A Novel Chemically-Modified Curcumin 2.24: Short-term Systemic Treatment for Natural Periodontitis in Dogs

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Abstract

Chronic periodontitis, a severe periodontal inflammatory disease, is initiated by the anaerobic microbial biofilm, however the tissue-breakdown is predominantly mediated by the host-response. The pathogenesis mediated by the latter is characterized by the generation of inflammatory mediators (e.g., cytokines), and the production of collagenolytic and tissue-destructive enzymes (especially the matrix metalloproteinases, MMPs) causing connective tissue and bone destruction. These biochemical changes, such as over-production of MMP-9, might be detected in the very-early stage of this disease/inflammatory process, even before apparent clinical manifestations. Interestingly, our pilot study of a short-term/1-month therapy of CMC2.24 (a novel chemically-modified curcumin) in the beagle dogs with naturally-occurring periodontitis indicated that, it did appear to significantly reduce both pro- and activated-MMP-9 in peripheral blood, compared to placebo group at early stage (1 month), prior to apparent clinical improvement at late stage (3 months). And therefore, MMP-9 may serve as an early and sensitive biomarker that can precede and predict future clinical changes in disease severity and response to treatment which we observed in our long-term study in this dog model of natural periodontitis.

Main Text

Deng et al (1) recently proposed a newer therapeutic strategy to treat naturally-occurring periodontitis (NOP) in beagle dogs, involving oral/systemic administration of a novel chemically-modified curcumin (CMC2.24; a phenylaminocarbonyl curcumin), for 3-months, as a host-response modulator. This pleiotropic therapy, based on a well-known natural product, was designed to reduce pathologic inflammatory/collagenolysis in oral and other diseases, and includes enhanced resolution of inflammation and inhibition of bone loss. In the current study, we determined whether shorter-term therapy with this novel compound, for 1-month, as an adjunct to mechanical debridement, could be effective in reducing NOP in beagles, an animal model with characteristics similar to human periodontitis. We also determined whether biochemical changes, in inflammatory mediators and tissue-destructive matrix metalloproteinases (MMPs), are affected by CMC2.24 treatment before clinical changes become apparent.

Interestingly, our results indicated that a short-term/1-month protocol, although it did not result in obvious clinical improvement at early stage, did appear to reduce both pro- and activated-MMP-9 in peripheral blood, which might serve as early and sensitive biomarkers that precede and predict future clinical changes in oral (and other) inflammatory diseases.

In brief, although a 1-month CMC2.24 treatment produced no detectable effect on inflammatory cytokines [e.g., Interleukin-1 beta (IL-1 β), Interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α): which were affected with longer-term/3-month therapy (1)) in the GCF and circulation of these dogs with NOP, an significant effect of CMC2.24 on both pro- and activated-MMP-9 was observed in the peripheral blood (Fig. 1). At baseline, the level of activated-MMP-9 in CMC2.24-treated group was 100% greater/higher ($p < 0.05$) than in the placebo-group. However, after 1-month therapy, although the activated-MMP-9 in the

placebo-group remained unchanged, the dogs treated with CMC2.24 showed a significant ($p < 0.05$) 50% reduction of activated-MMP-9 down to the unchanged levels in placebo-group. In addition, the total- and pro-MMP-9 values in the CMC2.24-treated group were also significantly reduced; in contrast, their baseline values were identical to placebo. These data suggest a possible protection by this novel curcuminoid, early-on in the disease/inflammatory process. The reduction of activated-MMP-9 can be partially due to the decreased levels of total-MMP-9, or this compound promotes the EXCESSIVE degradation of pro-MMP-9 by proteinases (e.g., trypsin), during the activation process, resulting in enzymatically-inactive small molecular weight fragments. In fact, this mechanism had been proposed previously by us to explain the loss of collagenolytic activity when pro-collagenase (pro-MMP-8) and pro-gelatinase (pro-MMP-9) were activated by pre-incubation with trypsin in the presence of doxycycline (due to $\text{Ca}^{2+}/\text{Zn}^{2+}$ binding by this tetracycline).(2) This novel mechanism was also described by Smith et al (3, 4) who stated that “doxycycline disrupts the hemopexin-like or catalytic domain of collagenase, and alters the conformation of pro-collagenase or collagenase by binding enzyme-associated Ca^{2+} , resulting in the MMPs becoming more susceptible to proteolysis and leading to irreversible loss of enzyme protein.” Since CMC2.24 has a cation-binding site similar to (and more potent than) the tetracyclines, a polyenolic assembly, it may exhibit a similar mechanism of disrupting the conformation of MMP-9, thus rendering this MMP susceptible to degradation to small-inactive fragments. Future experiments are needed to validate this proposed mechanism.

