1-Tetradecanol Complex Reduces Progression of *Porphyromonas gingivalis*–Induced Experimental Periodontitis in Rabbits

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Background: It has been recently shown that monounsaturated fatty acids inhibit endothelial activation and reduce tissue responsiveness to cytokines. The present study has been planned to investigate topical application of a novel monounsaturated fatty acid complex (1-tetradecanol complex) for prevention of *Porphyromonas gingivalis*–induced periodontitis in rabbits.

Methods: Experimental periodontitis was induced in New Zealand white rabbits with silk sutures tied around the mandibular second premolars bilaterally, followed by the topical application of 10^9 colony forming units (CFU) of *P. gingivalis*. 1-Tetradecanol complex (1-TDC) was topically applied at 1- and 10-mg/ml concentrations in five animals in each group, whereas control animals received olive oil vehicle (five animals) three times per week for 6 weeks. Negative controls included ligature alone (14 animals) or ligature + *P. gingivalis* (non-treatment; 15 animals). Rabbits were sacrificed after 6 weeks, and mandibular block sections were obtained; tissues were decalcified and embedded in paraffin. Thin sections (5 μm) were stained with hematoxylin and eosin or tartrate-resistant acid phosphatase. Macroscopic and histologic evaluation of samples was followed by the characterization of cellular inflammatory infiltrate and quantitative histomorphometric measurements.

Results: Treatment with both concentrations of 1-TDC and vehicle resulted in significant prevention of macroscopic periodontal inflammation and bone loss (75%; $P < 0.05$) compared to the non-treatment (ligature + *P. gingivalis*) group, where significant periodontal tissue destruction characterized by attachment and bone loss was detected. However, there was no statistically significant difference between the vehicle and both 1-TDC groups. Histologically, 1-TDC inhibited inflammatory cell infiltration and prevented osteoclastogenesis, whereas treatment with vehicle did not show the same effect as in the 1-TDC groups; the difference between vehicle and the higher concentration of 1-TDC (10 mg/ml) was statistically significant.

Conclusion: Topical application of an esterified monounsaturated fatty acid complex (1-TDC) was found promising in preventing bone loss, inflammatory cell infiltration, and connective tissue destruction in the rabbit periodontitis model. *J Periodontol 2007;78:924-932.*

KEY WORDS

Alveolar bone loss; fatty acids; inflammation; prevention.

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Periodontal disease is a local inflammation initiated by dental plaque bacteria. In chronic periodontitis, the presence of periodontal pathogens such as *Porphyromonas gingivalis* and *Tannerella forsythensis* are necessary for initiation of inflammation; however, the progression of periodontal disease depends on the host response to various bacterial products and components.[1] The destruction observed in periodontal disease is the result of an improperly regulated innate immune response to bacterial infection characterized by the recruitment of inflammatory cells, generation of proinflammatory proteins such as cytokines and chemokines, and activation of osteoclasts.[1-4] Several inflammatory mediators including cytokines (i.e., interleukin [IL]-1β and IL-6) and lipid mediators are associated with periodontal disease.[5] Among the lipid mediators are the arachidonic acid–derived products, including leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2).[6,7] Indeed, some of the pathophysiologic events that occur in periodontal diseases, particularly early events, can be explained

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by the activities of these lipid mediators. For example, LTB_4, a well-appreciated and potent chemoattractant, also initiates the accumulation of leukocytes within inflamed sites, stimulates the release of granule-associated enzymes, and was found to stimulate bone resorption.[8] LTB_4 exerts chemotaxis,[9] induction of leukocyte aggregation,[10] degranulation,[11] and superoxide generation.[12]

Fatty acids have been proposed to reduce chronic inflammation in individuals with arthritis by reducing the release of LTB_4 from stimulated neutrophils and IL-1 from monocytes.[13,14] Among these fatty acids, omega-3 polyunsaturated fatty acids (PUFAs) are essential fatty acids provided by dietary sources and exert anti-inflammatory effects that limit the inflammatory cascade.[15] Their attenuating effect on triglyceride levels that ultimately impairs the synthesis of eicosanoids has been shown to be effective in the treatment of various inflammatory conditions such as psoriasis and rheumatoid arthritis.[16-18] Experience with PUFA supplementation in other chronic immune-mediated inflammatory conditions such as rheumatoid arthritis,[19] systemic lupus erythematosus,[20] Sjogren syndrome,[17] ulcerative colitis,[21] psoriasis,[22] and atopic dermatitis[23] initiated studies on periodontal diseases, which share several important common pathways with these inflammatory conditions.[24] However, when dietary supplements of omega-3 PUFA were used to treat or prevent gingival inflammatory conditions including gingivitis and periodontitis, no significant differences were found in controlling the gingival inflammation, most probably because of the lack of sufficient concentration of the systemic use of omega-3 PUFA in the local environment.[25] On the other hand, the high epithelial penetration ability of fatty acids[26] makes it probable that local application is useful for the treatment of local oral inflammatory diseases. Indeed, topical application of omega-3 PUFA has been shown to be successful in the treatment of other inflammatory diseases, such as psoriasis,[27] as well as experimental periodontitis in animal models.[28-30]

Parallel to the findings with PUFAs, there is evidence suggesting that the substitution of monounsaturated fatty acids (MUFAs), another group of fatty acids, instead of saturated fatty acids (SFAs), may favorably affect cardiovascular risk.[31-33] MUFAs are distinguished from the other fatty acid classes on the basis of having only one double bond, whereas PUFAs have two or more double bonds and SFAs have none. Epidemiologic studies that controlled for a number of potentially confounding variables have reported protective effects of MUFAs against coronary heart disease (CHD).[34-37] Moreover, evidence from controlled clinical studies has shown that MUFAs favorably affect a number of risk factors for CHD, including plasma lipids and lipoproteins, factors related to thrombogenesis, in vitro low-density lipoprotein (LDL) oxidative susceptibility, and insulin sensitivity.[38-41] Likewise, experimental evidence suggests that MUFA diets favorably influence blood pressure, coagulation, endothelial activation, inflammation, and thermogenic capacity.[42,43]

