Archives of Oral Biology 81 (2017) 61–68

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Is caffeic acid phenethyl ester more protective than doxycycline in experimental periodontitis?

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ARTICLE INFO

Keywords:
Caffeic acid phenethyl ester-CAPE
Doxycycline
Periodontitis
Cytokines
Oxidative stress
Apoptosis

ABSTRACT

Background and objectives: Host modulation therapies (anti-inflammatory drugs, bone-stimulating agents, anti-proteinase etc.) target the inhibition or stabilization of tissue breakdown. The aim of the present study was to evaluate the effects of caffeic acid phenethyl ester (CAPE) and/or low dose doxycycline (LDD) administrations on alveolar bone loss (ABL), serum cytokines and gingival apoptosis, as well as the levels of oxidants and anti-oxidants in rats with ligature-induced periodontitis.

Material and methods: The animals were randomly divided into five groups: Group C (periodontally healthy), Group PC (Periodontitis + CAPE), Group PD (Periodontitis + LDD), Group PCD (Periodontitis + CAPE + LDD), Group P (Periodontitis). Experimental periodontitis was induced for 14 days. Levels of ABL, and the serum cytokines, interleukin (IL)-1 β, IL-6, tumor necrosis factor-α (TNF-α) and IL-10 were assessed as were the levels of the oxidants and anti-oxidants, malondialdehyde (MDA), glutathione (GSH) and glutathione peroxidase (GSH-Px), and levels of gingival apoptosis.

Results: The lowest ABL levels was evident in the PC group, among the experimental groups. There was also less inflammatory infiltration in the PC group than the PD group. IL-1β, IL-6, and IL-10 were lower in the PC group and higher in the P group in comparison to the levels in the other experiment groups. TNF-α levels in the PD group were higher than levels in the PC and PCD groups. The PC and PCD groups did not differ from the C group in regard to MDA levels. The highest GSH-Px level was found in the PC group. Gingival apoptosis in the PC group was not only lower than the PD and PCD groups, but also lower than in the C group.

Conclusion: The present study suggests that CAPE has more anti-inflammatory, anti-oxidant and anti-apoptotic effects than LDD, with no additive benefits of a CAPE + LDD combination being evident in rats with periodontitis.

1. Introduction

Periodontitis, is a chronic inflammatory disease caused by dental plaque bacteria and their products as well as the associated host immune response. The interaction between dental plaque bacteria and the host immune response induces the production of cytokines and enzymes that contribute to excessive amounts of reactive oxygen species (ROS) (Dahiya et al., 2013). Serum levels of pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α) in periodontitis have been shown to increase (Andrukhov et al., 2011) or remain unchanged (Yamazaki et al., 2005). In addition, IL-10, an anti-inflammatory cytokine, is also increased in periodontitis (Andrukhov et al., 2011).

The hyperfunction of polymorphonuclear leukocytes (PMNLs), which are inflammatory cells, induces ROS production (Dahiya et al., 2013). Levels of lipid peroxidation (LPO) products are the most common indicants of oxidants and oxidative damage. Malondialdehyde (MDA) arises as consequence of the peroxidation of polyunsaturated fatty acids in cells (Dahiya et al., 2013). The imbalance between the pro-oxidant and anti-oxidant system leads to further oxidative damage and the eventual destruction of periodontal tissue. Glutathione (GSH) and glutathione peroxidase (GSH-Px), ubiquitous antioxidant and anti-

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http://dx.doi.org/10.1016/j.archoralbio.2017.04.017
Received 26 September 2016; Received in revised form 9 March 2017; Accepted 17 April 2017

oxidant enzyme respectively, afford protection against LPO products. Due to the scavenging of the excessive LPO products generated at inflammatory sites, increased GSH and GSH-Px activities may be observed in periodontitis (Dahiya et al., 2013).

Apoptosis also plays an important role in many pathophysiological mechanisms, such as the regulation of the host immune response and mitosis-induced cell proliferation. Apoptotic processes can be stimulated by a variety of factors, including radiation exposure (Eriksson & Stigbrand, 2010), cytokines, bacterial and viral infections, and immune cells, as well as alterations in growth factors, nutrients, and the extracellular matrix (Thompson, 1995). Oxidative stress can also stimulate apoptosis. The eliminations of free radicals and ROS can prevent apoptosis and reduce oxidative DNA damage, as well as upregulating the expression of anti-oxidative enzymes (Proksch et al., 2014; Sinai et al., 2004). Gamonal, Bascones, Acevedo, Blanco, and Silva (2001) showed apoptotic processes to be increased in chronic periodontitis patients. As such, apoptotic processes may also play a role in the pathogenesis of periodontitis.

Host modulation therapies (HMTs) target the inhibition or stabilization of tissue breakdown, including by inhibiting or modulating the pro-inflammatory aspects of the host response, as well as increasing the levels of regenerative or preventive responses (Bhatavadekar & Williams, 2009). HMTs can be classified as anti-inflammatory drugs, bone-stimulating agents (bisphosphonates) and anti-proteinase, such as low-dose doxycycline (LDD) (Yağan, Kesim, & Liman, 2014). New types of HMTs that also modulate the host response, include caffeic acid phenethyl ester (CAPE) (Choi et al., 2015).

Doxycycline (DOX) is a member of the family of tetracycline antibiotics and exhibits potent immunomodulatory activities in several disease models (Yağan et al., 2014). Aside from its anti-microbial properties, LDD also has anti-inflammatory (Castro et al., 2016) and anti-oxidant effects (Yağan et al., 2014). LDD administration significantly downregulates mRNA expressions of pro-inflammatory cytokines in rats with periodontitis (Castro et al., 2016). LDD reduces oxidant enzyme levels *via* ROS scavenging and collagenolytic activity, with clinically proven benefits as an adjunctive treatment for periodontitis (Yağan et al., 2014).

