The effect of sub-antimicrobial dose-doxycycline periodontal therapy on serum inflammatory biomarker C-reactive protein levels in post-menopausal Women: A 2-year, double-blinded, randomized clinical trial

RAJESH S. KOPPIKAR, SNEH V. AGRAWAL

Abstract

Background: Periodontitis has been reported to be associated with coronary artery disease. Research is needed to determine whether therapies that improve periodontal health also reduce systemic marker of inflammation associated with both diseases. Aim: To determine whether sub-antimicrobial dose-doxycycline (SDD) therapy can reduce systemic serum inflammatory biomarker C-reactive protein (CRP) in post-menopausal women who have chronic periodontitis. Settings and Design: The study randomly assigned 128 eligible post-menopausal women with chronic periodontitis to a 90-day, twice-daily regimen of SDD or placebo tablets evaluated for 2 years, as an adjunct to periodontal maintenance therapy. Materials and Methods: The study assayed blood samples for inflammatory mediators at baseline, 1 year, and 2 years. CRP was measured using a high-sensitivity enzyme-linked immunosorbent assay. Results: SDD treatment reduced median high-sensitivity CRP by 18% (primary outcome = 0.02). Conclusion: Ninety-day SDD regimen in post-menopausal women significantly reduced the serum inflammatory biomarker CRP over a 2-year period.

Keywords: C-reactive protein, coronary artery disease, periodontitis, sub-antimicrobial dose-doxycycline

Introduction

Inflammation increasingly is recognized as a clinically significant factor in the initiation, progression, and ultimate instability of atherosclerotic plaques in patients with coronary artery disease (CAD). In this regard, chronic periodontitis is a common chronic inflammatory disease that has been claimed to have an association to CAD. Therapeutic strategies that resolve inflammation associated with both of these diseases and that affect CAD’s onset and progression are needed because CAD is a leading cause of death in post-menopausal women; novel therapies to reduce systemic inflammation should be studied in this patient population.

In the pathogenesis of atherosclerotic CAD, specific elements of the inflammatory process have been identified as risk factors and risk markers. For example, investigators have identified C-reactive protein (CRP) and matrix metalloproteinase-9 as important in CAD pathogenesis and as serum markers of disease activity. To date, the mainstay of pharmacological therapy to modulate these and other inflammatory mediators has been the statins; originally approved by the US Food and Drug Administration (FDA) for their lipid-lowering effects. In particular, doxycycline at a low dose, i.e., sub-antimicrobial dose-doxycycline (SDD) in humans modulates CRP activity and reduces severity of inflammatory diseases such as periodontitis.

The patient cohort consisted of post-menopausal women with chronic periodontitis, who are at risk of developing CAD, with no history of myocardial infarction, angina, or stroke. This study reports results from a 2-year clinical trial; the objective of which was to determine whether long-term SDD therapy can reduce systemic serum inflammatory biomarker in post-menopausal women who have chronic periodontitis.

Materials and Methods

Study design

This study was a 2-year, double-blinded, randomized clinical trial. A total of 128 subjects who gave their informed consent participated in the study and were assigned to study group (Periostat; Galderma Laboratories). Twenty milligrams of doxycycline hyclate were given twice daily for 3 months and same molecule/drug was administered to all study group (participants) and control group (placebo) after oral prophylaxis. All subjects were assigned to regular periodontal...
maintenance therapy (patients education, motivation, and scaling and root planing every 6 months). Serum samples were collected at baseline, 1-year, and 2-year period. No other active periodontal therapy was performed over the study period. A total of 113 participants consented to participate and completed the trial (SDD group, n = 51; placebo group, n = 62).

**Inclusion criteria**
The participants were post-menopausal women, aged between 45 years and 70 years with generalized, moderate to advanced chronic periodontitis having at least 30% of sites having clinical attachment loss >4 mm and at least two sites with probing depths and clinical attachment loss greater than or equal to 5 mm together with bleeding on probing, who were not on hormone replacement therapy and not undergoing any periodontal therapy. They were in good general health with no history of myocardial infarction, angina, or stroke.

**Exclusion criteria**
Patients with history of allergy or sensitivity to tetracycline group of drugs, diabetes, and osteoporosis at either the lumbar spine or the femoral neck were not considered. Exclusions also included patients undergoing regular drug therapy that would affect the inflammatory or immune response and patients receiving active periodontal therapy within the past year.

**Serum inflammatory mediator (CRP) analyses**
Specimen samples were analyzed in three batches. All samples from an individual patient in the same batch were analyzed and treatment assignments were masked and balanced among the batches by means of the block-randomized design.