Recently, 1-tetradecanol complex (1-TDC), a novel MUFA mixture, which contains a blend of esterified MUFAs, has been shown to inhibit endothelial activation and reduce tissue responsiveness to cytokines. Preliminary results revealed that 1-TDC significantly inhibited thromboxane A_2 production in human recombinant embryonic kidney (HEK)-293 cells through the inhibition of thromboxane synthase receptor (MDS Pharma Services-Taiwan, Pharmacology Laboratories, Taipei, Taiwan, unpublished data), which may suggest inhibiting platelet aggregation through the cyclooxygenase pathway. Preliminary data have also revealed that 1-TDC inhibited the growth of *Actinomyces viscosus* in vitro (MDS Pharma Services, unpublished data). Based on these preliminary data and the reported high epithelial penetration ability of fatty acids,[26] which may eliminate the previously reported limitations in the efficacy of systemic administration of these fatty acid compounds, we studied the preventive effects of a topical formulation of 1-TDC on experimental periodontitis in a previously established rabbit model.[4,30]

MATERIALS AND METHODS

Animal Model and Experimental Design

The test agent was the esterified MUFA composition, 1-TDC,[‡] which was given in two different concentrations (1 or 10 mg/ml). The vehicle was pure olive oil, which was the carrier used for the test agent and the placebo. Fifteen male New Zealand white rabbits (3.5 to 4.0 kg each) were purchased,[§] equilibrated, and housed at the Laboratory Animal Science Center at Boston University Medical Center. The study was approved by Boston University Medical Center Institutional Animal Care and Use Committee (BUMC IACUC) prior to study initiation (IACUC protocol # AN-14580). In addition, the BUMC Institutional Biohazard Committee (IBC) approved the use of *P. gingivalis* in this animal model to induce periodontal disease (IBC protocol # 06-010). Animals were randomly assigned into three treatment groups: vehicle group (five animals): ligature + *P. gingivalis* + vehicle; test group 1 (five animals), ligature + *P. gingivalis* + 1-TDC at a 1-mg/ml concentration; test group 2 (five animals), ligature + *P. gingivalis* + 1-TDC at 10-mg/ml concentration. Furthermore, in a separate experiment, the established negative controls including ligature alone

‡ Imagenetix, San Diego, CA.
§ Pine Acre Farms, Norton, MA.

(14 animals) and ligature + *P. gingivalis* (non-treatment; 15 animals) were prepared. On the first day of the experiment, weight was recorded, and rabbits were anesthetized using 40 mg/kg intramuscular (IM) ketamine‖ and 5 mg/kg IM xylazine.¶ Ligatures (3-0 braided silk suture)# were placed around the second premolars on both sides of the mandible. Multiple applications (every other day for a period of 6 weeks) of carboxymethylcellulose gel containing *P. gingivalis* and subsequently topical medications (1-TDC or placebo) to the ligatures were carried out under inhalation anesthesia** (4% induction and then 2% maintenance). *P. gingivalis* (strain A7436) was grown using standard procedures; 10^9 CFU was mixed with carboxymethylcellulose†† to form a thick slurry and applied topically to the ligated teeth to induce periodontitis as previously described.[30] After *P. gingivalis* application, test agents were applied topically to the same areas for 6 weeks. At these times, the sutures were also checked, and lost sutures were replaced. Animals were monitored by daily health check including food and fluid intake, urination, weight gain or loss, and general behavior. At the end of the 6 weeks, animals were sacrificed using an overdose (120 mg/kg, intravenously) of pentobarbital;‡‡ the mandible of each rabbit was dissected free of muscles and soft tissue, keeping the attached gingiva intact with the alveolar bone. The mandible was split in half from the midline between the central incisors. The left half was taken for morphometric analysis of the bone, and the right half was used for histologic evaluation.

Morphometric Analysis

One half of the sectioned mandible was defleshed by immersing in 10% hydrogen peroxide (3 to 4 days; room temperature). The soft tissue was carefully removed, and the mandible was stained with methylene blue for good visual distinction between the tooth and the bone. The bone level around the second premolar was measured directly by a 0.5-mm calibrated periodontal probe. Mean crestal bone levels were calculated for each tooth as the average of the bone level at buccal and lingual aspects. Similarly, for the proximal bone level, measurements were made at the mesial and distal aspects of the tooth. The measurements were taken from both the buccal and lingual side on both proximal aspects of the second premolar, and the mean proximal bone level was calculated. The sectioned mandible was mounted and photographed using an inverted microscope at ×10. The captured image was also analyzed as above, and the mean crestal bone level around the tooth was calculated in millimeters using imaging software.§§

Radiographic Analysis

The percentage of the tooth within the bone was calculated radiographically using a modified Bjorn technique.[4] The radiographs were taken with a digital radiograph.‖‖ To quantify bone loss, the length of the tooth from the cusp tip to the apex of the root was measured, as was the length of the tooth structure outside the bone, measured from the cusp tip to the coronal extent of the proximal bone. From this, the percentage of the tooth within the bone was calculated. Bone values are expressed as the percentage of the tooth in the bone (length of tooth in bone × 100/total length of tooth). As dictated by the BUMC IACUC, we used historical data for healthy tooth bone levels of the rabbit that represent ~90.2% ± 0.3% tooth length within the bone radiographically.[4] Using this information, the percent bone loss was calculated for each animal compared to healthy conditions where no bone loss detected,[4] and the mean bone loss (mean ± SD) was used to represent each of the groups for comparisons.

Histologic Analysis

For histologic analysis, the other half of the mandible was immersed in a volume of decalcification solution¶¶ equal to at least 10 times the size of section; solution was replaced every 24 hours for 2 weeks. Decalcification was confirmed by serial radiographs, which were taken every other day. After decalcification, the tissues were rinsed for 3 minutes in flowing deionized water and kept in formalin for ≥24 hours before embedding in paraffin. Thin sections (5 μm) were cut, and sections were either conventionally stained with hematoxylin and eosin (H&E) to identify the cellular composition of the inflammatory infiltrate or with tartrate-resistant acid phosphatase (TRAP) to detect osteoclastogenesis.

