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Effects of Tempol, a membrane-permeable radical scavenger, in a rodent model periodontitis

Rosanna Di Paola[1], Emanuela Mazzon[1], Daniele Zito[1], Daniele Maiere[1], Domenico Britti[2], Tiziana Genovese[1] and Salvatore Cuzzocrea[1]

[1]Department of Clinical and Experimental Medicine and Pharmacology, Torre Biologica, Policlinico Universitario, Messina, Italy; [2]Department of Clinical Veterinary Science, University of Teramo, Teramo, Italy

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Abstract

Background: Recent studies have demonstrated that Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl), a cell membrane-permeable radical scavenger, exerts protective effects in various models of inflammation and shock. Reactive oxygen species (ROS) plays a pivotal role in the induction of genes involved in physiological processes as well as in the response to inflammation.

Aim: We have investigated the effect of Tempol in a rat model of periodontitis.

Materials and Methods: Periodontitis was induced in rats by placing a 2/0 braided silk ligature around the lower left first molar. At day 8, the gingivomucosal tissue encircling the mandibular first molar was removed for evaluation of neutrophils infiltration, tissue permeability, nitrotyrosine formation, poly-(ADP-ribose) polymerase (PARP) activation, radiography and histology.

Results and conclusions: Legation significantly induced an increased neutrophil infiltration and a positive staining for nitrotyrosine formation and PARP activation. Ligation significantly increased Evans blue extravasation in gingivomucosal tissue and alveolar bone erosion as evaluated by radiography analysis. Intraperitonial injection of Tempol (10 mg/kg daily for 8 days) significantly decreased all of the parameters of inflammation as described above. This suggests that antioxidant therapies, which interfere with ROS, may be of benefit in the treatment of periodontitis.

Key words: alveolar bone loss; free radical; lipid peroxidation; neutrophil infiltration; nitrotyrosine; periodontal diseases; reactive oxygen species

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Human periodontal diseases are inflammatory disorders that give rise to tissue damage and loss, as a result of the complex interactions between pathogenic bacteria and the host's immune response. It is likely that the role of reactive oxygen species (ROS) is common to both bacterial and host-mediated pathways of tissue damage. The formation of a ROS have been demonstrated to play an important role in the pathogenesis of large number of pathological conditions (Halliwell 1991) such as rheumatoid arthritis (McCord 1974), acute respiratory distress syndrome (Tate & Repine 1983), AIDS (Droge et al. 1988) and, more recently, periodontal disease (Kimura et al. 1993). ROS, such as the superoxide and hydroxyl radical, are integral reaction products of normal cellular metabolism. The more current terminology aggressive periodontitis is an inflammatory disease with severe periodontal destruction, occurring in the early 20s, teens or before. Some reports have evaluated polymorphonuclear cell functions in patients affected by early-onset periodontitis, but the results are discordant. Some authors reported a defective chemotactic response to formyl-met-leu-phe (Suzuki et al. 1984) and to complement-derived C5a (Genco et al. 1986). Contrary to the above-mentioned studies, chemotaxis of early-onset periodontitis polymorphonuclear cells has been reported to be normal or increased (Repo et al. 1990). Recently, Garrett et al. (1990) demonstrated that ROS, and particularly superoxide, are also intermediate species in the activation of osteoclasts at the site of bone resorption. Superoxide has been localized at the ruffled border space of osteoclasts, suggesting that it may be involved in bone matrix degradation (Key et al. 1994, Chapple 1997).

Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl) is a water-soluble analogue of the spin label TEMPO, which is widely used in electron spin resonance spectroscopy. Tempol is a stable piperidine nitroxide of low molecular weight (MW: 172), which permeates biological membranes. There is now good evidence that Tempol exerts beneficial effects in animal models of shock, ischaemia–reperfusion injury,

inflammation, hypertension, diabetes and endothelial cell dysfunction. Various studies have demonstrated that Tempol attenuates the effects of superoxide anions (O_2^-) in vitro (Reddan et al. 1992, Laight et al. 1997), as well as the formation of hydroxyl radicals ($OH^\cdot$) (Mitchell et al. 1990) and attenuates the cytotoxic effects of hydrogen peroxide (H_2O_2), which is mediated by OH (Bowes et al. 1999). Tempol also inhibits the peroxynitrite-mediated nitration of phenolic compounds in the presence of a large molar excess of peroxynitrite, suggesting a catalytic-like mechanism (Carroll et al. 2000).

