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Green tea polyphenol epigallocatechin-3-gallate and cranberry proanthocyanidins act in synergy with cathelicidin (LL-37) to reduce the LPS-induced inflammatory response in a three-dimensional co-culture model of gingival epithelial cells and fibroblasts

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ABSTRACT

Objectives: The human antimicrobial peptide cathelicidin (LL-37) possesses anti-inflammatory properties that may contribute to attenuating the inflammatory process associated with chronic periodontitis. Plant polyphenols, including those from cranberry and green tea, have been reported to reduce inflammatory cytokine secretion by host cells. In the present study, we hypothesized that A-type cranberry proanthocyanidins (AC-PACs) and green tea epigallocatechin-3-gallate (EGCG) act in synergy with LL-37 to reduce the secretion of inflammatory mediators by oral mucosal cells.

Methods: A three-dimensional (3D) co-culture model of gingival epithelial cells and fibroblasts treated with non-cytotoxic concentrations of AC-PACs (25 and 50 μg/ml), EGCG (1 and 5 μg/ml), and LL-37 (0.1 and 0.2 μM) individually and in combination (AC-PACs + LL-37 and EGCG + LL-37) were stimulated with *Aggregatibacter actinomycetemcomitans* lipopolysaccharide (LPS). Multiplex ELISA assays were used to quantify the secretion of 54 host factors, including chemokines, cytokines, growth factors, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs).

Results: LL-37, AC-PACs, and EGCG, individually or in combination, had no effect on the regulation of MMP and TIMP secretion but inhibited the secretion of several cytokines. AC-PACs and LL-37 acted in synergy to reduce the secretion of CXC-chemokine ligand 1 (GRO-α), granulocyte colony-stimulating factor (G-CSF), and interleukin-6 (IL-6), and had an additive effect on reducing the secretion of interleukin-8 (IL-8), interferon-γ inducible protein 10

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(IP-10), and monocyte chemoattractant protein-1 (MCP-1) in response to LPS stimulation. EGCG and LL-37 acted in synergy to reduce the secretion of GRO-α, G-CSF, IL-6, IL-8, and IP-10, and had an additive effect on MCP-1 secretion.

Conclusion: The combination of LL-37 and natural polyphenols from cranberry and green tea acted in synergy to reduce the secretion of several cytokines by an LPS-stimulated 3D co-culture model of oral mucosal cells. Such combinations show promising results as potential adjunctive therapies for treating inflammatory periodontitis.

1. Introduction

Periodontitis is a multifactorial chronic inflammatory disease of polymicrobial origin that causes the destruction of the tooth-supporting tissues, including the periodontal ligament and alveolar bone.[1] It is initiated by a limited number of Gram-negative bacteria that colonize the subgingival area and that activate the host immune response.[1] More specifically, *Aggregatibacter actinomycetemcomitans* has been strongly associated with the aggressive form of periodontitis.[2] The lipopolysaccharide (LPS) of this bacterial species is considered as a major virulence factor since it can promote the adhesion to and invasion of oral mucosal cells and, consequently, the activation of the host immune response, resulting in the secretion of large amounts of pro-inflammatory cytokines and matrix metalloproteinases (MMPs), which modulate periodontal tissue destruction.[3–5]

Gingival epithelial cells are the primary physical barrier to tissue invasion by periodontopathogens such as *A. actinomycetemcomitans*, while gingival fibroblasts are the main cells in the periodontal connective tissue and play an important role in periodontal tissue repair and inflammatory processes induced by periodontopathogens.[6] Both cell types are able to secrete antimicrobial peptides, which are small cationic molecules that play significant roles in the innate host immune response to infections and that possess a broad activity spectrum against pathogens.[7–10] Cathelicidins are an important class of mammalian antimicrobial peptides, and hCAP18 is the only cathelicidin found in humans.[11] Its C-terminal end is proteolytically cleaved to generate LL-37, an active 37-amino-acid peptide beginning with two leucine residues.[11,12] LL-37 is expressed by several cell types, including monocytes, neutrophils, and epithelial cells, and possesses pleiotropic effects.[11,12] LL-37 reduces *Porphyromonas gingivalis*- and LPS-induced interleukin-6 (IL-6), interleukin-8 (IL-8), nitric oxide (NO), and tumour necrosis factor-alpha (TNF-α) production by human gingival fibroblasts[13] and bone marrow-derived macrophages (BMDM).[14] It has also been reported that LL-37 reduces the secretion of pro-inflammatory cytokines such as IL-6 and IL-8 by oral cells by inhibiting ligand recognition of TLRs and by binding to LPS, thus preventing the interaction between LPS and the CD14 receptor.[15,16] LL-37 has been detected in both gingival crevicular fluid and saliva.[17,18] LL-37 levels in gingival crevicular fluid are lower in diseased periodontal sites than in healthy sites.[19,20]

Given the multifactorial aetiology of periodontitis, a combination of drugs may produce a better therapeutic outcome through synergistic effects that will make it possible to achieve similar or superior treatment outcomes with lower doses of therapeutic agents.[21] Phytochemicals are promising bioactive molecules for preventing and treating oral diseases since they act on both pathogens and the host inflammatory response.[22] Cranberry (*Vaccinium macrocarpon*) polyphenols have received attention as potential therapeutic agents for the prevention of many human diseases, including cancer, cardiovascular diseases, and infectious diseases.[23,24] Cranberry proanthocyanidins possess unusual structures with A-type linkages as well as a second ether linkage between an A-ring of the lower unit and the C-2 ring of the upper unit (O7 C2).[25] In recent years, a number of studies have provided evidence of the beneficial effect of cranberry proanthocyanidins on periodontal disease through their capacity to inhibit biofilm formation, tissue-destructive enzymes, and inflammatory cytokine secretion by immune and mucosal cells.[26–29] Green tea (*Camellia sinensis*) polyphenols are mostly catechins, the most predominant being epigallocatechin-3-gallate (EGCG), which possesses a number of beneficial pharmacological properties.[30,31] EGCG inhibits the growth of major periodontopathogens, interferes with osteoclast formation, and reduces the secretion of pro-inflammatory cytokines by gingival fibroblasts, epithelial cells, and endothelial cells.[32–36] Hirasawa et al.[37] reported that the local delivery of green tea catechins into periodontal pockets can enhance the success of conventional periodontal treatments.

To the best of our knowledge, there are no findings reported in the literature on potential synergistic effects between polyphenols and LL-37 with respect to anti-inflammatory properties. The aim of the present study was to evaluate the synergistic interactions of AC-PACs and EGCG when used in combination with LL-37 on the inhibition of pro-inflammatory mediator, matrix metalloproteinase (MMP), and tissue inhibitor of metalloproteinase (TIMP) secretion by a three-dimensional (3D) co-culture model of gingival epithelial cells and fibroblasts stimulated with *A. actinomycetemcomitans* LPS.

2. Materials and methods

2.1. LL-37, A-type cranberry proanthocyanidins (AC-PACs), epigallocatechin-3-gallate (EGCG), and LPS

A synthetic LL-37 peptide (H-LLGDFFRKSKEKIGKEFKRIVQ-RIKDFLRNLVPRTES-OH) (Biomatik, Cambridge, ON, Canada) was dissolved in sterile UltraPure™ DNase/RNase-free distilled water (Life Technologies Inc., Canada) at a concentration of 1 mM and was stored at −20 °C until used. AC-PACs, kindly provided by A. Howell (Rutgers, The State University of New