Statistical Analysis

The data obtained by direct measurements during morphologic assessment and by histomorphometric measurements were used in multiple statistical analyses. Mean values for linear and area measurements were used to determine the changes in bone level. Ratio calculations were used, and multiple comparisons within groups were made using analysis of variance (ANOVA) with Bonferroni correction. Statistical comparisons were made between the two control groups (ligature alone, N = 14 animals; ligature and *P. gingivalis*, N = 15 animals) and treatment groups to test the effectiveness of the vehicle and two different concentrations of esterified fatty acid formulation.

‖ Ketaset, Fort Dodge Animal Health, Fort Dodge, IA.
¶ Anased, LLOYD Laboratories, Shenandoah, IA.
# Sharpoint, Surgical Specialties, Reading, PA.
** Isoflurane, Hospira, Lake Forest, IL.
†† Sigma-Aldrich, St. Louis, MO.
‡‡ Pentobarbital Euthanasia-5 Solution, Veterinary Laboratories, Lenexa, KS.
§§ Image-Pro Plus 4.0, Media Cybernetics, Silver Spring, MD.
‖‖ Schick Technologies, Long Island City, NY.
¶¶ Immunocal, Decal, Tallman, NY.

RESULTS

Macroscopic Analysis

Figures 1 and 2 show the gingival tissue and defleshed bone specimens from buccal and lingual aspects of mandibles of rabbits treated either by vehicle or 1-TDC applied at two different concentrations. All three applications resulted in a statistically significant ($P<0.05$) preventive effect on bone loss compared to the ligature + *P. gingivalis* group (non-treatment). However, no obvious differences were detected between the test application groups and vehicle. Topical delivery of two different doses of 1-TDC before *P. gingivalis* application did not show any difference in the prevention of gingival inflammation or bone destruction compared to vehicle group, and no apparent dose-dependent effect was detected between 1-TDC groups. Figure 3 shows the quantitative analyses of defleshed bone specimens. The quantitative findings further showed that all three groups (vehicle and 1-TDC groups) demonstrated inhibition of bone loss compared to the non-treatment group ($P<0.05$), with no significant difference between the vehicle and test groups.

Figure 1.
Macroscopic evaluation of soft tissue changes around second premolars where a ligature and P. gingivalis were applied to induce periodontitis over a 6-week period. Bacterial challenge together with ligature placement led to significant soft tissue changes in this rabbit model versus ligature alone. The red arrows show the buccal surface where there is a detectable gingival inflammation. In treatment groups where vehicle and 1-TDC (in two different concentrations) were preventively applied before periodontal disease induction, the gingival inflammation was minimal.

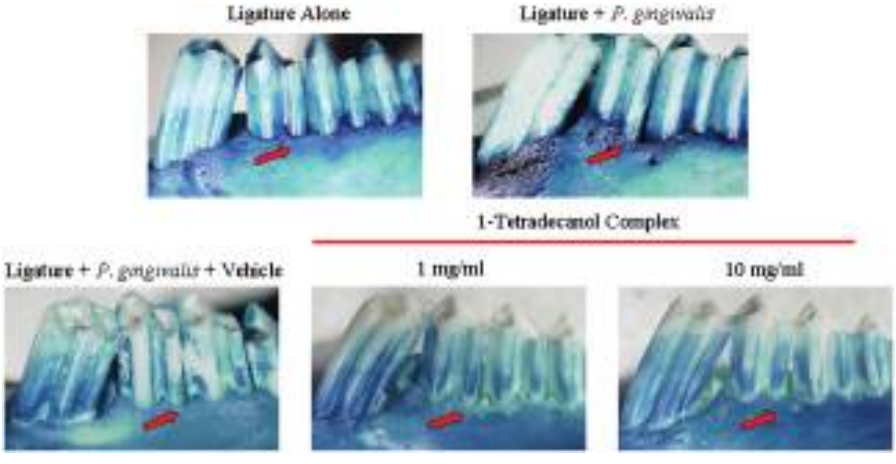

Figure 2.
Macroscopic evaluation of hard tissue changes on defleshed bone specimens that were stained with methylene blue to indicate the changes in bone level in the areas where periodontitis was induced. Similar to soft tissue changes, the red arrows show detectable bone loss around the ligated teeth in non-treatment group compared to the ligature-alone group. Conversely, in treatment groups (vehicle and 1-TDC), the hard tissue changes were minimal.

Radiographic Analysis

Figure 4 shows the radiographic images of the animals in all five groups and shows comparison of the percentage of bone loss between groups. Radiographically, the vehicle and both 1-TDC groups presented obvious inhibition of bone loss versus the non-treatment group, where clinical periodontal destruction was confirmed with significant bone loss around the second premolar. Statistical analysis of the radiographic measurements revealed that the difference in bone loss between 1-TDC treatment and non-treatment was statistically significant, whereas the vehicle group failed to show statistical significance. However, 1-TDC application did not show any statistically significant difference compared to the vehicle group.