Following the observation (Sledzinski et al. 1995) that Tempol exerts beneficial effects in a rodent model of pancreatitis, there is now good evidence that Tempol reduces the degree of inflammation and the associated tissue injury in animal models of diseases associated with local or systemic inflammation. These include rodent models of carrageenan-induced pleurisy, colitis, zymosan-induced multiple organ injury and uveoretinitis.

In this study, we investigate the effect of Tempol in a rat experimental model of periodontitis, and highlight potential mechanism(s) through which Tempol exerts its protective effects.

Materials and Methods

Surgical procedure

Male Sprague–Dawley rats (280–400 g) were lightly anaesthetized with surgical doses of sodium pentobarbitone (35 mg/kg). Sterile, 2/0 black braided silk thread was placed around the cervix of the lower left first molar and knotted mesially as previously described (Gyorfi et al. 1994, Di Paola et al. 2004). After the rats had recovered from the anaesthetic, they were allowed to eat commercial laboratory food and drink tap water ad libitum. Animal care and protocol was in compliance with Italian regulations on protection of animals used for experimental and other scientific purpose (D.M. 116192) as well as with the EEC regulations (O.J. of E.C. L 358/1 12/18/1986). Animals, and the study protocol were approved by the Institutional Animal Care and User Committee of the University of Messina.

Experimental groups

Rats were randomly allocated into the following groups: *Ligature+vehicle group*: rats were subjected to ligature-induced periodontitis for 8 days and animals received vehicle (saline solution) intraperitoneally (i.p.; daily treatment for 8 days). *Ligature+Tempol group*: rats were subjected to ligature-induced periodontitis for 8 days and animals received Tempol (10 mg/kg i.p., daily for 8 days). At different time points, the rats ($N = 10$ from each group for each parameter) were sacrificed in order to evaluate the various parameters as described below. The dose of Tempol used in the present studies was taken from previous studies showing a dose-dependent efficacy without side effect in models of acute inflammation (Cuzzocrea et al. 2000a).

Measurement of arterial blood pressure indirectly in conscious rat

The mean arterial blood pressure in conscious rats was measured by a Blood Pressure Recorder (UGO BASILE, Biological Research Apparatus, Comerio, Italy). After a 1-week period, rats were treated as described above, and blood pressure was measured before and after treatments (30 min. after the last administration). To measure arterial blood pressure, rats were housed for 30 min. in a warmed room (28–30°C) and then a tail cuff, consistently about 2 cm from the base of the tail, was placed and arterial blood pressure was measured. Heart rate was detected by a pulse rate counter placed after the tail cuff.

Measurement of vascular permeability by Evans blue extravasations

Vascular permeability was determined as described previously (Gyorfi et al. 1994). Extravasated Evans blue in the excised gingivomucosal tissue samples was extracted with 1 ml formamide for 48 h at room temperature for spectrophotometric determination at 620 nm and expressed as microgram per gram gingivomucosal tissue (Gyorfi et al. 1994).

Measurement of alveolar bone loss

In the same set of experiments, the distance from the cemento-enamel junction of the first lower molars to the alveolar crest was measured with a modification of the method by Crawford et al. (1978). Recordings were made along the median axis of the lingual surface of the mesial and mediolingual roots of the lower first left and right molars as described previously (Di Paola et al. 2004). These measurements were performed by an independent investigator who was unaware of the treatment regimens. The alveolar bone loss induced by the ligature was expressed as a difference between the left and the right side.

Histological examination

For histopathological examination, biopsies of gingivomucosal tissue were taken 8 days after the ligature induction of periodontitis. The tissue slices were fixed in 10% neutral-buffered formaldehyde for 5 days, embedded in paraffin and sectioned. The sections were stained with haematoxylin and eosin. The total number of infiltrating leucocytes (e.g., neutrophils and mononuclear cells) in cortical interstitial spaces was assessed quantitatively by counting the number of polymorphonuclear cells in 20 high-power fields.

Radiography

Mandibles were placed on a radiographic box at a distance of 90 cm from the X-ray source. Radiographic analysis of normal and ligated mandibles was performed by an X-ray machine (Philips X12, Milan, Italy) with a 40 kW exposure for 0.01 s. A radiographic examination 8 days after ligature placement revealed bone matrix resorption in the lower first left after ligation as described previously (Di Paola et al. 2004).

