test group and the control group at 2 hours post-dosing on day 1. These data seem to support the *in vitro* work described previously.

The local inflammatory mediators studied in this clinical trial have been implicated in the progression of destructive periodontal diseases. Due to the fact that the progression of periodontal disease represents a chronic inflammatory response, reduction of inflammatory mediators remains a treatment goal. Triclosan is well-studied component of a dentifrice which has been on the market for years with a proven safety profile. The data from this clinical trial indicate statistically significant reductions for IL-1β after dosing with triclosan compared to control. In addition, there were consistent tendencies for greater elevations of the local inflammatory mediators (RANKL, PGE_2, and IL-6) in the control group versus triclosan group. It is important to note that these local mediator changes were measured without any significant changes in clinical indices, such as PI, GI, or extend PD scores. Our clinical data suggest support for Mustafa's *in vitro* work showing reduced production of IL-1β with topical application of triclosan.

Conclusion

The data from this RCT suggest short term local inhibition of inflammatory mediators, such as IL-1β, following topical application of triclosan dentifrice in gingivitis subjects. These changes occurred independent of any measurable changes in clinical plaque or gingivitis scores over a 29-day dosing period; therefore, these changes appear to be secondary to direct pharmacodynamic affects of triclosan on host expression of these inflammatory mediators locally in GCF in gingivitis subjects.

ASSESMENT OF SERUM INFLAMMATORY MEDIATORS IN GINGIVITIS POPULATION

ABSTRACT

Patients with periodontal disease have local and systemic elevations of inflammatory mediators. An objective of this investigation was to explore systemic inflammatory levels in gingivitis subjects over the confines of a 4-week clinical trial. **Methods:** This was a single center, double blinded, randomized, controlled trial with two arms: 1) gingivitis subjects using 0.3% Triclosan/copolymer/fluoride dentifrice (bid); 2) gingivitis subjects using placebo dentifrice (bid). Serum analyses of inflammatory mediators were performed (pre-dosing) at days 1, 8, 15, 22 and 29. Periodontal probing exams were performed at baseline (prior to randomization) and day 29. Serum concentrations of CRP, IL-6 and 8-iso-$PGF_2\alpha$ were measured using ELISA techniques. **Results:** While no inter-group differences in serum biomarkers were detected, data from this clinical trial show that levels of CRP were elevated in gingivitis subjects overall. CRP mean values ranged from 1.91±1.57(mg/l) to 2.26±1.70(mg/l). These levels are similar to those reported for subjects with end-stage, destructive periodontitis; furthermore, the levels in these subjects may confer a moderate risk for future CVD. Elevated levels of IL-6 and 8-iso-$PGF_2\alpha$ were also in the range that has been reported in patients with periodontitis. **Conclusions:** The serum levels of inflammatory mediators were elevated in this trial population of gingivitis subjects. Gingivitis may represent a

critical infection and inflammatory burden that may stimulate systemic inflammatory responses.

Introduction

Despite the widespread acceptance that the primary etiology of periodontal disease is a bacterial biofilm, it is often the host inflammatory response that determines the course of the disease process. A bacterial challenge in one patient may result in a limited inflammatory response, while another patient may respond with an abnormally high inflammatory response.[1] Polymorphonuclear neutrophils (PMN), lymphocytes, macrophages, and plasma cells lead the local inflammatory response to etiologic gram-negative bacteria and their products. Immune cells, as well as fibroblasts, osteoblasts, and other cells located in the periodontium, are capable of releasing abnormally high amounts of inflammatory mediators in response to the pathogenic biofilm around teeth. This robust response may be reflected with local elevations in inflammatory mediators such as PGE_2 and IL-1β in periodontal tissues and GCF.[2-4]

Patients with periodontal disease not only exhibit increases in local inflammatory mediators, but also have increased serum levels of C-reactive Protein (CRP), IL-1, IL-6, and other inflammatory markers when compared to healthy controls.[49-51] In addition, increased levels of serum CRP are associated with elevated levels of infection with periodontal pathogens.[50] Inflammatory processes have been implemented in many pathologic processes in the human body, such as, atherosclerosis, diabetic control, and rheumatoid arthritis.[52] An elevation of inflammatory mediators triggered by periodontal diseases and concomitant bacteremias and endotoxemias could plausibly exacerbate

pathologic inflammatory processes elsewhere in the body.[53] Systemic inflammation from periodontal infection has been linked to increased odds of cardiovascular events, delivery of pre-term low birth weight babies, and poor diabetic control.[53-55] Thus, reducing the systemic inflammatory burden caused by periodontal infection may be an important therapeutic or preventive goal for maintaining systemic health. Interventions aimed at reducing the local periodontal infection may also suppress serum inflammatory responses.[56, 57]

The majority of the clinical studies conducted to date document serum inflammatory mediators in periodontitis subjects. Currently, there is little evidence on these mediators in human subjects with gingivitis. Due to the possible effect that these inflammatory mediators seen locally and systemically may have on other disease processes in the body, it is important to document levels in health and at various levels of periodontal disease. The aim of this investigation is to measure and document the levels of inflammatory mediators in serum in human gingivitis subjects within the confines of a four-week clinical trial of topical triclosan.

Materials and Methods

Patient Population and Study Design

This was a double-blinded, randomized, single-center clinical trial conducted at the University of North Carolina School of Dentistry. The protocol was approved by the Biomedical IRB, and informed consent was obtained from each subject. Clinical measurements were taken by two examiners who were assessed for intra- and inter-examiner reliability prior to study initiation as described previously.

Forty-eight subjects were recruited for this study. Subjects were adult males or

females 18 to 60 years old. Subjects presented with generalized, moderate plaque-associated gingivitis which was determined by having at least 4 teeth with 4-5mm probing depth and 30% of sites bleeding to gentle probing. Subjects presented with at least 20 teeth in the functional dentition excluding third molars. In general, subjects were medically healthy and were not taking any medications known to affect periodontal disease or inflammation.

The study design for this double armed phase IV clinical trial featured a 21-day screening period and a 29-day treatment period.

Investigational Procedures, and Parameters

During screening, subjects gave written informed consent and received oral and periodontal evaluations. Within 21 days of screening examination, subjects initiated the treatment period. On day 1, vital signs were taken, concomitant medication changes were recorded, and an oral soft tissue examination performed. The dental examiner clinically measured the PI, GI, percent BOP, PD and CAL for all existing teeth (excluding third molars) at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, distolingual).[23, 24]

There were two treatment groups in this study. The placebo group used a dentifrice containing 0.243% (1100 PPM) sodium fluoride, while the experimental group used an approved dentifrice containing 0.3% triclosan, 2% copolymer and 0.243% (1100 PPM) sodium fluoride (Total™; Colgate-Palmolive; Piscataway, NJ). The patient was instructed to administer the randomized dentifrice twice daily via the modified Bass brushing technique for three minutes.[25] In addition, a subject diary was given to the patient to record adverse events or changes in medication.

On days 8, 15, 22, and 29 vital signs and changes in medications were recorded. Oral soft tissue examinations were preformed as well. The dental examiner collected whole blood (pre-dosing), and the study dentifrice was applied via a three-minute administration.

Additionally on Day 29, the examiner clinically re-measured probing paramenters, PI, GI, and percent BOP. Study diaries were collected, and returned (unused) dentifrice was weighed.