Histomorphometric Analyses

Histomorphometric measurements were performed at the ligated site of each tooth on the H&E-stained sections. Linear measurements were made at three points: crestal, mid, and apical third of the alveolar bone. To quantitatively analyze periodontal disease progression with 1-TDC treatment compared to vehicle and non-treatment groups, the mean value (± SD) of the linear distance was calculated for each group. Linear distance was described as the horizontal distance between the tooth surface and

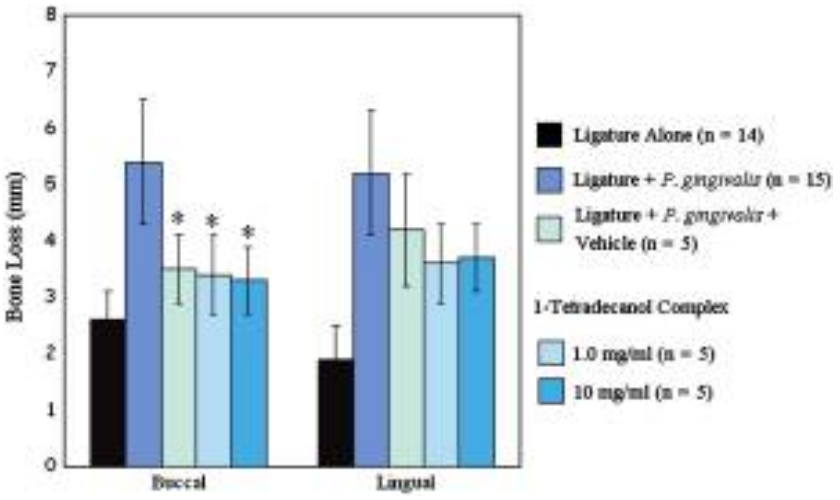

Figure 3.
*Quantitative analyses of bone loss on defleshed bone specimens. Clinical bone loss was calculated using the distance between alveolar crest and cusp tip of the tooth. Statistical analysis showed a significant difference in bone loss between non-treatment (*P. gingivalis *and ligature group) and vehicle and the two 1-TDC treatment groups. However, there was no significant difference between any of the treatment groups. *P <0.05 compared to ligature* + P. gingivalis.

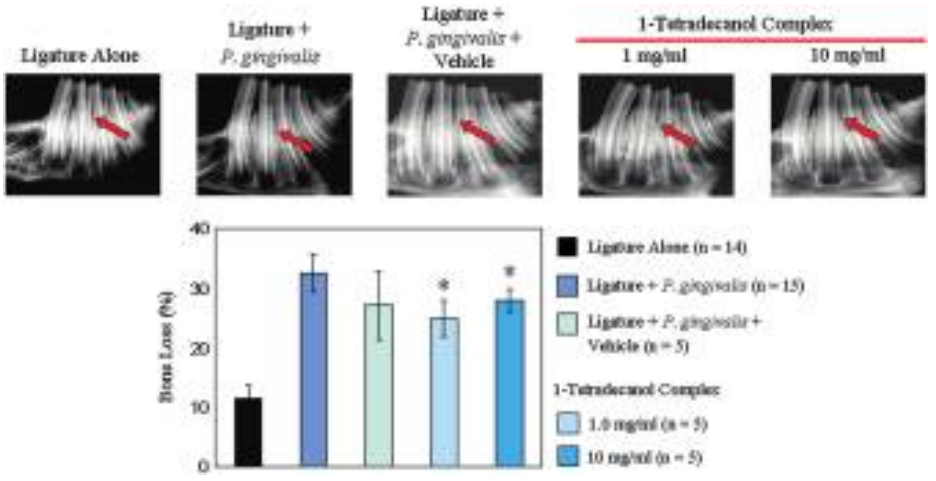

Figure 4.
*Radiographic analyses of hard tissue components. The non-treatment group clearly showed the amount of bone loss that was observed clinically, and it was significantly different compared to the ligature-alone group. Treatment groups showed a slight difference between the vehicle and both test groups; however, this difference was not statistically significant between any of the groups shown. Conversely, there was statistically significant inhibition in bone loss associated with animals in both 1-TDC treatment groups compared to the non-treatment group. *P <0.05 compared to ligature* + P. gingivalis. *Arrows indicate radiographic bone level.*

the alveolar bone at three chosen levels: the apical, middle, and coronal third of the root (Fig. 5).

1-TDC application at both concentrations showed significantly less bone loss compared to the non-treatment group ($P = 0.02$); however, compared to the vehicle group, only the higher concentration of 1-TDC showed a statistically significant difference (Fig. 5). The difference between vehicle and non-treatment groups was not statistically significant.

Histologic Observations

Figure 6 shows the histologic changes in periodontal inflammation and in response to different treatments. In the non-treatment group, local *P. gingivalis* administration in addition to ligature placement led to significant bone resorption and increased inflammation. The H&E-stained sections of the ligated and diseased sites showed disrupted connective tissue layers with irregular fiber arrangement. Numerous blood vessels and inflammatory cells were localized adjacent to the basal layer in the connective tissue. Dense inflammatory infiltration spread to the lamina dura of the alveolar bone, leading to bone destruction, and the alveolar borders were extremely ragged. The non-ligated sites showed no evidence of bone loss. Simultaneous vehicle application at the same time of the disease induction showed a limited prevention on the development of periodontitis where the inflammatory cell infiltration and bone loss were less but still detectable.

Treatment with 1-TDC at 1 and 10 mg/ml, however, showed significant prevention in both bone loss and inflammatory changes in rabbits where periodontal disease was induced by *P. gingivalis* and ligature placement (Fig. 6). In these groups, the H&E-stained sections showed intact epithelium, dense, connective tissue fibers, fewer blood vessels, and reduced inflammatory cells. The preventive effect of 1-TDC treatment was comparable to the non-treatment and vehicle groups, whereas both concentrations of 1-TDC showed similar prevention with regard to inflammatory cellular infiltration and bone loss induced by periodontal inflammation.