CAPE has been extensively investigated for its polyphenolic activity, particularly the effects of its components, propolis. CAPE has anti-oxidant, anti-tumoral, anti-inflammatory, immunomodulatory and properties (Akyol et al., 2012). CAPE reduces pro-inflammatory cytokines (Orban, Mitsiades, Burke, Tsokos, & Chrousos, 2000). CAPE also shows anti-apoptotic activity that is mediated, at least in part, by its inhibition of nuclear factor kappa B (NF-kB) (Lu et al., 2002). Moreover, CAPE significantly inhibits ROS production during inflammatory processes (Akyol et al., 2012). Combined HMT treatments are an area of considerable interest in periodontal therapy. DOX and bisphosphonate can synergistically inhibit periodontal breakdown (Lee et al., 2004; Yaffe, Herman, Bahar, & Binderman, 2003). Yaffe et al. (2003) reported that the local application of a combined therapy, using a tetracycline and bisphosphonate, reduced rat alveolar bone loss (ABL). His-Ming Lee et al. (2004) showed that combining LDD with non-steroidal anti-inflammatory drugs enhanced the efficacy of periodontal treatment. To date, there have been no studies investigating the efficacy of LDD combined with CAPE in the management of periodontitis. The aim of the present study was to evaluate the effects of LDD and CAPE on ABL and gingival apoptosis, as well as the serum levels of cytokines and pro-oxidant and anti-oxidant markers, in an experimental periodontitis rat model.

2. Materials and methods

Forty-eight male Wistar albino rats, weighing 200 ± 20 g, were used for the experimental procedure. The experimental protocol of the study was approved by the ethical committee of the School of Medicine, Süleyman Demirel University (Protocol no: 2012-26-09). Animals were maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory animals prepared by the Süleyman Demirel University. The rats were randomly divided into the following five groups: Group C (periodontally healthy, n = 8), Group P (periodontitis, n = 10), Group PC (periodontitis + CAPE, n = 10), Group PD (periodontitis + LDD, n = 10), Group PCD (periodontitis + CAPE + LDD, n = 10). The rats were housed under standard environmental condition. Temperature (24.0 ± 0.6 °C) and a 12/12-h light/dark cycle, with lights on at 07.30 h, were maintained throughout the experimental period. Standard rat food and tap water were available ad libitum for the duration of the experiment.

Experimental periodontitis was induced in rats under general anaesthesia by the intraperitoneal injection of ketamine,[1] (90 mg/kg of body weight) and xylazine[1] (10 mg/kg of body weight) by placement of sterile 3–0 silk ligatures[2] in a subgingival position around the maxillary 2nd molars in all groups for 14 days, except Group C. All rats were periodontally healthy because of the exclusion of rats with 0.5 mm periodontal probing depths. CAPE[3] 10 μmol/kg/day, was intraperitoneally administered (Sud'Ina et al., 1993), with LDD,[4] 10 mg/kg/day, being administered by oral gavage (Bezerra, Brito, Ribeiro, & Rocha, 2002; Yağan et al., 2014), over the experimental 14 day period.

After serum samples collections were made, rats were sacrificed. Maxillae were excised and separated between the central incisors and along the midline symmetrically. Right maxillary halves were utilized for histomorphometry analysis, whilst left maxillary halves were utilized for histopathological evaluations. Apoptosis was investigated on gingival tissue collected around the maxillary right 2nd molar.

After the right maxilla halves were fixed in 3% H_2O_2 for 24 h, right halves were stained with 1% methylene blue for one minute, in order to determine the cemento-enamel junction (CEJ) (Kinane, 2001). ABL was measured by recording the distance between CEJ and the alveolar bone crest (ABC) using a stereomicroscope (×4 magnification). ABL measurements were performed at three points in the buccal area (Grauballe et al., 2005). Morphometric analysis of the ABL was performed with standardized digital photography[5] and images were analysed with software programme.[6] The measurements were repeated twice at separate times by the same examiner.

For histopathological evaluation, left maxillae halves were fixed in 10% buffered formalin solution and decalcified with a 0.1 M EDTA solution for 2 weeks. The specimens were then washed, dehydrated and embedded in paraffin. Serial sections of the paraffin blocks (5 μm thickness) were cut in the mesio-distal direction along the long axis of the tooth and were stained with haematoxilin and eosin (H & E). Evaluations were made by a light microscope at ×40 magnification. Histopathological scoring criteria, modified from Leitão et al., (2005), were used. Inflammatory cells infiltrations, alveolar bone resorption, degeneration and destruction of the cementum were evaluated. Morphometric analyses of ABL and PMNLs infiltration were made using the Database Manual Cell Sens Life Science Imaging Software System.[7] The number of PMNLs in the junctional epithelium and connective tissue subjacent to the epithelium (in 0.05 mm × 0.05 mm area) were examined and counted under a magnification of ×40 (Yoshinaga et al., 2014).

[1] Pfizer, Kent, UK.
[2] 3.0, Doğsan, Istanbul, Turkey.
[3] Sigma-Aldrich, St. Louis MO, USA.
[4] Doksilin LA, Provet, Istanbul,Turkey.
[5] Leica MZ6, Wetzlar, Germany.
[6] ImageJ 1.46r, Bethesda MD, USA.
[7] Olympus Co., Tokyo, Japan.