As detailed in the first half of this thesis, 16 GCF samples were also collected from each subject (pre-dosing and post-dosing) on days 1, 8, 15, 22, and 29 and stored at 180 degrees Celsius. Whole blood (5 ml) was collected from subjects (pre-dosing) for analysis of serum mediators. Following collection, blood samples were allowed to clot for 2 hours at room temperature before centrifuging (20 minutes at approximately 2000 x g). Serum was decanted into a sample vial that was pre-labeled and bar-coded with unique identifiers for study, subject and specimen identifier. Serum vials were stored at -80°C until analysis.

Laboratory Analyses

Serum concentrations of hsCRP, IL-6 and 8-iso-$PGF_2\alpha$ were measured using ELISA techniques.[58] Briefly, 96-well plates (Linbro, ICN/Flow, Costa Mesa, CA) were coated with rabbit antibodies to human CRP, IL-6 and 8-iso-$PGF_2\alpha$ (Calbiochem, La Jolla, CA) at a concentration of 1 μg/ml. Samples were diluted 1:500 and 1:1000 and incubated for three hours and then washed. Biotinylated rabbit antibodies to the same human biomarkers (NHS-LC-Biotin reagent, Pierce, Rockford, IL) were added and incubated for 2 hours. The plates were washed and incubated overnight with strep-avidin-

alkaline phosphatase (Zymed, San Francisco, CA). The ELISA was developed with p-nitrophenylphosphate (Sigma, St. Louis, MO). Each 96-well plate included a full set of duplicate standards and appropriate controls. Standard curves for each assay run were computed and sample concentrations calculated using the OD value for each sample. Standard curves and controls from daily runs were used to generate particular control parameters for quality control evaluation. Serum concentrations of CRP, IL-6 and 8-iso-$PGF_2\alpha$ reported in mg/L, pg/ml, and ng/ml respectively. Similar immunological assays were performed for GCF quantification of local inflammatory mediators (PGE_2, IL-1β, TNF-α, IL-6, RANK-L).

Statistical Plan

All subjects randomized and treated were include in the analyses under the intent-to-treat rule. Patient demographics, clinical parameters and baseline (day 1, pre-dosing) mediator levels were compared overall between the groups using t-tests for continuous variables and chi-square, Fisher's exact tests, or ANCOVA for categorical variables. Treatment comparisons for serum biomarker concentrations were based on analyses using the log ten base scale and calculation of geometric means. An ANCOVA was used to compare different inflammatory mediators in the placebo group and the experimental group at different day time points to look for any significant differences between the two groups. Finally, mean values were calculated for pooled groups for total population (n=48).

Results

Table 1 summarizes the demographics for the 48 subjects enrolled in the trial. There were no statistical differences for any of the demographic data between the two

groups using chi-square and t-tests. The demographics have been detailed above. Briefly the mean age ranged from 34 -40 years old in the two groups. Experimental groups were predominantly female means ranging from 70.1% in the placebo group and 66.6% in the experimental group. The race of the subjects included African Americans, Hispanics, and Caucasians. Notably, there was an observed trend for a greater number of African Americans in the placebo group (p=.09. The Body Mass Index (BMI) was 26±5.7 for the placebo group and 25.2±7.0.

Mean and extent clinical measures are reported in Table 2. The baseline mean PD for placebo group and triclosan group were 2.52mm and 2.54 respectively. The baseline mean GI was 1.05 and 1.10 for groups A and B respectively. Finally, the baseline mean PI was 0.96 for the placebo group and 0.99 for the experimental group. There were no significant differences in the clinical measurements when using an ANCOVA analysis.

The means and medians values for the serum inflammatory mediators measured for all subjects, the placebo group and the experimental group are reported in Table 6. Overall, the mean values for IL-6 ranged from 1.72±.85(pg/ml) to 2.22±1.50(pg/ml) for all subjects over 29 days of the clinical trial. The mean values for 8-iso-$PGF_2\alpha$ ranged from 594.9±531.0(ng/ml) to 838.6±452.4(ng/ml) for all subjects. The CRP mean values range from 1.91±1.57(mg/l) to 2.26±1.70(mg/l) for all participants in the trial over the course of the four weeks. An ANCOVA for the log transformed serum markers by visit and group showed only a statistical significance for baseline levels of 8-iso-$PGF_2\alpha$ between control group and triclosan group; the control group was lower at baseline

(p=.03). However, paired t-tests revealed no significant changes within groups over the course of the four weeks.

Since Il-6 and 8-iso-$PGF_2\alpha$ were measured both systemically and locally, we conducted a Pearson correlation test to asess if there was a correlation between local inflammatory markers measured in the GCF and inflammatory markers in the serum. For the two inflammatory markers that were measured in both the GCF and serum, 8-iso-$PGF_2\alpha$ and IL-6, no statistically significant correlation was detected for 8-iso-$PGF_2\alpha$ and IL-6.

Discussion

The data from this clinical study indicate that serum inflammatory markers may be elevated in gingivitis subject. CRP is an acute phase reactant primarily produced in the liver with pro-inflammatory properties. Accordingly, acute phase reactants can bind and activate complement, neutralize pathogens, and stimulate repair and regeneration.[59] CRP has been classified as a member of the pentraxin family of innate immune response proteins.[60] CRP has been utilized as a marker for cardiovascular disease (CVD) and the marker has been used to assess risk. Recently, a consensus conference with American Heart Association (AHA) and the Centers for Disease Control (CDC) released guidelines for CRP levels based on the available evidence on the role of inflammation in the pathogenesis of atherosclerosis.[61] The consensus reports that subjects with CRP concentrations less than 1mg/l are considered to be at low risk. Patients with concentrations in the 1-3mg/l range are assigned a “medium risk” level and those with more than 3mg/l in serum CRP are considered “high risk” for future cardiovascular disease and events.[61] Due to the fact that there is an association between CVD and

periodontal disease, which is thought to be based on inflammation, several studies have looked at CRP levels in periodontitis patients. A recent meta-analysis showed that the majority of 18 studies demonstrated elevated CRP levels in periodontitis patients verses controls. Indeed, the majority of the reviewed studies showed CRP levels to be >2.1mg/l.[59] These levels observed in medically healthy individuals may indicate an increased risk of CVD.[60]

The mean CRP values in the overall gingivitis population of this clinical trial ranged from 1.91±1.57(mg/l) to 2.26±1.70(mg/l). This evidence suggests that subjects who were diagnosed with gingivitis in this clinical trial have CRP levels that would place them in a category for moderate risk for future CVD based on the AHA consensus report.[61] CRP values have been found to be slightly elevated in healthy controls previously (patients with minimal or no attachment loss). A recent review reported that healthy controls (no periodontal disease) CRP values ranged from 0.87 – 2.46m. This range can possibly be attributed to BMI fluctuations, CVD, infection, or injury.[62] However, the periodontitis patients in this review reported elevations of 2.40 - 9.12mg/l; the elevations in this population versus control may be attributed to inflammation associated with periodontitis.[62]

Several clinical studies have evaluated periodontal therapy on CRP levels in periodontitis subjects. D'Aiuto and coworkers conducted a clinical trial on 94 subjects with severe generalized periodontitis. Six months after non-surgical treatment, CRP levels were reduced 0.5mg/l from baseline values in this population.[56] A second study on 40 patients showed a range of 0.4 mg/l - 0.6 mg/l reduction in CRP over three follow-up visits for subjects in the intensive treatment group (ITG) which consisted of local

antibiotics and scaling and root planing (SRP).[57] A third study in a population of 60 severe generalized periodontitis subjects demonstrated a mean reduction of CRP by 0.5mg/l in the two months after SRP and the ITG reduced mean CRP by 0.8mg/l.[63] A recent meta-analysis showed an average reduction of 0.5mg/l after periodontal therapy.[59] These trials and meta-analysis indicate that treatment of periodontitis patients with aggressive periodontal therapy can lower CRP levels.

