Comparative Evaluation of Inhibitory Effect of Curcumin and Doxycycline on Matrix Metalloproteinase-9 Activity in Chronic Periodontitis

Abstract

Background and Objectives: The pathogenesis of inflammatory periodontal diseases essentially involves degradation of extracellular matrix molecules, and collagen breakdown and matrix metalloproteinases (MMPs) are proteases primarily involved in this process. It is known that doxycycline downregulates MMP activity. Curcumin has anti-inflammatory effect and also downregulates MMP activity. Thus, a study was conducted to evaluate the anti-inflammatory effect of curcumin by its inhibition of MMP-9 activity and compare the same with doxycycline, which has known anti-collagenase activity. Subjects and Methods: Gingival tissue samples were obtained from thirty patients diagnosed with chronic periodontitis. The tissue extracts were treated with Curcumin and doxycycline and inhibition of MMP-9 analyzed by gelatinzymography. Results showed that MMP-9 activity was significantly decreased by both the drugs. Curcumin showed 61.01% reduction in the MMP-9 activity at 1500 µg/ml concentration and doxycycline showed 59.58% reduction in the MMP-9 activity at 300 µg/ml concentration. Conclusion: The current study showed that curcumin has inhibitory effect on polymorphonuclear leukocyte-type MMP-9 involved in matrix degradation in periodontitis. Since Curcumin has a potent anti-inflammatory effect, it may have therapeutic potential as a host modulation agent in periodontal diseases.

Keywords: Anti-inflammatory agents, host modulation therapy, matrix metalloproteinases, periodontitis

Introduction

Periodontitis is a chronic inflammatory disease characterized by periodontal tissue destruction, resulting from complex interaction between bacteria and host immune system. The host immune response identifies invading pathogens and tries to neutralize or kill the microorganisms. In the process, it elaborates variety of pro-inflammatory mediators, cytokines, and proteolytic enzymes such as matrix metalloproteinases (MMPs) which cause soft and hard tissue destruction seen in periodontitis. Thus, this host response which is essentially protective by intent paradoxically can also result in tissue damage. Recent therapeutic efforts are focusing on altering or modulating this host response, leading to emergence of a new comprehensive treatment strategy combining traditional periodontal therapies with host modulatory therapy. To date, subantimicrobial dose doxycycline is the only Food and Drug Administration approved systemic therapy, prescribed as a host response modifier in treatment of periodontal disease which downregulates the activity of MMPs.

MMPs are the primary proteases involved in periodontal tissue destruction and synthesized by a variety of infiltrating inflammatory cells (i.e., neutrophils and macrophages), resident cells, and some bacteria. Numerous studies have substantiated the relationship between MMPs and periodontal diseases, with findings of significantly higher MMP-9 (neutrophil-derived MMPs) levels in patients with periodontitis compared to healthy controls. Doxycycline is the most potent tetracycline for inhibition of these MMPs.

Since ancient times, Ayurvedic drugs are being used for the treatment of various...
Curcuma longa (turmeric) is a widely used herb which is distributed throughout tropical and subtropical regions of the world and widely cultivated in India, China, and Indonesia. It has also long been used in both Ayurvedic medicine and Chinese medicine as an anti-inflammatory agent. The active constituent in turmeric is curcumin. Curcumin is known to have an anti-inflammatory effect. Studies show that curcumin limits the activity of two enzymes, lipoxygenase, and cyclooxygenase-2 that are involved in promoting and maintaining inflammation. Studies have also shown that curcumin downregulates the MMP-9 activity. Thus, it may have a potential role in the treatment of inflammatory periodontal disease. Its anti-inflammatory activity in periodontal diseases has not been extensively investigated.

With this background, the study was conducted to evaluate the anti-inflammatory effect of curcumin by its inhibitory effect on MMP-9 activity and compare the same with doxycycline, which is known to have an anticollagenase activity.

Subjects and Methods

Subjects

A total of thirty sites from thirty participants (males and females), above 35 years of age, diagnosed clinically as having generalized moderate-to-severe chronic periodontitis (based on American Academy of Periodontology classification 1999) with pocket depths ranging from 5 to 8 mm were selected for the study. The patients had no history of previous dental treatment and antibiotic or anti-inflammatory drug therapy for the past 6 months. Patients with any history of systemic diseases/conditions, pregnant patients, and smokers were excluded from the study. An informed consent was obtained from the patients before their enrollment into the study, and the study has been independently reviewed and approved by the Institutional Ethical Board. Postcollection of samples, the patients who participated in this study received the standard treatment protocol for chronic periodontitis.