It's worth noting that studies have indicated that MMP-9 helps mediate connective tissue breakdown and alveolar bone loss during periodontitis.(5–8) Thus, treatment with MMP-inhibitors may effectively suppress tissue breakdown, and decrease severity of periodontitis, as well as other chronic inflammatory diseases such as diabetes, rheumatoid arthritis and osteoporosis.(2) Previously, we mentioned that MMPs have been found to degrade the insulin receptor which would be expected to suppress normal glucose metabolism/control.(5, 9–13) Another possible mechanism of action of MMP-inhibitors is pH-dependent. During the MMP-8 activation process either with APMA or trypsin, when doxycycline was added, the activated-MMP-8 was excessively degraded into smaller-molecular weight inactive fragments (< 30 kDa).(2, 14) Thus, the reduced level of activated-MMP-9 in this 1-month study by CMC2.24 could be explained (at least in part) by this fragmentation theory; longer-term therapy with CMC2.24 could then result in additional biochemical as well as clinical improvements.

Summary

In conclusion, this preliminary 1-month study indicates that orally-administered CMC2.24 is a rapidly effective and potent inhibitor of host-derived MMPs and that this early effect, with longer periods of treatment, ultimately results in clinical improvements in periodontal and other diseases. The potential importance of the current observations are supported by previous, longer-term/3-months studies which demonstrated significant improvements in clinical (as well as biomarker) measurements of this chronic inflammatory disease.

Abbreviations

CMC2.24

Chemically-modified curcumin 2.24

IL-1 β

Interleukin-1 beta

IL-6

Interleukin-6

MMPs

Matrix metalloproteinases

NOP

Naturally-occurring periodontitis

TNF- α

Tumor necrosis factor-alpha

Declarations

Ethics approval and consent to participate

Protocols for the animal studies were approved by Stony Brook University's Institutional Animal Care and Use Committee (IACUC #896357). Animals were housed in the Division of Laboratory Animal Resources (DLAR) at Stony Brook University, with care provided by the center's personnel. All procedures were conducted at the same location. This facility follows the Animal Welfare Act (USDA enforced), the Public Health Service Act (OLAW enforced), and NY State law (DOH enforced), and is an AAALAC International accredited facility.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Jie Deng was the principle experiment conductor, and had the substantial contributions to the acquisition, analysis, and interpretation of data, and was a major contributor in writing the manuscript. Lorne M. Golub, Hsi-Ming Lee and Ying Gu had the substantial contributions to the conception, design of the work, acquisition, interpretation of data and substantively revised the manuscript. Heta Dinesh Bhatt helped to perform the experiment, and had the contributions to the acquisition and data analysis. Hou-Lin Hong helped the statistical data analysis. Thomas Zimmerman is the chief of the Division of Laboratory Animal Resources (DLAR). He and his team performed the animal caring work and the general anaesthesia. Lorne M. Golub and Francis Johnson are also listed as the inventors on several related Patents, including CMC2.24. In addition, both Lorne M. Golub and Francis Johnson are minor shareholders in Traverse Biosciences, Inc., and Joseph Scaduto is the president and major shareholder in Traverse Biosciences Inc. They received grants from NIH/NIDCR (Phase II STTR Award 2R42DE024946-02) and from Traverse Biosciences Inc. Also, Traverse Biosciences Inc. has exclusively licensed patents from the Research Foundation for the State University of New York (RF/SUNY) covering the structure and use of the chemically-modified curcumins for the purpose of commercialization in human and animal health.

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Figures

Figure 1

The effects of orally administered CMC2.24 or placebo on MMP-9 in peripheral blood (plasma), collected at baseline (left side) and 1-month (right side), measured by gelatin zymography and scanned densitometrically. Grey bar: Placebo Group; black bar: CMC2.24 Treatment Group. Each value represents the Mean (n=4 dogs/per group) ± Standard Error (S.E.M.). # indicates p<0.05, values of CMC2.24 treatment group at each time period compared to placebo group. * indicates p<0.05, values of CMC2.24 treatment group (activated-MMP-9) at 1-month compared to its own baseline. Std: Standard. Tx: Treatment of CMC2.24.