IL-6 is another inflammatory cytokine that can serve to mediate connective tissue destruction by enhancing osteoblast differentiation and activation, and fibroblast expression of matrix metalloproteinases.[64] IL-6 is an important inflammatory mediator that has been shown to induce hepatocytes to produce acute phase reactants such as CRP.[65] Several studies have reported serum levels of IL-6 in periodontitis patients. Loos and co-workers found that 42% of controls and 53% of periodontitis patients had measureable IL-6 levels in serum.[49] Like the CRP, the IL-6 levels correlated to the extent of disease. Another group, Buhlin and co-workers, reported elevated IL-6 plasma levels versus healthy controls; in addition, there were measurable IL-6 levels in aggressive periodontitis patients which correlated with severity of attachment loss.[66]

Mean levels of IL-6 for the gingivitis subjects in the present report ranged from 1.72±.85 (pg/ml) to 2.22±1.50(pg/ml). These values are similar to values reported in the literature for patients with periodontal disease. D'Aiuto and co-workers reported median baseline values of 1.8pg/ml and values of 1.6pg/ml after SRP.[56] Another group, Offenbacher and coworkers, reported baseline mean IL-6 values ranging from 0.47-1.22pg/ml in pregnant subjects with at least one PD greater than 5mm.[38] Mendall and coworkers reported median values of 1.73pg/ml from a random sample of 198 men aged

50-69, for which periodontal status was not documented but that were correlated with CVD risk.[67] At least one clinical trial has shown a reduction of IL-6 levels with periodontal therapy.[68] The levels of the gingivitis subjects in the present trial correlate closely with the levels in the cohort study reported by Mendall et al. Future clinical trials may look at gingivitis subjects and see if periodontal therapy can reduce IL-6 levels which may have implications towards systemic health.

8-iso-$PGF_2\alpha$ is a member of eicosanoid family called isoprostanes. Isoprostanes are thought to form nonenzymatically by free radical-mediated peroxidation of AA, while prostaglandins are produced as a result of COX enzyme activity.[69] However, there is some evidence that suggests that COX activity may also contribute to isoprostane activity in some tissues.[69] 8-iso-$PGF_2\alpha$ has been shown to be elevated in patients with atherosclerosis, acute myocardial infarction, Alzheimer's disease, and has been implicated in thrombus development at sites of vascular injury.[69] There have been reports in the literature of elevated 8-iso-$PGF_2\alpha$ in patients with periodontal disease. For instance, serum levels of 8-iso-$PGF_2\alpha$ were associated with an increase in PD greater than or equal to 4mm with third molars.[44] In addition, Boggess and coworkers showed that pregnant subjects with an IgM response to periodontal pathogens combined with elevated 8-isoprostane levels resulted in a significantly increased risk for preterm birth.[70] The mean 8-iso-$PGF_2\alpha$ values reported by Boggess et al., 121 ng/ml for a cohort of 640 pregnant subjects, were lower than our reported mean value for 8-iso-$PGF_2\alpha$, which ranged from 594.9±531.0(ng/ml) to 838.6±452.4(ng/ml) for all subjects. The author is not aware where other values of 8-iso-$PGF_2\alpha$ have been reported in periodontal populations.

A Pearson correlation test for levels of 8-iso-$PGF_2\alpha$ and IL-6 in the GCF and serum showed that there is no correlation between the levels locally and systemically. This finding suggests that there is no spill-over of these inflammatory markers from the GCF into the serum in gingivitis subjects.

The data from this short clinical trial of 48 subjects with gingivitis and no professional intervention suggest no change in serum inflammatory levels with the treatment twice daily of topical application of triclosan dentifrice or placebo dentifrice. The data suggest that serum values for biomarkers like CRP, IL-6 and 8-iso-$PGF_2\alpha$ are elevated in this gingivitis population comparable to what has been reported in periodontitis populations. Indeed, the levels of CRP that are seen in this population are in the range which has been classified as moderate risk for future CVD. These data suggest that gingivitis may impose an inflammatory burden with systemic consequences in otherwise medically healthy subjects.

Conclusion

Serum levels of CRP are elevated in the 48 subjects in this investigation to levels that confer moderate risk of future CVD. Serum IL-6 and 8-iso-$PGF_2\alpha$ may also be elevated and stable in gingivitis subjects over four weeks. Gingivitis may represent a critical infection/inflammatory burden accompanied by elevated serum responses.

Table 1.
Demographics

	Placebo	The experimental group
Age (years) ± SD	39.9±12.5	34.0±12.1
Height (inches)	67.5±4.0	67.5±4.0
Weight (pounds)	171.9±39.9	164.2±49.0
BMI	26.5±5.7	25.2±7.0
Male(%)	29.9	33.3
Female(%)	70.1	66.6
African American(%)	70.1	38.0
Hispanic(%)	4.9	3.7
Caucasian(%)	25.0	58.3

Table 2.
Mean (Standard Error) Clinical Measures by Day and Group (ANCOVA)

	Baseline	Day 29
Mean PD		
Placebo	2.52 (0.04)	2.47 (0.05)
Triclosan	2.54 (0.04)	2.55 (0.04)
p-value	0.80	0.21
Extent PD >=4mm (%)		
Placebo	9.73 (1.07)	8.82 (1.15)
Triclosan	9.59 (1.05)	8.70 (1.07)
p-value	0.56	0.94
Mean GI		
Placebo	1.05 (0.04)	0.99 (0.04)
Triclosan	1.10 (0.04)	1.06 (0.04)
p-value	0.31	0.20
Extent GI >=2 (%)		
Placebo	7.15 (3.13)	8.02 (3.36)
Triclosan	12.66 (3.06)	8.66 (3.13)
p-value	0.21	0.89
Mean PI		
Placebo	0.96 (0.03)	0.93 (0.04)
Triclosan	0.99 (0.03)	0.99 (0.03)
p-value	0.55	0.30
Extent PI >=2 (%)		
Placebo	2.68 (1.52)	2.99 (1.63)
Triclosan	5.79 (1.49)	4.47 (1.52)
p-value	0.15	0.51
Extent BOP (%)		
Placebo	48.1 (1.95)	45.1 (2.09)
Triclosan	51.9 (1.91)	44.7 (1.95)
p-value	0.31	0.88