Myeloperoxidase activity

Myeloperoxidase activity, an indicator of polymorphonuclear leucocyte (PMN) accumulation, was determined as described previously (Mullane et al. 1985). At the specified time, gingivomucosal tissue tissues were obtained and weighed, and each piece was homogenized in a solution containing 0.5% (w/v) hexadecyltrimethyl-ammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7) and centrifuged for 30 min. at $20{,}000 \times g$ at 4°C. An aliquot of the supernatant was then allowed to react with a solution of 1.6 mM tetramethylbenzidine and 0.1 mM H_2O_2. The rate of change in absorbance was measured spectrophotometrically at 650 nm. Myeloperoxidase activity was defined as the quantity of enzyme degrading 1 μmol/min. of peroxide at 37°C and was expressed in milliunits per gram of wet tissue.

Immunohistochemical localization of nitrotyrosine and poly-(ADP-ribose) polymerase (PARP)

Tyrosine nitration, a specific ''footprint'' of peroxynitrite formation, and evidence of PAR formation (an indicator of PARP activation of a DNA repair enzyme) were detected as previously described (Di Paola et al. 2004) in gingivomucosal tissue sections by immunohistochemistry. At the end of the experiment, tissues were fixed in 10% (w/v) phosphate buffer saline-buffered formaldehyde, and 8 μm sections were prepared from paraffin-embedded tissues. After deparaffinization, endogenous peroxidase was quenched with 0.3% (v/v) H_2O_2 in 60% (v/v) methanol for 30 min. The sections were permeablized with 0.1% (w/v) Triton X-100 in phosphate-buffered saline for 20 min. Non-specific adsorption was minimized by incubating the section in 2% (v/v) normal goat serum in phosphate-buffered saline for 20 min. Endogenous biotin or avidin binding site was blocked by sequential incubation for 15 min. with avidin and biotin (DBA, Milan, Italy). The sections were then incubated overnight with primary anti-nitrotyrosine antibody (1:1000 dilution), primary anti-PAR (1:500 dilution) with control solutions including buffer alone or non-specific purified rabbit IgG. Specific labelling was detected with a biotin-conjugated goat anti-rabbit IgG and avidin–biotin peroxidase complex (DBA). In order to confirm that the immunoreaction for the nitrotyrosine was specific, some sections were also incubated with the primary antibody (anti-nitrotyrosine) in the presence of excess nitrotyrosine (10 mM) to verify the binding specificity. To verify the binding specificity for PARS, some sections were also incubated with only the primary antibody (no secondary) or with only the secondary antibody (no primary). Immunocytochemistry photographs ($n = 5$ photos from each sample collected from all rats in each experimental group) were assessed by densitometry analysis by using Optilab Graftek software on a Macintosh personal computer.

Materials

Primary anti-nitrotyrosine antibody was from Upstate Biotech (DBA). All other reagents and compounds used were obtained from Sigma Chemical Company (Sigma, Milan, Italy).

Data analysis

All values in the figures and text are expressed as mean ± standard error of the mean of n observations, where n represents the number of animals studied. Data sets were examined by one- and two-way analysis of variance, and individual group means were then compared with Bonferroni's or Student's unpaired t-test. A p-value less than 0.05 was considered significant. In the experiments involving histology or immunohistochemistry, the figures shown are representative of at least three experiments performed on different experimental days.

Results

Effects of Tempol on neutrophils infiltration, nitrotyrosine formation and PARP activation in periodontitis

Myeloperoxidase activity was significantly elevated ($p < 0.001$) at 8 days after the ligature (Fig. 1a), and Tempol treatment significantly reduced ligature-induced increase of myeloperoxidase activity (Fig. 1a). No significant changes of myeloperoxidase activity were observed in the gingivomucosal tissues from the contra-lateral side (Fig. 1a). Sections of gingivomucosal tissues from the contra-lateral side did not reveal any immunoreactivity for nitrotyrosine or for anti-PARP (data not shown) within the normal architecture. At 8 days following ligation, a positive staining for nitrotyrosine (Fig. 2a, e) and for anti-PARP (Fig. 2b, b1, e) was found in the gingivomucosal tissues from ligature-operated rats. Tempol (10 mg/kg, i.p.) reduced the staining for both nitrotyrosine and anti-PARP (Fig. 2c, d, e, respectively).