Assistants trained in phlebotomy drew non-fasting blood samples at baseline, 1-year, and 2-year appointments. Serum was obtained using standard technique and stored it at −80°C until analysis. CRP was measured using a high-sensitivity enzyme-linked immunosorbent assay. The assay had a sensitivity of 0.1 mg/L.

**Results**

**Participants**
One hundred and twenty-eight post-menopausal women, aged between 45 years and 70 years with chronic periodontitis.

**Statistical analyses**
The average 2-year change in CRP was a decrease of 2.5 mg/L for participants in the SDD group and a decrease of 0.5 mg/L for participants in the placebo group, with a standard deviation (SD) of 3.0 mg/L. A total sample of 50 participants per group resulted in 90% power to detect a true difference of 2 mg/L in the mean change in CRP across the 2-year period, assuming an SD of 3.0 mg/L and a two-sided 0.05 α level. We modeled the follow-up biomarker measure (outcome) as a function of study drug with adjustment for the baseline biomarker measure, visit (1 year or 2 years), assay batch, and randomization stratification factors (independent variables).

A secondary, per-protocol analysis included only measurements up to the time at which lack of protocol adherence occurred (e.g., initiation of significant concomitant use of medications or pill count adherence rate below 80%) (SDD group, n = 29; placebo group, n = 25). Tests of interactions were used to perform pre-specified sub-group analyses on the basis of smoking status, time since menopause, study medication regimen adherence, and significant concomitant medication use. No formal adjustment to the α level for multiple tests was made.

**Intent-to-treat analyses**
SDD reduced median CRP levels by 18% compared with placebo across the 2-year protocol, which was statistically significant (ratio of medians [SDD relative to placebo], 0.82; 95% confidence interval [CI], 0.70-0.97; P = 0.02) [Table 1]. The mean CRP level at baseline, although descriptively higher among SDD-group participants than among placebo-group participants, did not differ significantly between treatment groups (P = 0.09). When the participants whose baseline CRP was greater than 9 mg/L (the maximum baseline level among placebo-group participants) (SDD-group participants, n = 4) were removed from the analysis set, the estimated difference in CRP changes across time between the treatment groups remained essentially the same (ratio of medians, 0.83; 95% CI, 0.71-0.97; P = 0.02).

**Discussion**

CAD is an important clinical sequel of menopause and results from a number of studies indicated that systemic inflammation contributes to CAD pathogenesis, with CRP being a robust diagnostic risk marker and risk factor. Out of 11 plasma measures (CRP, serum amyloid A, Lp (a) lipoprotein, apolipoprotein A-I, apolipoprotein B-100, total cholesterol, high density lipids, low density lipids [LDL],

| Table 1: Statistical averages of C-reactive protein levels |
|--------------------------------------------------------|
| Inflammatory biomarker CRP (mg/L) | Placebo group (n=62) | SDD group (n=51) | Estimated effect | 95% CI | P value |
| Median | Mode | SD | Median | Mode | SD | 0.82 | 0.7-0.97 | 0.02 |
| Base line | 2.89 | 3.33 | 1.82 | 3.41 | 4.35 | 3.92 | 0.82 | 0.7-0.97 | 0.02 |
| 1 year | 2.97 | 3.45 | 2.37 | 2.72 | 3.54 | 3.21 | 0.82 | 0.7-0.97 | 0.02 |
| 2 years | 2.80 | 3.20 | 1.99 | 2.70 | 3.47 | 2.79 | 0.82 | 0.7-0.97 | 0.02 |

CRP: C-reactive protein; CI: Confidence interval; SDD: Sub-antimicrobial dose-doxycycline
soluble intercellular adhesion molecule-1, interleukin-6 [IL-6], plasma homocysteine) CRP was the most significant predictor of the risk of cardiovascular events when measured with a widely available, standardized commercial assay; therefore, CRP was used as a preferred inflammatory biomarker in our study.[12]

Results from this 2-year randomized clinical trial suggest potential benefits of SDD in improving inflammatory biomarker levels in post-menopausal women, including a reduction in serum CRP. Status for chronic periodontitis was not taken since the purpose of the study was just to find out the effect of SDD periodontal therapy on serum inflammatory biomarker CRP levels. The study never intended to investigate the effect of SDD on periodontal health parameters such as gingival inflammation, probing depth and hence baseline plaque index, gingival index, and pocket depth have not been recorded.