Table 3.
GCF Mean (Standard Error) and Median Values Day 1

	Pre-dosing	1-hour Post-dosing	2-hour Post-dosing	3-hour Post-dosing	6-hour Post-dosing
PGE_2 (pg/ml)					
Group A					
Mean	252.1(266.3)	459.7(608.2)	700(985.8)	427.8(564.5)	569.5(826.5)
Median	176.3	252.2	341.2	273.9	193.7
Group B					
Mean	354.9(446.1)	300.7(297.3)	637.3(965.9)	514.9(514.3)	605.4(819.0)
Median	201.0	183.3	238.0	293.0	211.7
IL-1β(pg/ml)					
Group A					
Mean	317.0(291.3)	330.2(354.4)	492.2(545.5)	410.4(396.9)	607.2(783.8)
Median	197.5	239.0	385.7	272.3	247.8
Group B					
Mean	414.0(372.0)	257.9(246.8)	326.0(307.7)	304.0(251.1)	643.3(733.1)
Median	267.4	151.2	201.6	215.3	477.8
IL-6(pg/ml)					
Group A					
Mean	1.9(4.6)	13.0(29.2)	12.8(23.3)	12.8(30.3)	26.5(81.8)
Median	0	0	0	0	0
Group B					
Mean	6.2(16.8)	10.8(19.2)	21.3(46.4)	11.6(22.7)	32.0(65.7)
Median	0	0	1.9	0	0
8-iso-$PGF_2\alpha$ (mg/l)					
Group A					
Mean	8.8(16.1)	763449(3.5e6)	9.2(17.0)	5.5(10.1)	65.6(250.8)
Median	2.4	3.2	3.8	0.04	2.9
Group B					
Mean	8.0(12.2)	3.2(4.5)	8.1(14.6)	15.8(44.7)	30.2(65.2)
Median	2.5	2.0	2.0	3.3	5.3
RANKL(pg/ml)					
Group A					
Mean	5.6(5.0)	4.0(3.5)	4.7(4.0)	4.1(2.9)	5.7(5.0)
Median	3.5	2.8	3.9	3.3	4.3
Group B					
Mean	4.9(5.3)	3.1(2.1)	4.2(4.2)	2.8(1.9)	5.7(5.0)
Median	3.5	2.7	3.7	2.7	2.65

Table 4.
GCF Mean (Standard Error) and Median Values Pre-dosing and Post-dosing for Days 8, 15, 22, 29.

	Day 8		Day 15		Day 22		Day 29	
	Pre-dosing	Post-dosing	Pre-dosing	Post-dosing	Pre-dosing	Post-dosing	Pre-dosing	Post-dosing
PGE_2(pg/ml) Placebo								
Mean	452.8 (445.6)	527.2 (803.0)	213.1 (165.4)	506.9 (646.5)	345.8 (373.6)	263.9 (362.5)	386.0 (511.5)	565.0 (756.0)
Median	353.7	185.1	157.5	203.1	144.3	120.8	137.1	336.8
Triclosan								
Mean	394.6 (383.8)	419.9 (736.1)	346.9 (394.3)	266.6 (251.0)	254.6 (190.1)	391.8 (444.0)	354.4 (418.3)	416.5 (532.3)
Median	222.8	146.4	237.7	190.4	201.6	218.9	184.8	262.8
IL-1β (pg/ml) Placebo								
Mean	424.8 (502.0)	451.4 (484.8)	288.3 (329.5)	266.5 (222.5)	250.6 (267.8)	317.0 (497.3)	390.3 (365.5)	166.9 (246.5)
Median	219.5	296.7	196.3	206.1	141.0	160.7	282.3	105.7
Triclosan								
Mean	603.6 (544.4)	393.0 (292.1)	704.7 (796.7)	541.0 (492.0)	734.2 (621.2)	383.3 (314.0)	453.1 (473.8)	344.3 (286.6)
Median	493.6	350.5	450.5	436.1	584.5	356.3	287.7	319.4
IL-6(pg/ml) Placebo								
Mean	0.6 (2.5)	6.4 (16.7)	2.7 (5.6)	4.6 (11.7)	1.9 (3.8)	2.7 (7.0)	1.0 (2.8)	4.4 (10.7)
Median	0	0	0	0	0	0	0	0
Triclosan								
Mean	2.4 (5.3)	3.6 (7.6)	4.34 (11.8)	7.1 (10.0)	2.7 (5.7)	3.1 (6.3)	1.9 (4.7)	6.5 (11.4)
Median	0	0	0	0	0	0	0	0

Table 5.
GCF Mean and Median Values Pre-dosing and Post-dosing for Days 8, 15, 22, 29

	Day 8		Day 15		Day 22		Day 29	
	Pre-dosing	Post-dosing	Pre-dosing	Post-dosing	Pre-dosing	Post-dosing	Pre-dosing	Post-dosing
8-iso-$PGF_2\alpha$ (mg/l) Placebo								
Mean	6.6 (13.4)	589857.4 (2.4E6)	5034.1 (21914.5)	15.0 (24.2)	12.8 (21.9)	12.6 (23.7)	6.4 (9.0)	251.3 (1057.8)
Median	.09	4.0	3.6	1.7	3.7	2.3	2.5	3.6
Triclosan								
Mean	27.8 (73.2)	16.6 (21.9)	7.9 (13.7)	5.3 (8.7)	10.1 (14.2)	4.0 (6.6)	17.7 (39.8)	7.3 (13.1)
Median	6.4	2.7	0.5	.0009	2.4	1.0	3.1	2.1
RANK-L(pg/ml) Placebo								
Mean	4.7 (4.9)	5.1 (2.9)	3.6 (7.6)	5.3 (3.6)	3.9 (2.7)	4.6 (2.5)	4.2 (3.2)	9.2 (14.5)
Median	3.2	5.0	3.5	4.1	2.9	3.6	3.6	4.6
Triclosan								
Mean	9.9 (22.4)	8.1 (14.5)	4.3 (2.4)	4.1 (2.7)	6.6 (7.8)	5.6 (7.3)	3.7 (3.2)	4.3 (3.6)
Median	4.3	3.8	5.3	3.6	2.7	2.7	2.8	3.6

Table 6.
Mean ±Standard Deviation and Median Serum Marker Values by Day and Group

	Day 1	Day 8	Day 15	Day 22	Day 29
Il-6 (pg/ml)					
All					
Mean	1.76±1.60	2.22±1.50	1.90±1.85	1.72±.85	1.92±1.43
Median	1.25	1.74	1.50	1.47	1.48
Placebo					
Mean	2.08±2.10	2.38±1.92	2.37±2.51	1.80±0.93	2.12±1.85
Median	1.42	1.67	1.65	1.47	1.38
Triclosan					
Mean	1.42±0.69	2.06±0.96	1.42±0.57	1.59±0.74	1.70±0.78
Median	1.17	1.83	1.27	1.6	1.51
D8Iso(ng/ml)					
All					
Mean	838.6±452.4	646.7±519.4	757.5±472.9	594.9±531.0	600.5±440.2
Median	867.76	597.76	610.31	594.80	604.74
Placebo					
Mean	680.2±512.6	573.4±545.2	663.0±476.2	528.1±555.6	645.3±450.6
Median	605.7	590.9	604.7	589.9	604.2
Triclosan					
Mean	999.1±321.6	720.1±503.2	852.0±468.8	528.1±555.6	553.1±437.4
Median	1197.2	600.7	1153.5	604.2	605.7
CRP(mg/l)					
All					
Mean	1.97±1.45	2.26±1.70	1.91±1.57	2.19±1.76	1.99±1.48
Median	2.05	2.27	1.51	1.69	2.31
Placebo					
Mean	2.11±1.60	2.46±1.48	1.68±1.48	2.35±1.75	2.01±1.42
Median	2.05	2.42	1.42	1.99	2.32
Triclosan					
Mean	1.83±1.30	2.06±1.93	2.13±1.67	1.97±1.82	1.97±1.58
Median	1.86	1.31	1.55	1.62	2.31