Effect of Tempol on plasma extravasations and bone destruction

Before the measurement of Evans blue extravasations, the mean arterial pressure of vehicle- and Tempol-treated animals was recorded. In agreement with previous studies (Cuzzocrea et al. 2000b), Tempol treatment did not affect the mean arterial blood pressure (vehicle treated: 119+5 mmHg; $n = 10$ and Tempol treated: 112+4 mmHg; $n = 10$). After Evans blue injection, in contrast to the contra-lateral side, in the gingival around the neck of the teeth a definite blue belt could be observed even in non-legated control animals (not shown). Legation significantly increased Evans blue extravasations in gingivomucosal tissue compared with the contra-lateral side (Fig. 1b). Tempol treatment prevented this increase in Evans blue extravasations, but did not change the Evans blue content of the contra-lateral side (Fig. 1b).

A radiographic examination of the mandibles at day 8 after ligature placement revealed bone matrix resorption in the lower left first molar region after legation (Fig. 3a). There was no evidence of pathology in the right first molar (data not shown). Tempol markedly reduced the degree of bone resorption in the lower left first molar region after legation (Fig. 3b). In addition, a significant alveolar bone loss between the lower first left and the right first molars induced by the left side ligature was observed in vehicle-treated rats (Fig 3c) Tempol treatment resulted in a significant inhibition of alveolar bone loss after legation (Fig. 3c).

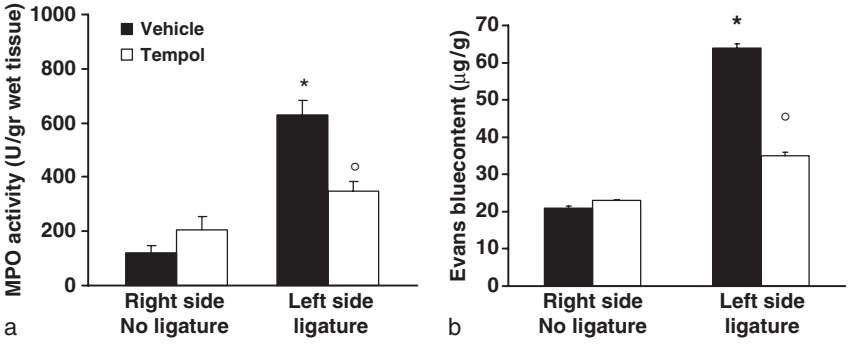

Fig. 1. Myeloperoxidase activity (a), and Evans blue content (b) in gingivomucosal tissue were significantly increased by ligature compared with the contra-lateral side. Tempol (10 mg/kg i.p., daily for 8 days) significantly reduced myeloperoxidase activity, and Evans blue content. Data represent the mean ± SEM from $n = 10$ rats for each group. *$p < 0.01$ *versus* non-legated; °$p < 0.01$ *versus* legated.