Osteoclastic Cell Activity

The TRAP-stained sections of the ligated sites of the non-treatment, vehicle, and test groups are shown

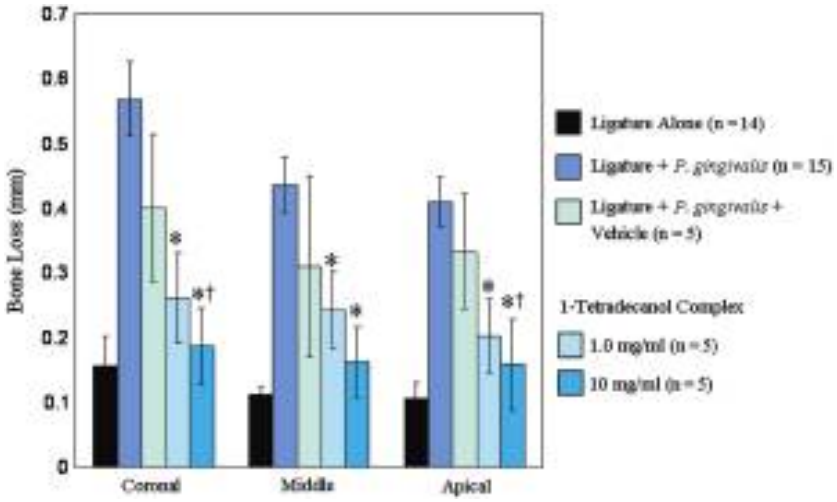

Figure 5.
*Histomorphometric measurements were performed at the ligated site of each tooth on the histologic sections. Linear measurements were made at three points: crestal, middle, and apical third of the alveolar bone. The ligated sites in the non-treatment group showed significant bone loss compared to all three treatment groups. Although there was no significant difference between the 1-TDC groups, the higher dose resulted in significant inhibition of bone loss compared to vehicle and non-treatment groups. *P <0.05 compared to ligature + P. gingivalis; †P <0.05 compared to ligature + P. gingivalis + vehicle.*

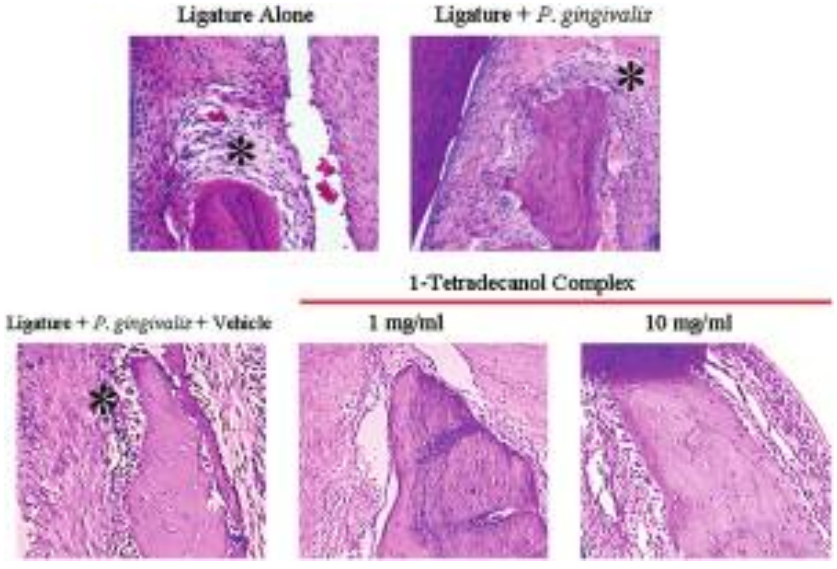

Figure 6.
Histologic changes in P. gingivalis*–induced periodontal inflammation and in response to different treatments. In the non-treatment group, local* P. gingivalis *administration in addition to ligature placement led to significant bone resorption and increased inflammation compared to ligature-alone and all three treatment groups. Vehicle application at the same time of the disease induction showed a limited prevention on the development of periodontitis where the inflammatory cell infiltration and bone loss were still detectable. Both doses of topical 1-TDC applications (1 and 10 mg/ml) showed a preventive effect on both bone loss and inflammatory changes in rabbits that receive* P. gingivalis *and ligature placement. *Inflammatory cellular infiltration.*

in the top panels of Figure 7. The non-treatment group (ligature + *P. gingivalis*) showed disrupted connective tissue and increased inflammatory cell infiltrate, especially at the alveolar bone borders. In the vehicle group, vehicle application during the experimental periodontitis showed limited prevention in osteoclastic activity where the $TRAP^+$ osteoclastic cells were still detectable (Fig. 7). The disrupted connective tissue and increased inflammatory cell infiltrate were obvious, especially at the alveolar bone borders. The alveolar bone borders were extremely ruffled, with increased numbers of irregular-shaped Howship resorptive lacunae presenting multinucleated osteoclastic activity.

Conversely, in 1-TDC groups, osteoclasts were in fewer numbers (Fig. 7). Although both doses had a preventive effect on bone resorptive activity induced by periodontitis, the higher dose showed significant prevention versus vehicle and the lower dose of the 1-TDC group ($P = 0.01$ and $P = 0.03$, respectively). Overall, the TRAP-stained sections in 1-TDC at the 10-mg/ml concentration showed intact epithelium and dense connective tissue layers with few blood vessels. Intact, regular, and well-defined alveolar bone borders were seen in most areas, except for a few TRAP-positive cells.

The number of osteoclasts at the apical, middle, and coronal thirds of the root was another variable that was compared between groups (Fig. 7). In the vehicle group, there was more osteoclastic activity detected in all sections compared to both 1-TDC groups. Both concentrations of 1-TDC suspended osteoclastic activity; the 10-mg/ml concentration had a statistically significant ($P = 0.02$) preventive effect of osteoclastic activity at the coronal and mid-third of the root compared to the non-treatment and vehicle groups.

DISCUSSION

In this study, we showed that local administration of an esterified MUFA mixture (1-TDC) inhibited *P. gingivalis*–induced periodontal inflammation in rabbits as evidenced by the reduction of inflammatory cell infiltration and bone