Table 7.
Mean (Standard Error) LOG Serum Markers by Day and Group (ANCOVA)

	Day1	Day8	Day15	Day22	Day29
IL6					
Placebo	0.43 (0.03)	0.48 (0.04)	0.47 (0.04)	0.43 (0.04)	0.45 (0.04)
Triclosan	0.37 (0.03)	0.47 (0.04)	0.37 (0.04)	0.40 (0.05)	0.42 (0.04)
p-value	0.19	0.85	0.14	0.64	0.59
D8iso					
Placebo	5.08 (0.29)	4.56 (0.36)	5.30 (0.36)	4.89 (0.32)	5.32 (0.31)
Triclosan	5.97 (0.29)	5.25 (0.36)	5.42 (0.36)	5.21 (0.38)	5.04 (0.32)
p-value	**0.03**	0.18	0.82	0.52	0.53
CRP					
Placebo	6.12 (0.11)	6.27 (0.15)	5.96 (0.15)	6.19 (0.13)	6.13 (0.13)
Triclosan	5.99 (0.12)	6.02 (0.15)	6.14 (0.15)	6.08 (0.16)	6.07 (0.13)
p-value	0.44	0.24	0.40	0.61	0.74

Figure 1. Mean Percent Change in GCF IL-1β from Pre-dosing Day 1

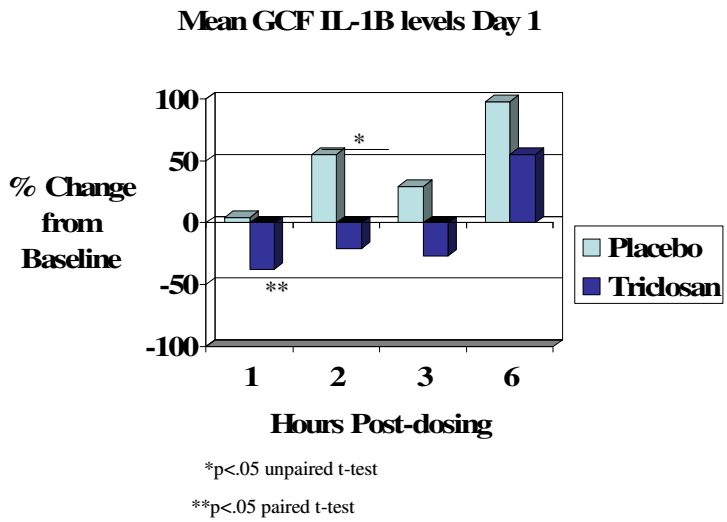


Figure 2. Mean Percent Change in GCF IL--1β from Pre-dosing Days 8-29

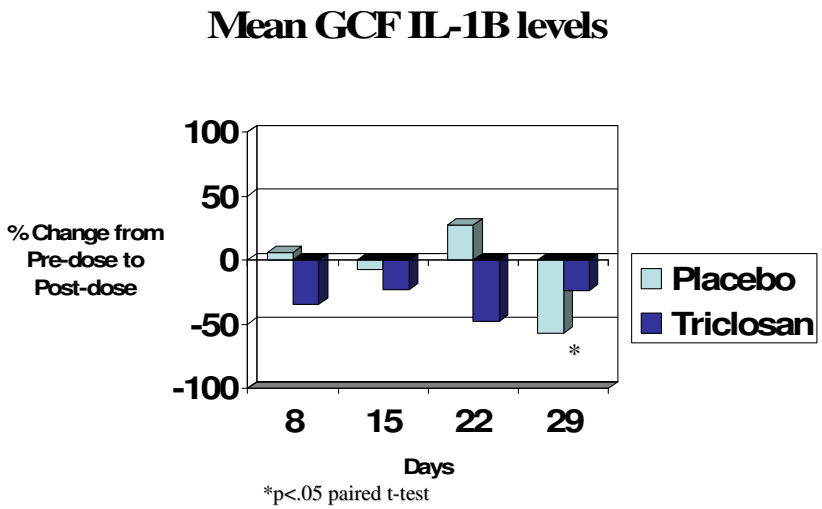
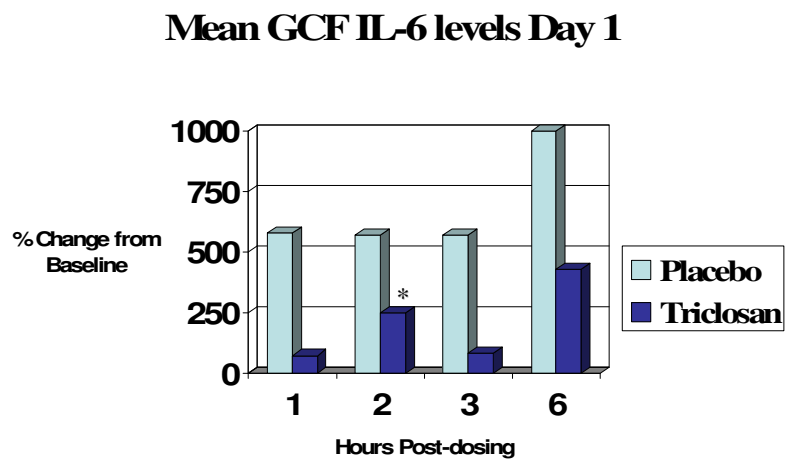
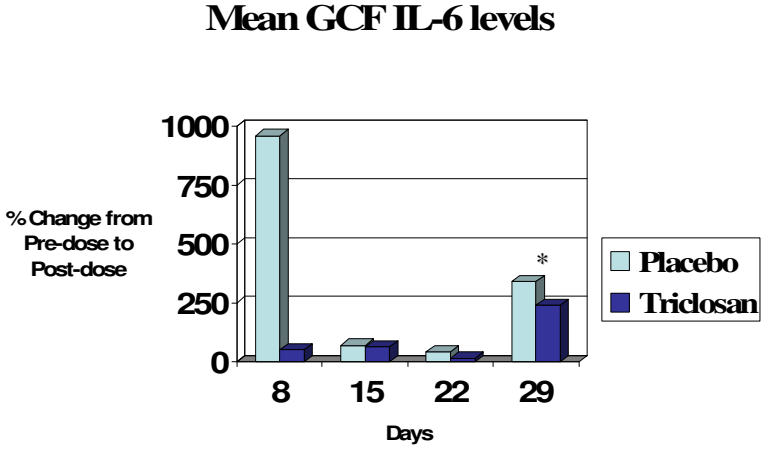