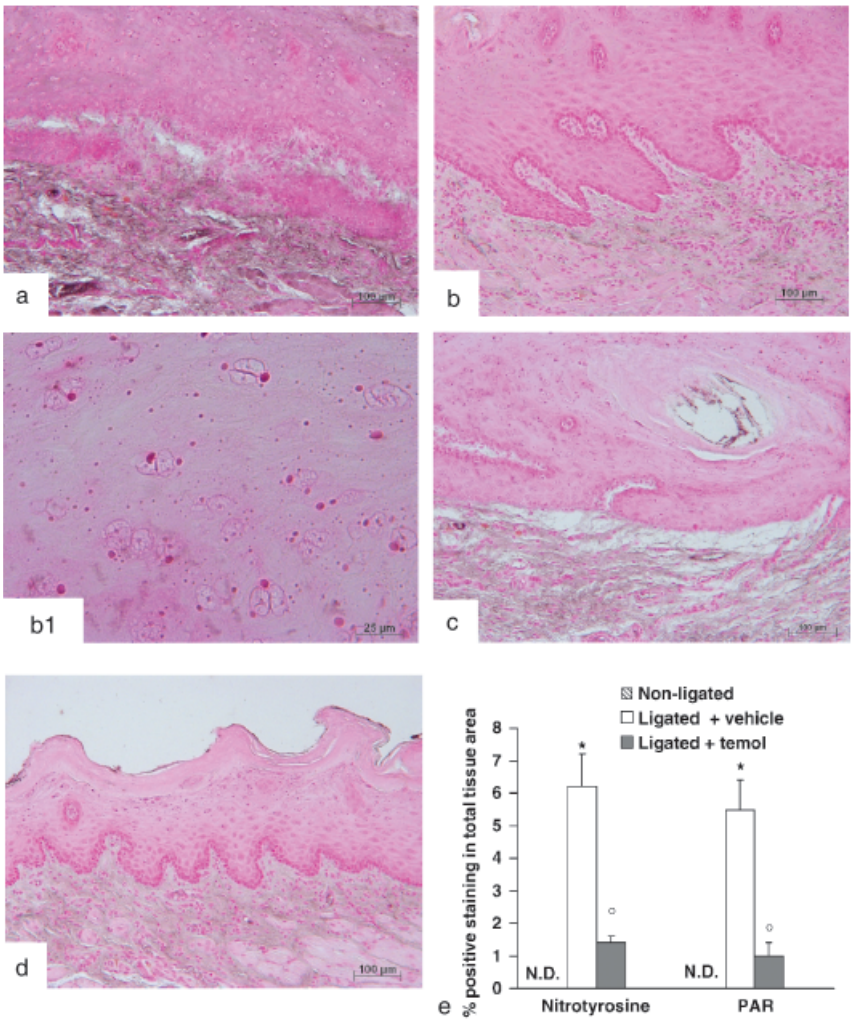

Fig. 2. Immunohistochemical staining for nitrotyrosine and poly-(ADP-ribose) polymerase (PARP) formation. Positive staining for nitrotyrosine (a) and PARP (b, b1) was observed in gingivomucosal tissue after ligature. In gingivomucosal tissue of Tempol (10 mg/kg i.p., daily for 8 days)-treated rats, no positive staining was observed both for nitrotyrosine (c) and PARP (d). Densitometry analysis of immunocytochemistry photographs (e; $n = 5$ photos from each samples collected from all rats in each experimental group) for PAR and nitrotyrosine from gingivomucosal tissue was assessed. The assay was carried out by using Optilab Graftek software on a Macintosh personal computer (CPU G3-266). The Figure is representative of at least three experiments performed on different experimental days. Densitometry data are expressed as percentage of total tissue area. $^{*}p < 0.01$ *versus* non-legated; $^{\circ}p < 0.01$ *versus* legated.

Effects of Tempol on ligature-induced periodontitis

When compared with gingivomucosal tissues sections taken from the contra-lateral side (Fig. 4a), histological examination of gingivomucosal tissues sections of ligature-operated rats showed oedema, tissue injury as well as infiltration of the tissue with inflammatory cells (Fig. 4b, b1). Tempol treatment reduced the degree of gingivomucosal tissues injury (Fig. 4c). Quantification of infiltrating polymorphonuclear cell into gingivomucosal tissue showed that there was only a minimal number of polymorphonuclear cells in tissue from the contra-lateral side (Fig. 4d). However, a large number of infiltrating polymorphonuclear cells were observed in the gingivomucosal tissue of legated rats (Fig. 4d). Tempol administration significantly reduced the numbers of polymorphonuclear cells infiltrating into gingivomucosal tissue (Fig. 4d).

Discussion

The inability to examine initiation and progression of periodontal disease and to assess certain therapies in humans has led to a great interest in the use of animal models in periodontal research. For every animal model of a human disease, there are inherent limitations. Among other important variations that could affect conclusions drawn from animal models, non-human animals differ from humans in anatomy, immune response and lifestyle. For the study of periodontal disease, mice (Lohinai et al. 2003), rats (Nassar et al. 2004), dogs (Shibli et al. 2003) and non-human primates (Oates et al. 2002) have been most frequently used. While primates closely resemble humans with regard to anatomy and physiology, their cost, size and maintenance requirements prohibit studies with large samples. However, rats and mice have been used for the study of periodontal disease (Lohinai et al. 2003, Di Paola et al. 2004, Nassar et al. 2004). Clinically healthy gingiva can be established and maintained in experimental animals, and gingivitis as well as periodontitis occur in these animals. It is possible to induce experimental periodontitis by placement of peridental silk ligatures or orthodontic elastics as well as by surgical removal of alveolar bone. Although the most appropriate model for studies of periodontal disease pathogenesis in experimental primates appears to involve the application of silk ligatures, some difficulties may occur in establishing periodontal breakdown by using this model. Many clinical, histological, microbiological and immunological characteristics of spontaneous and experimental marginal inflammation in most experimental animals are similar to those in humans.