Method of collection of samples

Gingival tissue samples were obtained under aseptic conditions after administration of local anesthesia from patients who were diagnosed with chronic periodontitis. Gingival tissue samples were obtained from sites with pocket depths ranging from 5 to 8 mm. Immediately following excision, these tissue specimens were briefly washed under cold distilled water and blotted dry. Then, the specimens were transferred into sterile plastic vials which contained phosphate buffer solution, pH 7.2, that acted as the transport medium. Specimens were then immediately carried to the laboratory where they were stored at −80°C until use.

In vitro study

Chemicals used

Pure extract of curcumin in powder form (Samy Labs, Bengaluru, India) and doxycycline, i.e., pure doxycycline hyclate in powder form (Aristo Pharmaceuticals Limited, Bengaluru, India) was used for the purpose of the study. The curcumin solution was prepared by dissolving 15 mg of curcumin in 10 ml of distilled water (1500 µg/ml). Preparation of doxycycline solution was by dissolving 3 mg of doxycycline in 10 ml of distilled water (300 µg/ml).

Extraction of matrix metalloproteinase-9

The frozen gingival tissue samples were allowed to thaw to room temperature. Then, each tissue sample was homogenized with 2.5% Triton X-100. This was followed by centrifugation of the homogenized samples for 30 min at 6000 rpm, at 4°C. The resulting supernatant was separated and used for analysis.

Detecting the optimal curcumin and doxycycline concentration

To determine the optimal inhibition of MMP-9 by curcumin, different concentrations of curcumin, i.e., 300, 500, 1000, and 1500 µg/ml were added to 50 µl of gingival tissue extract and incubated at room temperature for 60 min. The addition of increasing concentrations of curcumin to the gingival tissue extract resulted in decreased MMP-9 activity as shown in Figure 1.

Assessing the MMP-9 activity by densitometric analysis demonstrated that the concentration of curcumin required to inhibit more than 50% of MMP-9 activity was 1500 µg/ml. Since curcumin showed more than 50% inhibition of MMP-9 activity at a concentration of 1500 µg/ml as compared to the control (without drug), this concentration was used to pretreat the gingival tissue extract in the study. The curcumin solution with a concentration of 1500 µg/ml was freshly prepared before processing of each sample of gingival tissue extract.

Figure 1: Effect of Curcumin at different concentrations on matrix metalloproteinase-9 activity (%)
Similarly, increasing concentrations of doxycycline, i.e. 50, 100, 200, and 300 µg/ml were added to 50 µl of gingival tissue extract and incubated at room temperature for 60 min. Assessing the MMP-9 activity by densitometric analysis demonstrated that the concentration of doxycycline required to inhibit more than 50% of MMP-9 activity was 300 µg/ml, and thus this concentration was used to pretreat the gingival tissue extract in the study. The doxycycline solution with a concentration of 300 µg/ml was freshly prepared before processing of each sample of gingival tissue extract.

Pretreatment of gingival tissue extract with curcumin and doxycycline

To compare the inhibition of MMP-9 activity, 50 µl of gingival tissue extract was preincubated with freshly prepared solution of 50 µl of curcumin (1500 µl/ml) and 50 µl of doxycycline (300 µl/ml) solution for 60 min at room temperature in separate vials. In addition, 50 µl of extract was also incubated with 50 µl of distilled water which was used as the control.

Assay for matrix metalloproteinase-9 activity

The presence of MMP-9 activity in collected samples was studied by gelatinzymography. Sodium dodecyl sulfate (SDS) poly acrylamide gel electrophoresis on 10% polyacrylamide containing 10% SDS copolymerized with 1 g/L gelatin was used to pretreat tissue extracts under nonreducing conditions without prior boiling. After electrophoresis, to regain the enzyme activity, the gels were rinsed with 2.5% Triton X-100 for 1 h to remove SDS, thus allowing the protein to denature. The gels were then immersed in a proteolysis buffer containing Tris-hydrochloride 50 m M/L (pH 7.6) and CaCl2 20 mM/L and incubated at 37°C for 16 h. The gels were subsequently stained Coomassie Blue (0.25% Coomassie Brilliant Blue R250, 40% methanol, and 10% acetic acid). Gels were destained (30% methanol, 10% acetic acid, and 60% water) until white bands appeared clearly from the blue background. These bands of gelatinlysis detected against the blue background as seen in Figure 2, represented enzymatic activities.