Figure 3. Mean Percent Change in GCF IL-6 from Pre-dosing Day 1



*p<.05 paired t-test

Figure 4. Mean Percent Change in GCF IL-6 from Pre-dosing Days 8-29



*p<.05 paired t-test

Figure 5. Mean Percent Change in GCF RANK-L from Pre-dosing Day 1

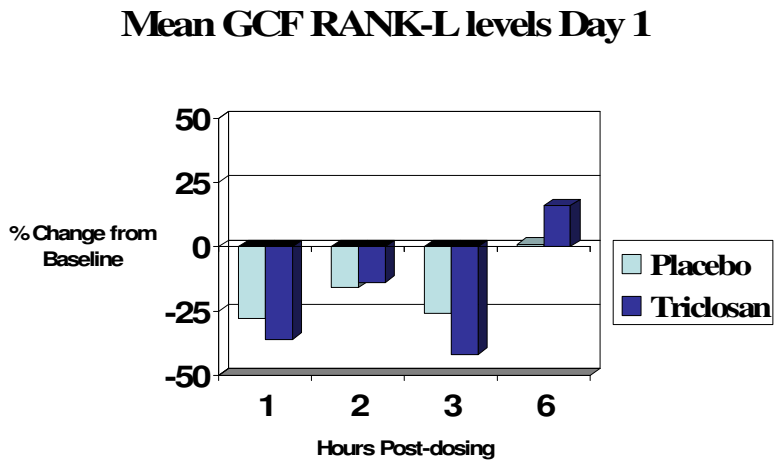
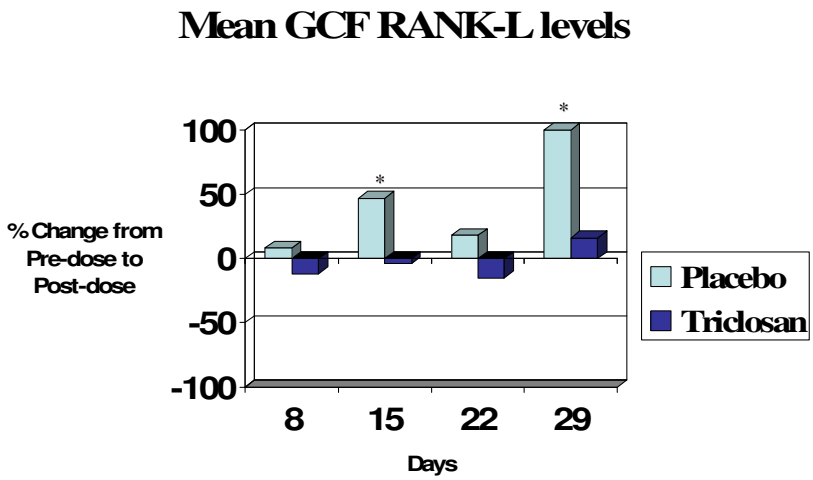
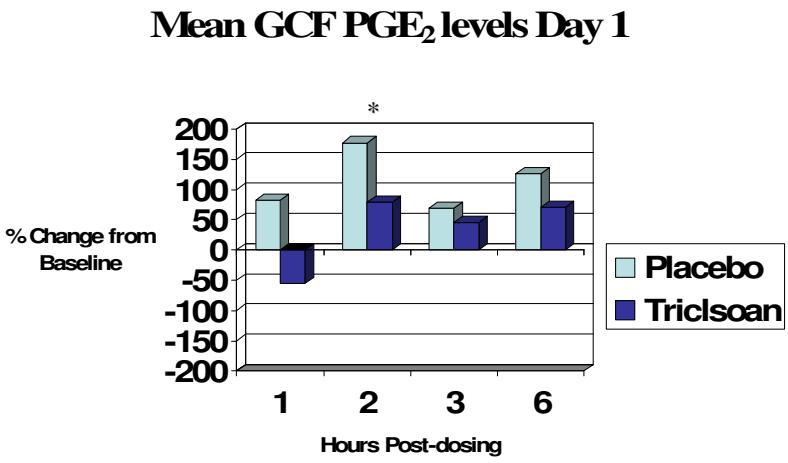


Figure 6. Mean Percent Change in GCF RANK-L from Pre-dosing Days 8-29



*p<.05 paired t-test

Figure 7. Mean Percent Change in GCF PGE_2 from Pre-dosing Day 1



*p<.05 paired t-test

Figure 8. Mean Percent Change in GCF PGE_2 from Pre-dosing Days 8-29

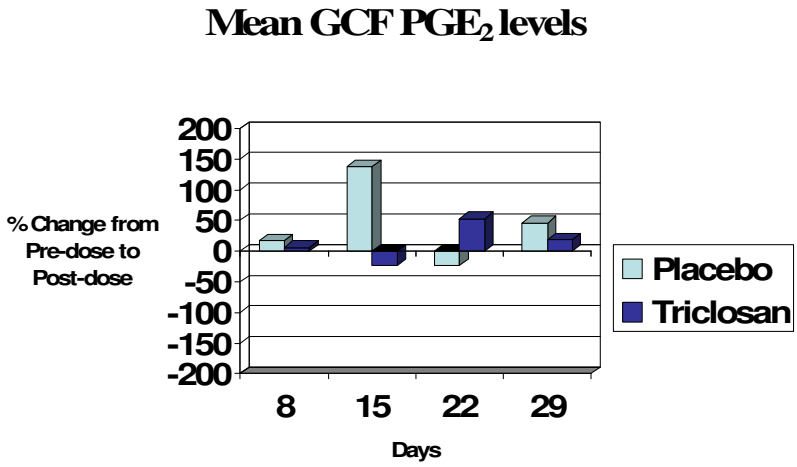


Figure 9.

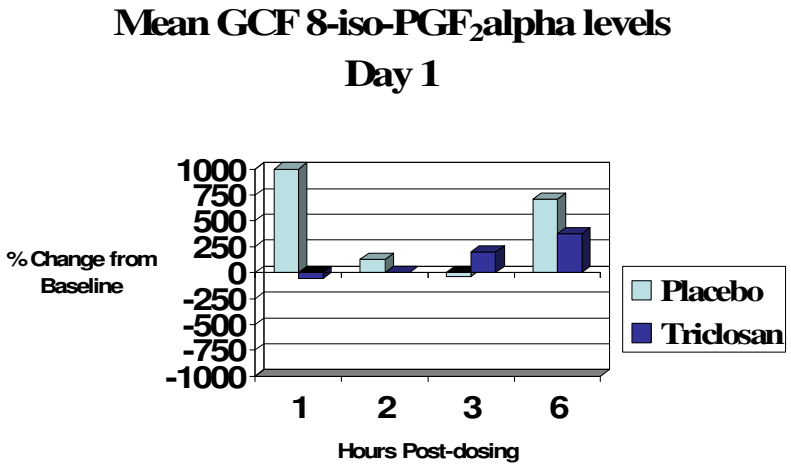
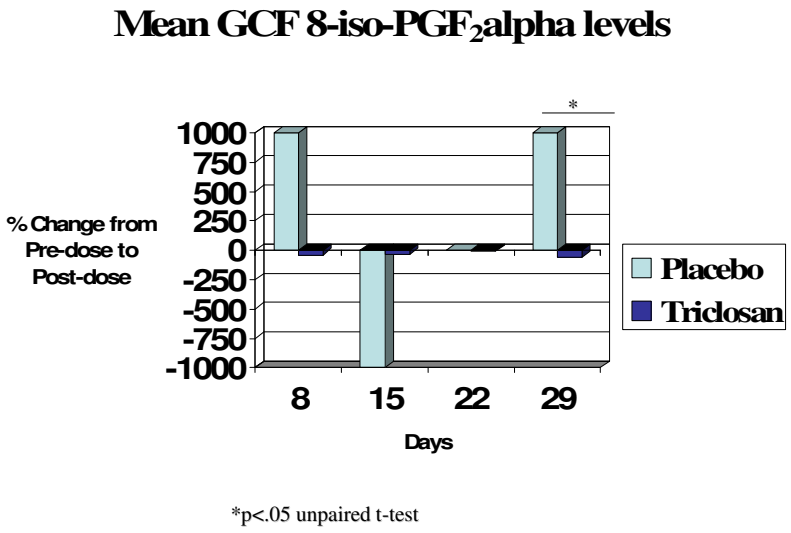


Figure 10. Mean Percent Change in GCF 8-iso-$PGF_2\alpha$ from Pre-dosing Day 1



*p<.05 unpaired t-test

References

1. Offenbacher S. Periodontal diseases: pathogenesis. *Ann Periodontol* 1996;1(1):821-878.

2. Offenbacher S, Farr DH, Goodson JM. Measurement of prostaglandin E in crevicular fluid. *J Clin Periodontol* 1981;8(4):359-367.