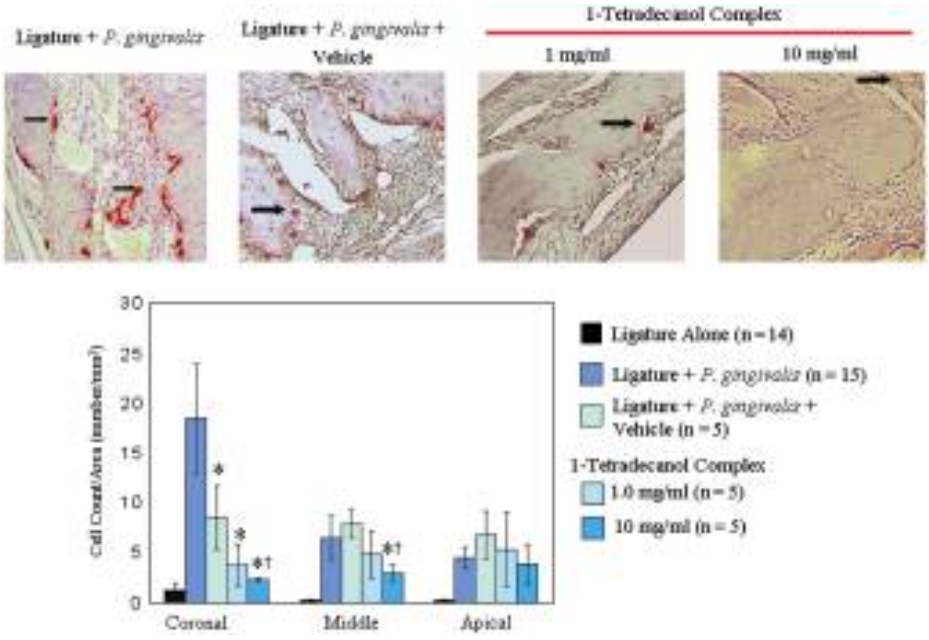

Figure 7.
TRAP-stained sections of ligature + P. gingivalis + *vehicle and two different applications of 1-TDC. The non-treatment group showed disrupted connective tissue and increased inflammatory cell infiltrate, especially at the alveolar bone borders. The vehicle application during the experimental periodontitis showed limited prevention in osteoclastic activity where the TRAP$^+$ osteoclastic cells were still detectable. Conversely, osteoclastic cells were at fewer numbers in both 1-TDC groups. The number of osteoclasts was counted at all three levels (coronal, middle, and apical) to detect osteoclastogenesis associated with inflammatory changes. The ligature* + P. gingivalis *group presented markedly increased number of osteoclasts (osteoclastic activity) at all three levels versus all three treatment groups (vehicle and two test groups), whereas comparable and significant differences were found between vehicle and the higher dose (10 mg/ml) of the fatty acid composition. *P <0.05 compared to ligature* + P. gingivalis; †P *<0.05 compared to ligature* + P. gingivalis + *vehicle. Arrows indicate osteoclastic activity.*

loss. Interestingly, the vehicle (olive oil, also a MUFA) also had preventive activity macroscopically, which was limited compared to 1-TDC; however, it failed to present the same activity on the histopathologic changes of periodontal inflammation. The rabbit model of periodontitis was previously shown to be a relevant model in which the physiology and pathology of periodontal tissues resemble humans with respect to proinflammatory and anti-inflammatory mechanisms.[4] To study the efficacy of topical application of 1-TDC in preventing the onset and progression of *P. gingivalis*–induced periodontitis, silk ligatures were placed around the second premolars, and the periodontitis-specific human pathogen, *P. gingivalis,* was applied. The test agents were compared to two negative control groups in which 14 animals received ligature application alone, and 15 animals received *P. gingivalis* in addition to the ligature. The ligature + *P. gingivalis* group exhibited periodontal tissue destruction, where bone loss and osteoclastic activity were detectable in macroscopic and histologic observations. Conversely, the ligature-alone group did not show any measurable soft and hard tissue destruction with the absence of *P. gingivalis*, indicating that bacterial challenge is necessary to initiate the host response. Topical 1-TDC application inhibits the pathologic changes in periodontal tissues. There were statistically significant histomorphometric differences between the non-treatment (ligature + *P. gingivalis*) and 1-TDC treatment groups. Although there was no significant difference between two concentrations of 1-TDC, the higher dose (10 mg/ml) showed a statistically significant difference compared to vehicle.

Certain fatty acids have the potential to attenuate inflammation by the synthesis of mediators of the 15-lipoxygenase pathways, which show opposite effects to the proinflammatory arachidonic acid metabolites such as LTB_4.[13,14] Fish oils are the main source of omega-3 fatty acids, where the major PUFA components are eicosapentaenoic acid (EPA) and docohexaenoic acid (DHA).[15] Soy oil is a rich source of omega-6 fatty acids, where linoleic acid is the major fatty acid component. Beneficial effects of PUFAs have been shown in many inflammatory conditions, including periodontal disease, through regulation of a variety of enzymatic processes.[44,45] Fatty acids can decrease the amount of arachidonic acid in cell membranes, reducing eicosanoid production through cyclooxygenase and lipoxygenase pathways.[46,47] The integration between arachidonic acid byproducts and their involvement with leukotriene and prostaglandins leads to the control of inflammation.[48,49] These mechanisms were also shown to play important roles in the development of periodontal inflammation. Thus, studies have shown the effects of dietary fish oil supplements in experimental and clinical periodontal inflammation models.[25,50] The systemic administration of fatty acids, however, failed to show any significant influence on gingival inflammation.[25] Although improvement in some clinical parameters has been shown, especially in gingival index and bleeding index, the results could not conclude that systemic administration of omega-3 PUFA were effective in preventing experimental gingivitis.[25] Because of the high epithelial penetration ability of fatty acids,[26]

local application may be a better route for treatment of oral inflammatory diseases than dietary supplementation[27] and in experimental periodontitis in animal models.[28-30,51]

Because the present formulation of 1-TDC has not been previously tested in a gingival inflammation model, there are several considerations that may be relevant. First, the concentrations used in the experiments may not have been optimal for in vivo models. Second, because olive oil has been shown to have anti-inflammatory properties in several clinical trials,[52-54] using olive oil as the vehicle may have confounded the actual results of 1-TDC. Third, the duration of the topical administration of the test agent used in this study may not be sufficient to show its complete anti-inflammatory effect. This is evidenced by the finding that, in the TRAP-stained sections, osteoclastic activity was significantly suspended in response to the high dose of 1-TDC application. Despite the confounders identified post hoc in this study, a trend toward improvement was seen in active treatment groups, especially with a higher dose.