Results

The presence of MMP-9 was studied. The enzymatic activities were detected as unstained bands on gelatin gel by zymography technique. To measure the relative MMP-9 levels, multiimage gel documentation systems were used to scan the clear zones, and the percentage of inhibition was analyzed. Significant differences were found in the MMP activity in treated groups compared to the control.

Table 1 summarizes the range, mean values, and the standard deviation values for curcumin and doxycycline. The mean values show 59.58% reduction in the MMP-9 activity with the addition of doxycycline and 61.01% reduction in the MMP-9 activity with addition of curcumin to the gingival tissue extract under identical conditions.

Table 2 summarizes comparison of MMP-9 inhibitory effect between doxycycline and curcumin.

The results obtained were analyzed statistically using student’s paired t-test.

Discussion

Research in the field of pathogenesis of periodontal disease has shown that various enzymatic activities which are directed toward the destruction of the pathogen in the periodontal connective tissue and MMPs are one group of enzymes which are responsible to a large extent. MMPs are normally tightly regulated, and disruption of this regulation leads to pathologic breakdown of connective tissues. Higher levels of MMPs in the periodontal tissues provoke an imbalance between the production and degradation of collagen, causing

| Drugs       | Range    | Mean±SD                  | P    |
|-------------|----------|--------------------------|------|
| Doxycycline | 55.4-63.4| 59.58±2.1596             | <0.0001 |
| Curcumin    | 55.2-66.2| 61.01±2.8916             |      |

Table 2: Comparison of matrix metalloproteinase-9 inhibitory effect between doxycycline and curcumin

| Drugs       | Mean | Mean difference | P   |
|-------------|------|-----------------|-----|
| Doxycycline | 59.58 | 1.42±2.43       | 0.003 |
| Curcumin    | 61.01 |                 |      |
tooth attachment loss. Especially, polymorphonuclear leukocyte (PMN)-derived MMPs (MMP-8 and MMP-9) are the main proteinases related to tissue destruction and remodeling events in periodontal diseases and numerous studies have substantiated this relationship, with findings of significantly higher MMP-9 levels in patients with periodontitis as compared to healthy controls.\[4,5,15\]

With this understanding, possibility of host modulation so as to reduce the destructive aspects of the host response and hence reduce damage to the periodontium was investigated. First group of drugs that showed this host modulation activity was tetracyclines. Studies have demonstrated that tetracycline could significantly inhibit collagenase activity in gingival crevicular fluid and gingival tissue, even at lower dosage than traditional antimicrobial dosages, i.e. subantimicrobial dosage.\[3\] Tetracyclines inhibit collagenases by binding to the Ca$^{2+}$ or Zn$^{2+}$ (cations) required for the activation of MMPs.\[16\] It is also possible that tetracyclines can inhibit synthesis of neutrophil-derived oxygen radicals, suppressing neutrophil migration and degranulation.\[17\] In addition, long-term treatment can result in other side effects such as anorexia, nausea, epigastric distress, and fatty liver apart from microbial resistance.

Ayurvedic drugs such as Neem, Triphala, Bakul etc. have been used therapeutically since ancient times to treat diseases including periodontal diseases. With the advent of modern synthetic drugs, their convenience of standardized dosage, dramatic efficacy in acute conditions, and most of all simplicity of usage, there was a decline in the use of the plant medicines. However, long-term treatment, with these synthetic drugs, has many adverse effects and they are also not cost effective. As Ayurvedic drugs are widely acclaimed for their minimal side effects and cost-effectiveness in India, they are now again being used extensively in treatment.

It has been shown that certain Ayurvedic medicines also have the host modulation effect, similar to that of tetracyclines. A few recent studies conducted on collagenase inhibition by herbal extracts suggest that, like tetracyclines, herbal extracts are also potent inhibitors of pathogenically elevated collagenase and hence may be used as an alternative adjunct in the management of periodontal diseases.\[18-20\]

Literature records the anti-inflammatory, antioxidant, antibacterial, and antiviral activities\[10\] of turmeric, which has curcumin as its active constituent. However, the potential of curcumin in downregulating the MMP-9 activity in periodontal disease has not been investigated.

With the above details in mind, the present study was conducted to evaluate the anti-inflammatory property of turmeric. In the present study, the anticollagenase activity of curcumin was compared with that of doxycycline which has proven anticollagenase activity.