3. Offenbacher S, Odle BM, Gray RC, Van Dyke TE. Crevicular fluid prostaglandin E levels as a measure of the periodontal disease status of adult and juvenile periodontitis patients. *J Periodontal Res* 1984;19(1):1-13.

4. Stashenko P, Jandinski JJ, Fujiyoshi P, Rynar J, Socransky SS. Tissue levels of bone resorptive cytokines in periodontal disease. *J Periodontol* 1991;62(8):504-509.

5. Lang NP, Adler R, Joss A, Nyman S. Absence of bleeding on probing. An indicator of periodontal stability. *J Clin Periodontol* 1990;17(10):714-721.

6. Axelsson P, Lindhe J. The significance of maintenance care in the treatment of periodontal disease. *J Clin Periodontol* 1981;8(4):281-294.

7. Axelsson P, Lindhe J. Effect of controlled oral hygiene procedures on caries and periodontal disease in adults. *J Clin Periodontol* 1978;5(2):133-151.

8. Axelsson P, Lindhe J, Nystrom B. On the prevention of caries and periodontal disease. Results of a 15-year longitudinal study in adults. *J Clin Periodontol* 1991;18(3):182-189.

9. Lindhe J, Nyman S. Long-term maintenance of patients treated for advanced periodontal disease. *J Clin Periodontol* 1984;11(8):504-514.

10. Williams RC, Jeffcoat MK, Howell TH, et al. Altering the progression of human alveolar bone loss with the non-steroidal anti-inflammatory drug flurbiprofen. *J Periodontol* 1989;60(9):485-490.

11. Williams RC, Jeffcoat MK, Kaplan ML, Goldhaber P, Johnson HG, Wechter WJ. Flurbiprofen: a potent inhibitor of alveolar bone resorption in beagles. *Science* 1985;227(4687):640-642.

12. Reddy MS, Geurs NC, Gunsolley JC. Periodontal host modulation with antiproteinase, anti-inflammatory, and bone-sparing agents. A systematic review. *Ann Periodontol* 2003;8(1):12-37.

13. Lindhe J, Rosling B, Socransky SS, Volpe AR. The effect of a triclosan-containing dentifrice on established plaque and gingivitis. *J Clin Periodontol* 1993;20(5):327-334.

14. Schiff T, Cohen S, Volpe AR, Petrone ME. Effects of two fluoride dentifrices containing triclosan and a copolymer on calculus formation. *Am J Dent* 1990;3 Spec No:S43-5.

15. Mann J, Vered Y, Babayof I, et al. The comparative anticaries efficacy of a dentifrice containing 0.3% triclosan and 2.0% copolymer in a 0.243% sodium fluoride/silica base and a dentifrice containing 0.243% sodium fluoride/silica base: a two-year coronal caries clinical trial on adults in Israel. *J Clin Dent* 2001;12(3):71-76.

16. Afflitto J, Fakhry-Smith S, Gaffar A. Salivary and plaque triclosan levels after brushing with a 0.3% triclosan/copolymer/NaF dentifrice. *Am J Dent* 1989;2 Spec No:207-210.

17. Modeer T, Bengtsson A, Rolla G. Triclosan reduces prostaglandin biosynthesis in human gingival fibroblasts challenged with interleukin-1 in vitro. *J Clin Periodontol* 1996;23(10):927-933.

18. Mustafa M, Wondimu B, Ibrahim M, Modeer T. Effect of triclosan on interleukin-1 beta production in human gingival fibroblasts challenged with tumor necrosis factor alpha. *Eur J Oral Sci* 1998;106(2 Pt 1):637-643.

19. Gaffar A, Scherl D, Afflitto J, Coleman EJ. The effect of triclosan on mediators of gingival inflammation. *J Clin Periodontol* 1995;22(6):480-484.

20. Lin YJ, Fung KK, Kong BM, DeSalva SJ. Gingival absorption of triclosan following topical mouthrinse application. *Am J Dent* 1994;7(1):13-16.

21. Mustafa M, Wondimu B, Hultenby K, Yucel-Lindberg T, Modeer T. Uptake, distribution and release of 14C-triclosan in human gingival fibroblasts. *J Pharm Sci* 2003;92(8):1648-1653.

22. Barkvoll P, Rolla G. Triclosan reduces the clinical symptoms of the allergic patch test reaction (APR) elicited with 1% nickel sulphate in sensitised patients. *J Clin Periodontol* 1995;22(6):485-487.

23. LOE H, SILNESS J. Periodontal Disease in Pregnancy. I. Prevalence and Severity. *Acta Odontol Scand* 1963;21:533-551.

24. SILNESS J, LOE H. Periodontal Disease in Pregnancy. Ii. Correlation between Oral Hygiene and Periodontal Condtion. *Acta Odontol Scand* 1964;22:121-135.

25. Gibson JA, Wade AB. Plaque removal by the Bass and Roll brushing techniques. *J Periodontol* 1977;48(8):456-459.

26. Offenbacher S, Barros SP, Singer RE, Moss K, Williams RC, Beck JD. Periodontal disease at the biofilm-gingival interface. *J Periodontol* 2007;78(10):1911-1925.

27. White RP,Jr, Offenbacher S, Phillips C, Haug RH, Blakey GH, Marciani RD. Inflammatory mediators and periodontitis in patients with asymptomatic third molars. *J Oral Maxillofac Surg* 2002;60(11):1241-1245.

28. Cancer Therapy Evaluation Program. Common Terminology Criteria for Adverse Events. Version 3.0(Available at: http://ctep.cancer.gov.libproxy.lib.unc.edu/forms/CTCAEv3.pdf. 1/21, 2008.)

29. Tatakis DN. Interleukin-1 and bone metabolism: a review. *J Periodontol* 1993;64(5 Suppl):416-431.

30. Matsuki Y, Yamamoto T, Hara K. Detection of inflammatory cytokine messenger RNA (mRNA)-expressing cells in human inflamed gingiva by combined in situ hybridization and immunohistochemistry. *Immunology* 1992;76(1):42-47.

31. Horowitz MC. Cytokines and estrogen in bone: anti-osteoporotic effects. *Science* 1993;260(5108):626-627.

32. Sauder DN, Mounessa NL, Katz SI, Dinarello CA, Gallin JI. Chemotactic cytokines: the role of leukocytic pyrogen and epidermal cell thymocyte-activating factor in neutrophil chemotaxis. *J Immunol* 1984;132(2):828-832.

33. Masada MP, Persson R, Kenney JS, Lee SW, Page RC, Allison AC. Measurement of interleukin-1 alpha and -1 beta in gingival crevicular fluid: implications for the pathogenesis of periodontal disease. *J Periodontal Res* 1990;25(3):156-163.

34. Ishihara Y, Nishihara T, Kuroyanagi T, et al. Gingival crevicular interleukin-1 and interleukin-1 receptor antagonist levels in periodontally healthy and diseased sites. *J Periodontal Res* 1997;32(6):524-529.

