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 10⁹ 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.

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. 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. Several inflammatory mediators including cytokines (i.e., interleukin [IL]-1β and IL-6) and lipid mediators are associated with periodontal disease. Among the lipid mediators are the arachidonic acid–derived products, including leukotriene B₄ (LTB₄) and prostaglandin E₂ (PGE₂). Indeed, some of the pathophysiologic events that occur in periodontal diseases, particularly early events, can be explained...
by the activities of these lipid mediators. For example, LTB₄, 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 LTB₄ 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 LTB₄ 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 Sjögren syndrome,¹⁷ ulcerative colitis,²¹ psoriasis,²² and atopic dermatitis²³ initiated studies on periodontal diseases, which share several important common pathways with these inflammatory conditions.²⁴ However, when dietary supplements of omega-3 PUFAs 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 PUFAs in the local environment.²⁵ On the other hand, the high epithelial penetration ability of fatty acids²⁶ makes it probable that local application is useful for the treatment of local oral inflammatory diseases. Indeed, topical application of omega-3 PUFAs 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 MUFAs 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 A₂ 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.
926

(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 x10. 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, 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.

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* Ketaset, Fort Dodge Animal Health, Fort Dodge, IA.
* Anased, LLOYD Laboratories, Shenandoah, IA.
* Sharpont, 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.

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 ($\pm$ SD) of the linear distance was calculated for each group. Linear distance was described as the horizontal distance between the tooth surface and...
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...
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
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. 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 LTB4. Fish oils are the main source of omega-3 fatty acids, where the major PUFA components are eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA). 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. Fatty acids can decrease the amount of arachidonic acid in cell membranes, reducing eicosanoid production through cyclooxygenase and lipoxygenase pathways. The integration between arachidonic acid byproducts and their involvement with leukotriene and prostaglandins leads to the control of inflammation. 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. The systemic administration of fatty acids, however, failed to show any significant influence on gingival inflammation. 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. Because of the high epithelial penetration ability of fatty acids,
local application may be a better route for treatment of oral inflammatory diseases than dietary supplementation\(^\text{27}\) and in experimental periodontitis in animal models.\(^\text{28-30,51}\)

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,\(^\text{52-54}\) 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 suppressed 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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1-Tetradecanol Complex Reduces Periodontal Inflammation


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