Research has shown that the predominant MMPs in inflamed gingival and periodontal tissues are PMN-type MMPs (MMP-8 and MMP-9). Elevated activity of gelatinases (MMP-2 and MMP-9) has also been found in inflamed gingival tissues from chronic periodontitis patients.\[5,21\] Thus, in the present study, we decided to use gingival tissue samples that were obtained from patients diagnosed clinically with chronic periodontitis.

The reduction in MMP-9 activity of each sample of gingival tissue extract when incubated with the drugs, i.e., doxycycline and curcumin, was expressed as percentage of reduction from the control (without drug). The mean values showed 59.58% reduction in MMP-9 activity with the addition of doxycycline and 61.01% reduction in MMP-9 activity with the addition of curcumin to gingival tissue extract under identical conditions. These results show that curcumin has a significant inhibitory effect on PMN-type MMP-9, and this inhibitory effect of curcumin is comparable to that of doxycycline when the percentage
of inhibition as compared to control was analyzed. Curcumin also showed a significant reduction in the MMP-9 activity with an average inhibition of about 61% which is also statistically highly significant ($P < 0.0001$) when compared to control (without drug). When MMP-9 inhibitory effect of curcumin was compared to that of doxycycline, the inhibitory effect of curcumin was also statistically significant ($P < 0.003$). In the present study since it was observed that curcumin at concentration of 1500 µg/ml showed more than 50% inhibition of MMP-9 activity, this concentration of curcumin was used as the optimal concentration which could be compared to 300 µg/ml of doxycycline. However, further studies need to be done to determine if a more significant MMP-9 inhibitory effect will be seen if curcumin concentration above 1500 µg/ml is used. Clinical trials have shown no significant toxicity even when curcumin was administered at doses as high as 8 g/day$^{[22]}$ and 12 g/day.$^{[23]}$ However, overall results show that curcumin has the ability to significantly inhibit the MMP-9 activity.

The present study suggested that curcumin could produce significant inhibition of MMPs at 1500 µg/ml concentration, which is well under the safe drug profile confirmed by toxicological studies.$^{[22,23]}$ Thus, this study shows that doxycycline and curcumin possess anticollagenase activity in vitro.

The finding of the present study is significant and confirms the use of curcumin in treating periodontal diseases. A recent in vitro study showed that curcumin modulates periodontal disease and had potent anti-inflammatory effects when systemically administered in ligature-induced periodontitis in rats.$^{[24]}$ Studies have shown that one of the main mechanisms for anti-inflammatory effects of curcumin may be the inhibition of nuclear factor kappa B (NF-κB)$^{[11,24]}$. The curcumin-mediated inhibition of MMP-9 gene expression appears to occur through NF-κB and activator protein-1 because their DNA binding activities were suppressed by curcumin.$^{[14,25]}$

When compared to tetracycline, curcumin has better anti-inflammatory effect, is more cost effective, and has minimal side effects and thus can be tried as a substitute for tetracycline as an anticollagenase agent. Curcumin also allows suppression of collagenase activity well within the safe dosage profile confirmed by toxicological studies.

The in vivo environment is substantially different, and inherent limitations of an in vitro replication may constrain our understanding of the systemic effects of curcumin. Further studies on cultured cells and also in vivo studies of curcumin are needed to define its toxicological profile before making it part of the therapeutic regimen in periodontal treatment.

### Conclusion

In the light of observations from the current study, it can be concluded that doxycycline and curcumin possess anticollagenase activity in vitro. Since curcumin has better anti-inflammatory effect compared to tetracycline, curcumin being more cost effective and with no side effects can be tried as a substitute for tetracycline as an anticollagenase agent. Thus, curcumin may have therapeutic potential as a host modulation agent in periodontal diseases.

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### Conflicts of interest

There are no conflicts of interest.