CONCLUSION

Although the mechanisms remain to be elucidated, topical application of 1-TDC was found promising in preventing bone loss, inflammatory cell infiltration, and connective tissue destruction in the rabbit periodontitis model, and these results warrant further studies to fully assess the potential effects of 1-TDC on periodontal inflammation and mechanisms of action.

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REFERENCES

1. Listgarten MA. Nature of periodontal diseases: Pathogenic mechanisms. *J Periodontal Res* 1987;22:172-178.
2. Genco RJ. Host responses in periodontal diseases: Current concepts. *J Periodontol* 1992;63(Suppl.):338-355.
3. Van Dyke TE, Serhan CN. Resolution of inflammation: A new paradigm for the pathogenesis of periodontal diseases. *J Dent Res* 2003;82:82-90.
4. Serhan CN, Jain A, Marleau S, et al. Reduced inflammation and tissue damage in transgenic rabbits overexpressing 15-lipoxygenase and endogenous anti-inflammatory lipid mediators. *J Immunol* 2003;171:6856-6865.
5. Champagne CM, Buchanan W, Reddy MS, Preisser JS, Beck JD, Offenbacher S. Potential for gingival crevice fluid measures as predictors of risk for periodontal diseases. *Periodontol 2000* 2003;31:167-180.
6. Offenbacher S, Williams RC, Jeffcoat MK, et al. Effects of NSAIDs on beagle crevicular cyclooxygenase metabolites and periodontal bone loss. *J Periodontal Res* 1992;27:207-213.
7. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: Summary of developments, clinical implications and future directions. *Periodontol 2000* 1997;14:216-248.
8. Garcia C, Boyce BF, Gilles J, et al. Leukotriene B4 stimulates osteoclastic bone resorption both in vitro and in vivo. *J Bone Miner Res* 1996;11:1619-1627.
9. Ternowitz T, Herlin T, Fogh K. Human monocyte and polymorphonuclear leukocyte chemotactic and chemokinetic responses to leukotriene B4 and FMLP. *Acta Pathol Microbiol Immunol Scand C* 1987;95:47-54.
10. Ford-Hutchinson AW, Bray MA, Doig MV, Shipley ME, Smith MJ. Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature* 1980;286:264-265.
11. Palmblad J, Malmsten CL, Uden AM, Radmark O, Engstedt L, Samuelsson B. Leukotriene B4 is a potent and stereospecific stimulator of neutrophil chemotaxis and adherence. *Blood* 1981;58:658-661.
12. Serhan CN, Radin A, Smolen JE, Korchak H, Samuelsson B, Weissmann G. Leukotriene B4 is a complete secretagogue in human neutrophils: A kinetic analysis. *Biochem Biophys Res Commun* 1982;107:1006-1012.
13. Curtis CL, Hughes CE, Flannery CR, Little CB, Harwood JL, Caterson B. n-3 fatty acids specifically modulate catabolic factors involved in articular cartilage degradation. *J Biol Chem* 2000;275:721-724.
14. Kremer JM. n-3 fatty acid supplements in rheumatoid arthritis. *Am J Clin Nutr* 2000;71(1 Suppl.):349S-351S.
15. Drevon CA. Marine oils and their effects. *Nutr Rev* 1992;50:38-45.
16. Kremer JM, Lawrence DA, Jubiz W, et al. Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. Clinical and immunologic effects. *Arthritis Rheum* 1990;33:810-820.
17. Belch JJ, Muir A. n-6 and n-3 essential fatty acids in rheumatoid arthritis and other rheumatic conditions. *Proc Nutr Soc* 1998;57:563-569.
18. Stammers T, Sibbald B, Freeling P. Fish oil in osteoarthritis. *Lancet* 1989;2:503.
19. Calder PC, Zurier RB. Polyunsaturated fatty acids and rheumatoid arthritis. *Curr Opin Clin Nutr Metab Care* 2001;4:115-121.
20. Das UN. Beneficial effect of eicosapentaenoic and docosahexaenoic acids in the management of systemic lupus erythematosus and its relationship to the cytokine network. *Prostaglandins Leukot Essent Fatty Acids* 1994;51:207-213.
21. Stenson WF, Cort D, Rodgers J, et al. Dietary supplementation with fish oil in ulcerative colitis. *Ann Intern Med* 1992;116:609-614.
22. Bittiner SB, Tucker WF, Cartwright I, Bleehen SS. A double-blind, randomised, placebo-controlled trial of fish oil in psoriasis. *Lancet* 1988;1:378-380.
23. Bjorneboe A, Soyland E, Bjorneboe GE, Rajka G, Drevon CA. Effect of dietary supplementation with eicosapentaenoic acid in the treatment of atopic dermatitis. *Br J Dermatol* 1987;117:463-469.
24. Alam SQ, Bergens BM, Alam BS. Arachidonic acid, prostaglandin E2 and leukotriene C4 levels in gingiva and submandibular salivary glands of rats fed diets containing n-3 fatty acids. *Lipids* 1991;26:895-900.