35. Zhong Y, Slade GD, Beck JD, Offenbacher S. Gingival crevicular fluid interleukin-1beta, prostaglandin E2 and periodontal status in a community population. *J Clin Periodontol* 2007;34(4):285-293.

36. Kornman KS, Crane A, Wang HY, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. *J Clin Periodontol* 1997;24(1):72-77.

37. Kusano K, Miyaura C, Inada M, et al. Regulation of matrix metalloproteinases (MMP-2, -3, -9, and -13) by interleukin-1 and interleukin-6 in mouse calvaria: association of MMP induction with bone resorption. *Endocrinology* 1998;139(3):1338-1345.

38. Offenbacher S, Lin D, Strauss R, et al. Effects of periodontal therapy during pregnancy on periodontal status, biologic parameters, and pregnancy outcomes: a pilot study. *J Periodontol* 2006;77(12):2011-2024.

39. Bostanci N, Ilgenli T, Emingil G, et al. Gingival crevicular fluid levels of RANKL and OPG in periodontal diseases: implications of their relative ratio. *J Clin Periodontol* 2007;34(5):370-376.

40. Silva N, Dutzan N, Hernandez M, et al. Characterization of progressive periodontal lesions in chronic periodontitis patients: levels of chemokines, cytokines, matrix

metalloproteinase-13, periodontal pathogens and inflammatory cells. *J Clin Periodontol* 2008;35(3):206-214.

41. Gemmell E, Marshall RI, Seymour GJ. Cytokines and prostaglandins in immune homeostasis and tissue destruction in periodontal disease. *Periodontol 2000* 1997;14:112-143.

42. Goldhaber P, Rabadjija L, Beyer WR, Kornhauser A. Bone resorption in tissue culture and its relevance to periodontal disease. *J Am Dent Assoc* 1973;87(5):1027-1033.

43. Offenbacher S, Odle BM, Van Dyke TE. The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. *J Periodontal Res* 1986;21(2):101-112.

44. Moss KL, Mauriello S, Ruvo AT, Offenbacher S, White RP,Jr, Beck JD. Reliability of third molar probing measures and the systemic impact of third molar periodontal pathology. *J Oral Maxillofac Surg* 2006;64(4):652-658.

45. Bradshaw DJ, Marsh PD, Watson GK, Cummins D. The effects of triclosan and zinc citrate, alone and in combination, on a community of oral bacteria grown in vitro. *J Dent Res* 1993;72(1):25-30.

46. Lindhe J, Rosling B, Socransky SS, Volpe AR. The effect of a triclosan-containing dentifrice on established plaque and gingivitis. *J Clin Periodontol* 1993;20(5):327-334.

47. Lindhe J, Rosling B, Socransky SS, Volpe AR. The effect of a triclosan-containing dentifrice on established plaque and gingivitis. *J Clin Periodontol* 1993;20(5):327-334.

48. Rosling B, Wannfors B, Volpe AR, Furuichi Y, Ramberg P, Lindhe J. The use of a triclosan/copolymer dentifrice may retard the progression of periodontitis. *J Clin Periodontol* 1997;24(12):873-880.

49. Loos BG, Craandijk J, Hoek FJ, Wertheim-van Dillen PM, van der Velden U. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *J Periodontol* 2000;71(10):1528-1534.

50. Noack B, Genco RJ, Trevisan M, Grossi S, Zambon JJ, De Nardin E. Periodontal infections contribute to elevated systemic C-reactive protein level. *J Periodontol* 2001;72(9):1221-1227.

51. Slade GD, Offenbacher S, Beck JD, Heiss G, Pankow JS. Acute-phase inflammatory response to periodontal disease in the US population. *J Dent Res* 2000;79(1):49-57.

52. Mendall MA, Patel P, Asante M, et al. Relation of serum cytokine concentrations to cardiovascular risk factors and coronary heart disease. *Heart* 1997;78(3):273-277.

53. Offenbacher S, Katz V, Fertik G, et al. Periodontal infection as a possible risk factor for preterm low birth weight. *J Periodontol* 1996;67(10 Suppl):1103-1113.

54. Genco R, Offenbacher S, Beck J. Periodontal disease and cardiovascular disease: epidemiology and possible mechanisms. *J Am Dent Assoc* 2002;133 Suppl:14S-22S.

55. Grossi SG, Genco RJ. Periodontal disease and diabetes mellitus: a two-way relationship. *Ann Periodontol* 1998;3(1):51-61.

56. D'Aiuto F, Parkar M, Andreou G, et al. Periodontitis and systemic inflammation: control of the local infection is associated with a reduction in serum inflammatory markers. *J Dent Res* 2004;83(2):156-160.

57. D'Aiuto F, Nibali L, Parkar M, Suvan J, Tonetti MS. Short-term effects of intensive periodontal therapy on serum inflammatory markers and cholesterol. *J Dent Res* 2005;84(3):269-273.

58. Ebersole JL, Machen RL, Steffen MJ, Willmann DE. Systemic acute-phase reactants, C-reactive protein and haptoglobin, in adult periodontitis. *Clin Exp Immunol* 1997;107(2):347-352.

59. Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *J Clin Periodontol* 2008;35(4):277-290.

60. Blake GJ, Ridker PM. Inflammatory bio-markers and cardiovascular risk prediction. *J Intern Med* 2002;252(4):283-294.

61. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003;107(3):499-511.

62. Loos BG. Systemic markers of inflammation in periodontitis. *J Periodontol* 2005;76(11 Suppl):2106-2115.

63. D'Aiuto F, Parkar M, Nibali L, Suvan J, Lessem J, Tonetti MS. Periodontal infections cause changes in traditional and novel cardiovascular risk factors: results from a randomized controlled clinical trial. *Am Heart J* 2006;151(5):977-984.

64. Kusano K, Miyaura C, Inada M, et al. Regulation of matrix metalloproteinases (MMP-2, -3, -9, and -13) by interleukin-1 and interleukin-6 in mouse calvaria: association of MMP induction with bone resorption. *Endocrinology* 1998;139(3):1338-1345.

65. Steel DM, Whitehead AS. The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. *Immunol Today* 1994;15(2):81-88.

66. Buhlin K, Gustafsson A, Pockley AG, Frostegard J, Klinge B. Risk factors for cardiovascular disease in patients with periodontitis. *Eur Heart J* 2003;24(23):2099-2107.

67. Mendall MA, Patel P, Asante M, et al. Relation of serum cytokine concentrations to cardiovascular risk factors and coronary heart disease. *Heart* 1997;78(3):273-277.

68. D'Aiuto F, Ready D, Tonetti MS. Periodontal disease and C-reactive protein-associated cardiovascular risk. *J Periodontal Res* 2004;39(4):236-241.

69. Khasawneh FT, Huang JS, Mir F, Srinivasan S, Tiruppathi C, Le Breton GC. Characterization of isoprostane signaling: Evidence for a unique coordination profile of 8-iso-PGF(2alpha) with the thromboxane A(2) receptor, and activation of a separate cAMP-dependent inhibitory pathway in human platelets. *Biochem Pharmacol* 2008;

70. Boggess KA, Moss K, Madianos P, Murtha AP, Beck J, Offenbacher S. Fetal immune response to oral pathogens and risk of preterm birth. *Am J Obstet Gynecol* 2005;193(3 Pt 2):1121-1126.