Periodontitis, a chronic inflammatory disease of periodontal, supports the protection against local microbial attack this inflammatory reaction may also damage the surrounding cells and connective tissue structures, including alveolar bone causing tooth loss (Lindhe & Nyman 1987). It has been recently demonstrated that the most frequent cause of periodontitis are bacteria. The toxins, enzymes and metabolites of the bacteria present in the dental plaque play a key role in the initiation of the inflammatory process (Paquette & Williams 2000). In the present study, a well-established rat model of acute periodontitis was utilized, which involves placing a ligature around the cervix of the mandibular first molar tooth, and a similar model has previously been used in several species (Oates et al. 2002, Lohinai et al. 2003, Shibli et al. 2003, Nassar et al. 2004). In this model, liga-

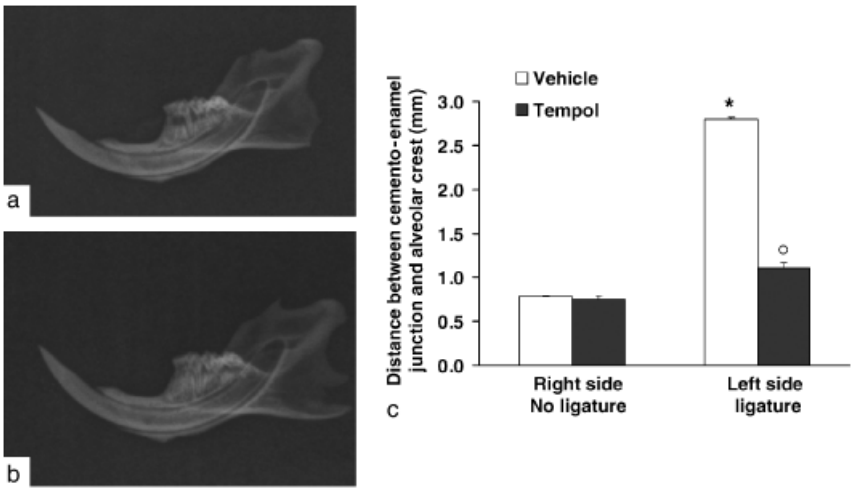

Fig. 3. The alveolar bone from legated (8 days) rats demonstrated alveolar bone resorption (a). Tempol treatment suppressed alveolar pathology in the rat alveolar bone (b). A significant increase in the distance between the cement-oenamel injunction and alveolar crest at mediolingulal root of the first molar was observed in ligature-treated rats (c). Tempol treatment significantly reduced the increase in the distance between the cemento-enamel injunction and alveolar crest (c). Radiographic figure is representative of at least three experiments performed on different experimental days. Data represent the data from 20 counts obtained from the gingivomucosal tissue of each treatment group. $^{*}p<0.01$ *versus* non-legated; $^{\circ}p<0.01$ *versus* legated.

tion acts as (i) a mechanical trauma on the dentogingival area, thereby reducing tissue integrity and allowing for intense host–plaque interaction and (ii) a plaque-formation-promoting factor, thus increasing the number of bacteria. Initiation of periodontal disease by bacteria is well documented, and the end result, destruction of the alveolar bone and other connective tissues, is readily observed. However, the molecular events that promote these alterations are not completely understood.

A further limitation to the experimental model utilized is that the induced periodontitis follows an acute course, during which tissue trauma and adjacent microbial accumulation accelerate the destructive process. Such pathways of acute inflammation are likely to differ from chronic periodontitis with respect to the balance of pro-inflammatory/anti-inflammatory cytokine network activity and indeed, that of ROS and antioxidant activity. Nevertheless, the model does provide an insight into potential modifications of biological pathways that may be anticipated to be relevant to chronic human disease and that may occur during future phase-II human clinical studies.

This study suggests a potential therapeutic application of Tempol for treatment of active inflammatory periodontal disease. In particular, we demonstrate that Tempol reduces (i) the development of ligature-induced periodontitis, (ii) the infiltration of the gingivomucosal tissues with polymorphonuclear cells, (iii) the degree of nitrotyrosine formation in the gingivomucosal tissues and (iv) the degree of gingivomucosal tissues injury (histology) in rats subjected to ligature-induced periodontitis. All of these findings support the view that ROS plays an important role in this model of periodontitis.