### References

1. Offenbacher S. Periodontal diseases: Pathogenesis. Ann Periodontol 1996;1:821-78.
2. Paquette DW, Williams RC. Modulation of host inflammatory mediators as a treatment strategy for periodontal diseases. Periodontol 2000 2000;24:239-52.
3. Preshaw PM, Hefi AF, Novak MJ, Michalowicz BS, Pihlstrom BL, Schoor R, et al. Subantimicrobial dose doxycycline enhances the efficacy of scaling and root planing in chronic periodontitis: A multicenter trial. J Periodontol 2004;75:1068-76.
4. Türköglu O, Barış N, Tervahartiala T, Şenarslan Ö, Sorsa T, Atilla G, et al. Evaluation of systemic levels of neutrophilic enzymes in patients with hypertension and chronic periodontitis. J Periodontol 2014;85:908-16.
5. Choi DH, Moon IS, Choi BK, Paik JW, Kim YS, Choi SH, et al. Effects of sub-antimicrobial dose doxycycline therapy on crevicular fluid MMP-8, and gingival tissue MMP-9, TIMP-1 and IL-6 levels in chronic periodontitis. J Periodontal Res 2004;39:20-6.
6. Tilakaratne A, Soory M. Anti-inflammatory actions of adjunctive tetracyclines and other agents in periodontitis and associated comorbidities. Open Dent J 2014;8:109-24.
7. Gobu LM, Wolff M, Roberts S, Lee HM, Leung M, Payonk GS, et al. Treating periodontal diseases by blocking tissue-destructive enzymes. J Am Dent Assoc 1994;125:163-9.
8. Surathu N, Kurumathur AV. Traditional therapies in the management of periodontal disease in India and China. Periodontol 2000 2001;56:14-24.
9. Beevers CS, Huang S. Pharmacological and clinical properties of curcumin. Botanics 2011;1:5-18.

10. Araújo CC, Leon LL. Biological activities of Curcuma longa L. Mem Inst Oswaldo Cruz 2001;96:723-8.

11. Ammon HP, Safayhi H, Mack T, Sabieraj J. Mechanism of antiinflammatory actions of curcumin and boswellic acids. J Ethnopharmacol 1993;38:113-9.

12. Rao CV. Regulation of COX and LOX by curcumin. Adv Exp Med Biol 2007;595:213-26.

13. Swarnakar S, Paul S. Curcumin arrests endometriosis by downregulation of matrix metalloproteinase-9 activity. Indian J Biochem Biophys 2009;46:59-65.

14. Cao J, Han Z, Tian L, Chen K, Fan Y, Ye B, et al. Curcumin inhibits EMMPRIN and MMP-9 expression through AMPK-MAPK and PKC signaling in PMA induced macrophages. J Transl Med 2014;12:266.

15. Marcaccini AM, Meschiari CA, Zuardi LR, de Sousa TS, Taba M Jr., Teofilo JM, et al. Gingival crevicular fluid levels of MMP-8, MMP-9, TIMP-2, and MPO decrease after periodontal therapy. J Clin Periodontol 2010;37:180-90.

16. Seymour RA, Heasman PA. Tetracyclines in the management of periodontal diseases. A review. J Clin Periodontol 1995;22:22-35.

17. Gabler WL, Creamer HR. Suppression of human neutrophil functions by tetracyclines. J Periodontal Res 1991;26:52-8.

18. Vijayalakshmi D, Dhandapani R, Jayaveni S, Jithendra PS, Rose C, Mandal AB, et al. In vitro anti inflammatory activity of aloe vera by down regulation of MMP-9 in peripheral blood mononuclear cells. J Ethnopharmacol 2012;141:542-6.

19. Santos J, La VD, Bergeron C, Grenier D. Inhibition of host- and bacteria-derived proteinases by natural anthocyanins. J Periodontal Res 2011;46:550-7.

20. Abraham S, Kumar MS, Sehgal PK, Nitish S, Jayakumar ND. Evaluation of the inhibitory effect of triphala on PMN-type matrix metalloproteinase (MMP-9). J Periodontol 2005;76:497-502.

21. Ciancio S, Ashley R. Safety and efficacy of sub-antimicrobial-dose doxycycline therapy in patients with adult periodontitis. Adv Dent Res 1998;12:27-31.

22. Gupta SC, Patchva S, Aggarwal BB. Therapeutic roles of curcumin: Lessons learned from clinical trials. AAPS J 2013;15:195-218.

23. Lao CD, Ruffin MT 4th, Normolle D, Heath DD, Murray SL, Bailey JM, et al. Dose escalation of a curcuminoid formulation. BMC Complement Altern Med 2006;6:10.

24. Guimarães MR, Coimbra LS, de Aquino SG, Spolidorio LC, Kirkwood KL, Rossa C Jr., et al. Potent anti-inflammatory effects of systemically administered curcumin modulate periodontal disease in vivo. J Periodontal Res 2011;46:269-79.

25. Kim SK, Kim YW, Youn HJ, Jung SH. Curcumin suppresses MMP-9 expression via inhibition of PKCa/MAPKs and NF-kB/ AP-1 activation in MCF-7 cells. Cancer Res 2012;72(24 Suppl).