25. Campan P, Planchand PO, Duran D. Pilot study on n-3 polyunsaturated fatty acids in the treatment of human experimental gingivitis. *J Clin Periodontol* 1997;24: 907-913.
26. Tanojo H, Bouwstra JA, Junginger HE, Bodde HE. In vitro human skin barrier modulation by fatty acids: Skin permeation and thermal analysis studies. *Pharm Res* 1997;14:42-49.
27. Dewsbury CE, Graham P, Darley CR. Topical eicosapentaenoic acid (EPA) in the treatment of psoriasis. *Br J Dermatol* 1989;120:581.
28. Vardar S, Buduneli E, Turkoglu O, et al. Therapeutic versus prophylactic plus therapeutic administration of omega-3 fatty acid on endotoxin-induced periodontitis in rats. *J Periodontol* 2004;75:1640-1646.
29. Vardar S, Buduneli E, Baylas H, Berdeli AH, Buduneli N, Atilla G. Individual and combined effects of selective cyclooxygenase-2 inhibitor and omega-3 fatty acid on endotoxin-induced periodontitis in rats. *J Periodontol* 2005;76:99-106.
30. Hasturk H, Kantarci A, Ohira T, et al. RvE1 protects from local inflammation and osteoclast-mediated bone destruction in periodontitis. *FASEB J* 2006;20: 401-403.
31. Mensink RP, Katan MB. Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. *Lancet* 1987; 1:122-125.
32. Grundy SM, Florentin L, Nix D, Whelan MF. Comparison of monounsaturated fatty acids and carbohydrates for reducing raised levels of plasma cholesterol in man. *Am J Clin Nutr* 1988;47:965-969.
33. Kris-Etherton PM. AHA Science Advisory. Monounsaturated fatty acids and risk of cardiovascular disease. American Heart Association. Nutrition Committee. *Circulation* 1999;100:1253-1258.
34. Kromhout D, de Lezenne Coulander C. Diet, prevalence and 10-year mortality from coronary heart disease in 871 middle-aged men. The Zutphen Study. *Am J Epidemiol* 1984;119:733-741.
35. Kromhout D, Menotti A, Bloemberg B, et al. Dietary saturated and trans fatty acids and cholesterol and 25-year mortality from coronary heart disease: The Seven Countries Study. *Prev Med* 1995;24:308-315.
36. McGee DL, Reed DM, Yano K, Kagan A, Tillotson J. Ten-year incidence of coronary heart disease in the Honolulu Heart Program. Relationship to nutrient intake. *Am J Epidemiol* 1984;119:667-676.
37. Garcia-Palmieri MR, Sorlie P, Tillotson J, Costas R Jr., Cordero E, Rodriguez M. Relationship of dietary intake to subsequent coronary heart disease incidence: The Puerto Rico Heart Health Program. *Am J Clin Nutr* 1980; 33:1818-1827.
38. Reaven P. Dietary and pharmacologic regimens to reduce lipid peroxidation in non-insulin-dependent diabetes mellitus. *Am J Clin Nutr* 1995;62(6 Suppl.): 1483S-1489S.
39. Reaven PD, Grasse BJ, Tribble DL. Effects of linoleate-enriched and oleate-enriched diets in combination with alpha-tocopherol on the susceptibility of LDL and LDL subfractions to oxidative modification in humans. *Arterioscler Thromb* 1994;14:557-566.
40. Reaven P, Parthasarathy S, Grasse BJ, Miller E, Steinberg D, Witztum JL. Effects of oleate-rich and linoleate-rich diets on the susceptibility of low density lipoprotein to oxidative modification in mildly hypercholesterolemic subjects. *J Clin Invest* 1993;91: 668-676.
41. Reaven P, Parthasarathy S, Grasse BJ, et al. Feasibility of using an oleate-rich diet to reduce the susceptibility of low-density lipoprotein to oxidative modification in humans. *Am J Clin Nutr* 1991;54:701-706.
42. Sirtori CR, Tremoli E, Gatti E, et al. Controlled evaluation of fat intake in the Mediterranean diet: Comparative activities of olive oil and corn oil on plasma lipids and platelets in high-risk patients. *Am J Clin Nutr* 1986;44:635-642.
43. Lopez-Segura F, Velasco F, Lopez-Miranda J, et al. Monounsaturated fatty acid-enriched diet decreases plasma plasminogen activator inhibitor type 1. *Arterioscler Thromb Vasc Biol* 1996;16:82-88.
44. Nieto N, Torres MI, Rios A, Gil A. Dietary polyunsaturated fatty acids improve histological and biochemical alterations in rats with experimental ulcerative colitis. *J Nutr* 2002;132:11-19.
45. Nettleton JA, Katz R. n-3 long-chain polyunsaturated fatty acids in type 2 diabetes: A review. *J Am Diet Assoc* 2005;105:428-440.
46. Kragballe K, Voorhees JJ, Goetzl EJ. Inhibition by leukotriene B5 of leukotriene B4-induced activation of human keratinocytes and neutrophils. *J Invest Dermatol* 1987;88:555-558.
47. Ziboh VA. Cutaneous essential fatty acids and hydroxy fatty acids: Modulation of inflammatory and hyperproloferative process. In: *Fatty Acids and Inflammatory Skin Diseases.* Schroder JM, ed. Basel, Switzerland: Birkhaeuser Verlag; 1999:55-57.
48. Prescott SM, Zimmerman GA, Morrison AR. The effects of a diet rich in fish oil on human neutrophils: Identification of leukotriene B5 as a metabolite. *Prostaglandins* 1985;30:209-227.
49. Lokesh BR, Black JM, German JB, Kinsella JE. Docosahexaenoic acid and other dietary polyunsaturated fatty acids suppress leukotriene synthesis by mouse peritoneal macrophages. *Lipids* 1988;23:968-972.
50. Eberhard J, Heilmann F, Acil Y, Albers HK, Jepsen S. Local application of n-3 or n-6 polyunsaturated fatty acids in the treatment of human experimental gingivitis. *J Clin Periodontol* 2002;29:364-369.
51. Vardar-Sengul S, Buduneli N, Buduneli E, et al. Dietary supplementation of omega-3 fatty acid and circulating levels of interleukin-1beta, osteocalcin, and C-reactive protein in rats. *J Periodontol* 2006;77:814-820.
52. Berbert AA, Kondo CR, Almendra CL, Matsuo T, Dichi I. Supplementation of fish oil and olive oil in patients with rheumatoid arthritis. *Nutrition* 2005;21: 131-136.
53. Beauchamp GK, Keast RS, Morel D, et al. Phytochemistry: Ibuprofen-like activity in extra-virgin olive oil. *Nature* 2005;437:45-46.
54. Camuesco D, Galvez J, Nieto A, et al. Dietary olive oil supplemented with fish oil, rich in EPA and DHA (n-3) polyunsaturated fatty acids, attenuates colonic inflammation in rats with DSS-induced colitis. *J Nutr* 2005; 135:687-694.

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