Some important pro-inflammatory roles for ROS include: endothelial cell damage and increased microvascular permeability, formation of chemotactic factors such as leukotriene B_4, recruitment of neutrophils at sites of inflammation, lipid peroxidation and oxidation, DNA single-strand damage and formation of peroxynitrite (Beckman et al. 1990, Deitch et al. 1990, Salvemini et al. 1999). Recent data from studies of human chronic periodontitis have demonstrated a deficiency in gingival crevicular fluid (GCF) and plasma total antioxidant defence mechanisms to ROS (Brock et al. 2004) and also reduced GCF concentrations of the key radical scavenger-reduced glutathione (Chapple et al. 2002) relative to matched non-periodontitis controls. While the latter studies may imply an antioxidant deficiency underpinning periodontal tissue damage, the authors acknowledge that the antioxidant deficiencies may also be secondary to excess ROS release in chronic periodontitis.

The results of the present study clearly demonstrated that Tempol exerted a significant inhibitory effect on the plasma extravasation and reduced the degree of bone resorption during periodontitis. Our study also confirmed earlier findings that one of the characteristic signs of inflammation, Evans blue extravasations, was higher on the legated side on the 8th day compared with the opposite side (Gyorfi et al. 1994). The hypothesis that highly reactive radicals may be responsible for the initial degradation of extracellular matrix components seen in periodontal disease is in agreement with Misaki et al. (1990) who demonstrated improved healing of gingival wounds in rats after i.v. application of SOD. In the present study, we report that ligature-induced periodontitis in the rat results in a significant infiltration of inflammatory cells in the gingivomucosal tissues. In previous studies, ROS i.e. superoxide, hydroxyl radical and peroxynitrite has been found to increase both neutrophil infiltration and adhesion (Beckman et al. 1990, Deitch et al. 1990, Salvemini et al. 1999). We also demonstrated in the present study that treatment with Tempol reduces this inflammatory cell infiltration as assessed by the specific granulocyte enzyme myeloperoxidase and moderates tissue damage as evaluated by histological examination. A possible mechanism by which Tempol attenuates polymorphonuclear cells infiltration is by down-regulating adhesion molecules ICAM-1 and P-selectin as demonstrated previously (Cuzzocrea et al. 2000a). These findings are in accordance with those of Berglundh & Lindhe (1993), who also found a significant increase in inflammatory cell infiltration in inflamed gingival as compared with a healthy one. Furthermore, we found that the tissue damage induced by ligature in vehicle-treated rats was associated with high levels of an intense immunostaining of nitrotyrosine formation, also suggesting that a structural alteration of gingivomucosal tissues had occurred, most probably because of the formation of highly reactive nitrogen derivatives. It has been demonstrated, in fact, that several chemical reactions, involving nitrite, peroxynitrite, hypochlorous acid and peroxidases, can induce tyrosine nitration and may contribute to tissue damage including gingivomucosal tissues (Lohinai et al. 1998). In addition to nitric oxide, peroxynitrite is also generated in ligature-induced periodontitis (Lohinai et al. 1998).

Therefore, in this study we clearly demonstrate that Tempol treatment significantly decrease the markers of ROS-