As it is SDD or in that case any local drug delivery system had to be used as an adjuvant to primary periodontal therapy. However, to assess the effect of SDD only, active periodontal treatment was not initiated as it would reduce inflammation. Administering this patient with primary therapy along with maintenance therapy was paramount to evaluate the possible effect of SDD in patient with periodontitis.

CRP is a systemic inflammatory biomarker that has been reported to be more predictive of cardiovascular events than elevated LDL cholesterol levels.[13] In patients at risk of developing CAD owing to abnormal lipid profiles, CRP may form a complex with elevated levels of LDL cholesterol that has been oxidized by the inflammatory process.[13]

The FDA approved SDD in 1998 as a safe and effective adjunct to scaling and root planing in the treatment of chronic periodontitis. Study results showed that there was a progressive decrease in the serum CRP level and there was no reversal in the serum CRP levels even after the cessation of the SDD regime; however, there were non-significant changes in CRP levels in the placebo group.

**Limitations of the Study**

The post-menopausal women in our study were in good general health, although all of them had chronic periodontitis, which generates elevated levels of pro-inflammatory cytokines[14] in the periodontium; these cytokines, particularly IL-6, are carried by the circulation to the liver where they induce expression of acute-phase proteins, notably CRP. Evidence indicates that tetracyclines, by means of non-antimicrobial mechanisms, can be effective in treating both pathogenic periodontitis and CAD pathways. However, a large multi-center clinical trial of longer duration will be necessary to determine whether SDD can reduce risk of CAD development in addition to reducing serum biomarkers of systemic inflammation.

**Conclusion**

Adjunctive SDD confers clinical benefit in post-menopausal women with periodontitis. A comprehensive treatment strategy is suggested, involving patient education and motivation, reduction of the bacterial burden by scaling and root planing (SRP), host response modulation with SDD, and periodontal risk factor modification.

SDD has a favorable safety profile and is relatively inexpensive, so it is a potentially attractive pharmaceutical approach to managing chronic systemic inflammation.

**References**

1. Libby P. Inflammation in atherosclerosis. Nature 2002;420:868-74.
2. Montecucco F, Mach F. Common inflammatory mediators orchestrate pathophysiological processes in rheumatoid arthritis and atherosclerosis. Rheumatology (Oxford) 2009;48:11-22.
3. Craig RG, Yip JK, So MK, Boylan RJ, Socransky SS, Haffajee AD. Relationship of destructive periodontal disease to the acute-phase response. J Periodontol 2003;74:1007-16.
4. Friedewald VE, Kornman KS, Beck JD, Genco R, Goldfine A, Libby P, et al. The American Journal of Cardiology and Journal of Periodontology Editors’ Consensus: Periodontitis and atherosclerotic cardiovascular disease. Am J Cardiol 2009;104:59-68.
5. Rees M, Stevenson J. British Menopause Society Council. Primary prevention of coronary heart disease in women. Menopause Int 2008;14:40-5.
6. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:836-43.
7. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 2002;347:1557-65.
8. Blankenberg S, Rupprecht HJ, Poirier O, Bickel C, Smieja M, Hafner G, et al. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. Circulation 2003;107:1579-85.
9. Ridker PM, Danielson E, Fonseca FA, Genest J, Goto AM Jr, Kastelein JJ, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008;359:2195-207.
10. Golub LM, Lee HM, Ryan ME, Giannobile WV, Payne J, Sorsa T. Tetracyclines inhibit connective tissue breakdown by multiple non-antimicrobial mechanisms. Adv Dent Res 1998;12:12-26.
11. Payne JB, Stoner JA, Numnikoski PV, Reinhardt RA, Goren AD, Wolff MS, et al. Subantimicrobial dose doxycycline effects on alveolar bone loss in post-menopausal women. J Clin Periodontol 2007;34:776-87.
12. Golub LM, Lee HM, Stoner JA, Sorsa T, Reinhardt RA, Wolff MS, et al. Subantimicrobial-dose doxycycline modulates gingival crevicular fluid biomarkers of periodontitis in postmenopausal osteopenic women. J Periodontol 2008;79:1409-18.
13. Rifai N, Tracy RP, Ridker PM. Clinical efficacy of an automated high-sensitivity C-reactive protein assay. Clin Chem 1999;45:2136-41.
14. Chang MK, Binder CJ, Torzewski M, Witztum JL. C-reactive protein binds to both oxidized LDL and apoptotic cells through recognition of a common ligand: Phosphorylcholine of oxidized phospholipids. Proc Natl Acad Sci U S A 2002:99:13043-8.