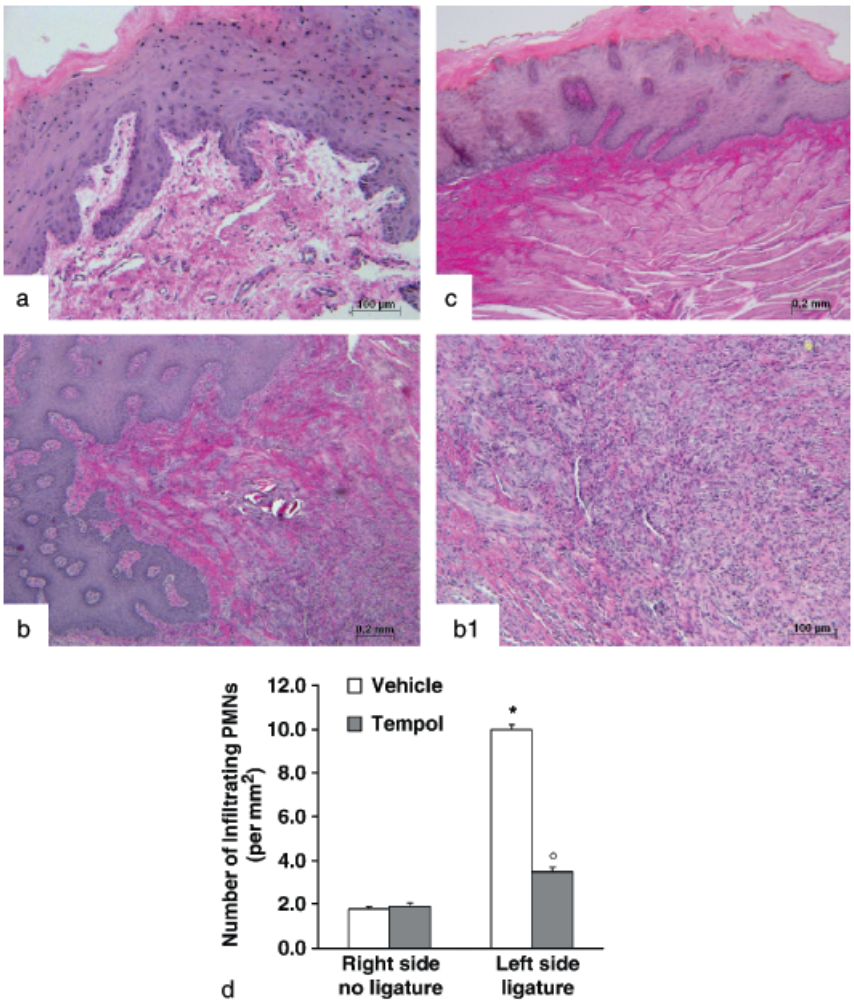

Fig. 4. Gingivomucosal section from non-ligature-treated rats (a) demonstrating no tissue damage. Inflammatory cells' infiltration and oedema were observed in gingivomucosal section from ligature-treated rats (b, b1). Significantly less oedema and inflammatory cells infiltration were observed in gingivomucosal section from ligature-treated rats that had been treated with Tempol (10 mg/kg i.p., daily for 8 days) (c). The total number of infiltrating leucocytes (e.g., neutrophils and mononuclear cells) in gingivomucosal tissue was assessed quantitatively by counting the number of polymorphonuclear cells in 20 high-power fields (D). The Figure is representative of at least three experiments performed on different experimental days. Data represent the mean ± SEM for 20 counts obtained from the gingivomucosal tissue of each treatment group. $^{*}p<0.01$ *versus* non-legated; $^{\circ}p<0.01$ *versus* legated.

mediated tissue damage. ROS produce strand breaks in DNA that triggers energy-consuming DNA repair mechanisms and activates the nuclear enzyme PARP. There is evidence that the activation of PARP may also play an important role in inflammation (Szabo 1997), and we demonstrate here that Tempol treatment reduced the activation of PARP during ligature-induced periodontitis in the gingivomucosal tissues. Thus, we propose that the anti-inflammatory effects of Tempol may be, at least in part, because of the prevention of the activation of PARP. In conclusion, this study provides the first evidence that Tempol causes a substantial reduction of ligature-induced periodontitis in the rat. Finally, our findings suggest that, taking into consideration the inherent limitations between experimental animal model and human disease, interventions, which may reduce the generation or the effects of ROS, may be useful in conditions associated with local or systemic inflammation like active periodontitis.

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Address:
Salvatore Cuzzocrea
Institute of Pharmacology
School of Medicine
University of Messina
Torre Biologica – Policlinico Universitario Via C Valeria – Gazzi
98100 Messina
Italy
E-mail: salvator@unime.it

Clinical Relevance

The chronic inflammatory disease of periodontal tissues is periodontitis, one of the most frequent human diseases. While periodontitis supports the protection against local microbial attack, this inflammatory reaction may also damage the surrounding cells and connective tissue structures, including alveolar bone causing tooth loss. In the present study, a well-established rat model of periodontitis was utilized, which involves a ligature around the cervix of the mandibular first molar tooth, and a similar model has previously been used in several species. In this model, legation induced periodontitis by: (i) a mechanical trauma on the dentogingival area, thereby reducing tissue integrity and allowing for intense host–plaque interaction and (ii) a plaque-formation-promoting factor, thus increasing the number of bacteria. Initiation of periodontal disease by bacteria is well documented, and the end result, destruction of the alveolar bone and other connective tissues, is readily observed. However, the molecular events that promote these alterations are not completely understood. In order to investigate the potential role of oxidative stress in the pathophysiology of periodontitis, we used Tempol, a membrane-permeable radical scavenger, which exerts protective effects in various models of inflammation and shock. Our findings suggest that interventions, which may reduce the generation or the effects of ROS, may be useful in conditions associated with local or systemic inflammation like periodontitis.